Guide to the quality and safety of TISSUES AND CELLS for human application

European Committee (Partial Agreement) on Organ Transplantation (CD-P-TO)

EDQM
4th Edition
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COUNCIL OF EUROPE
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Guide to the quality and safety of tissues and cells for human application

4th Edition

European Directorate for the Quality of Medicines & HealthCare (EDQM)

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Foreword

Founded in 1949, the Council of Europe is the oldest and largest of all European institutions and now numbers 47 member states.* One of its founding principles is that of increasing co-operation between member states to improve the quality of life for all Europeans. Within this context of intergovernmental co-operation in the field of health, the Council of Europe has consistently selected ethical problems for study. One of the most important of these ethical issues relates to the prohibition of financial gain from the human body and its parts, including blood, organs, tissues and cells.

Transplantation-related activities at the Council of Europe are co-ordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM). This directorate is a key European organisation involved in the harmonisation, co-ordination, standardisation, regulation and quality control of medicines, blood transfusion, organ, tissue and cell transplantation, pharmaceuticals, pharmaceutical care, consumer health, cosmetics and food packaging.

Transplant and reproductive medicine have progressed during recent decades in ways that could not have been imagined previously. As with organs, the demand for some tissues and cells far outweighs the available supply. This has important consequences because human tissues and cells for human application can restore essential functions or, in some cases, save lives. However, as with all material of human origin, they carry risks of disease transmission that must be controlled by application of scrupulous donor selection and testing criteria, and also by ensuring that comprehensive quality systems are in place.

The *Guide to the quality and safety of tissues and cells for human application*, published first in 2013 and now in its 4th edition, collates the most up-to-date information to provide healthcare professionals with a comprehensive overview of the most recent advances in the field as well as technical guidance on ensuring the quality and safety of human tissues and cells applied to patients. To increase safety for the recipients of human tissues and cells, it is essential that professionals involved in identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood collection centres, fertility clinics, tissue establishments processing and storing tissues and cells, testing laboratories, organisations responsible for human application, inspectors auditing these establishments and Health Authorities responsible for tissues and cells for human application all have easy access to this information. This Guide aims to support professionals at a practical level and improve

* Albania, Andorra, Armenia, Austria, Azerbaijan, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Republic of Moldova, Monaco, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Russian Federation, San Marino, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom.

the rate of successful and safe clinical application of tissues and cells.

This Guide includes recommendations considered to be minimum standards that align with the principles set out in the various relevant European Union (EU) directives. Thus, the Guide provides technical support both to EU member states that have implemented the directives and to those non-EU states that are considering their adoption. These minimum standards state 'what must be done'; however, this Guide goes further by providing additional technical advice based on good manufacturing practice (GMP) guidelines and on best practice consistent with current scientific knowledge, expert opinion and the results of many international projects. It describes background information that should be considered and it informs policy decisions and educational initiatives by explaining the 'why and how'. It also refers to recent developments that may be reflected in future updates of EU legislation, where necessary and relevant, thereby providing advance information and recommendations regarding developments in the field. Throughout this Guide, use of the word 'must' indicates mandatory compliance in alignment with Council of Europe recommendations and resolutions as well as with EU directives, whereas the use of the word 'should' indicates recommended compliance in accordance with commonly accepted good practice.

Whereas tissue establishments in EU member states are required to comply with legislation derived from EU directives, this Guide has a broader scope and is intended to facilitate ongoing improvements in the donation, procurement, testing, processing, preservation, storage and distribution of tissues and cells through education and the provision of non-binding recommendations for all Council of Europe member states, including those in the EU, but also beyond the Council of Europe borders. At any given time, implementation of these recommendations among member states and individual tissue establishments may vary, and alternative procedures, practices and equivalent standards of quality and safety based on a careful risk-based assessment may be in place.

Unless otherwise stated, the guidelines apply only to human tissues and cells intended for transplantation or clinical use (including medically assisted reproduction). Tissues and cells used for research, as well as tissues and cells procured and applied in the same medical procedure, do not fall under the scope of the present Guide.

In addition to all the new content in this 4th edition, chapters have been updated and extended. This responds to an increased interest in the field of tissues and cells, not only from professionals with regard to their potential applications and quality standards but also from Health Authorities, since the rapid development of novel processing methods and clinical applications requires the establishment of well-defined quality and safety criteria on which to base regulatory requirements. Therefore, in this 4th edition great efforts have been made to respond to these needs, bringing the number of chapters from 29 in the previous edition to 35, in addition to the new Parts D (tissue and cell monographs) and E (Good Practice Guidelines for tissue establishments).

As a consequence of all the new content, this Guide has been divided into five parts.

- Part A (Chapters 1-16) contains general requirements applicable to all tissue establishments and organisations involved in the donation, procurement, testing, processing, preservation, storage and distribution of tissues and cells. Some of the main changes include:

– **Chapter 1: Introduction** has been updated to include new developments in the field, including laws, regulations and collaborative projects.

– **Chapter 2: Quality management, risk management and validation** now defines the responsibilities of key personnel managing the quality system and has expanded the guidance on protection of data, risk-management methodologies and root-cause analysis.

– **Chapter 4: Donor evaluation** aims to improve readability and clarity by separating the assessment of potential donors into autologous and allogeneic donors. The chapter also develops the guidance for the evaluation of malignancies, highlighting criteria that should be met in order to accept potential donors.

– **Chapter 5: Donor testing – markers for infectious diseases** now develops in a more comprehensive manner the guidance on how to interpret results of mandatory tests. The chapter has also been revised by updating the advice on algorithms for testing of parameters for HIV, HBV and HCV and how to manage the presence of antibodies against hepatitis C virus in the absence of viral RNA. Of particular importance is the recommendation for the widest possible introduction of PCR techniques that have been assessed both qualitatively and quantitatively. A new appendix to this chapter also elaborates recommendations for testing of parameters for syphilis infection and how to interpret results.

– **Chapter 6: Procurement** expands the guidance on risk assessment during the recovery proce-

dure for different tissues and cells and expands the requirements for environmental air quality during the procurement procedure when processing with a specific algorithm.
- **Chapter 7: Premises** has been adapted to include new insights, technologies, expectations and risk-management concepts defined by international standards such as ISO14644-1 and 2 and EU GMP Annex 1. Concepts such as a validation master plan for the qualification of cleanrooms and a monitoring plan to gain assurance of aseptic processes have been updated. The terminology used in this chapter has also been thoroughly revised to improve clarity.
- For this edition, processing and storage have been developed into separate chapters. **Chapter 8: Processing** now expands the information on decellularisation techniques and methods and agents used. It also provides guidance on processing validation and the disposal of discarded tissues and cells.
- **Chapter 9: Storage and release** now describes in more detail the different types of storage and storage validation; it also includes the requirements for storage facilities and expands the section on release. Special attention has been given to the disposal of tissues and cells that have been discarded.
- **Chapter 10: Principles of microbiological testing** has been revised to provide more guidance on testing for bacterial endotoxins and bioburden testing. Additional guidance has been provided on the correct use of microbiological test methods described in the *European Pharmacopoeia*.
- **Chapter 12: Organisations responsible for human application** now includes the latest legal requirements regarding the protection of personal data. It emphasises the need for close co-operation between tissue establishments, organisations responsible for human application and Health Authorities in responding to the introduction of novel processes and clinical applications in the field of tissues and cells. It also notes that distribution to individuals should be avoided and draws attention to the role of registers with data on clinical outcomes when assessing the safety and effectiveness of therapies involving human tissues and cells.
- **Chapter 13: Computerised systems** has expanded the information on V-model diagrams for computer system implementation, illustrating design flow and documents related to specific phases (life-cycle documentation), together with the responsibility relationship between user and supplier, and how to handle software life-cycle activities. In addition, guidance on data protection has been updated to meet the requirements of new EU legislation.
- **Chapter 14: Coding, packaging and labelling** and **Chapter 15: Traceability** now provide more examples and structured guidance.
- **Chapter 16: Biovigilance** has been enhanced to define the three levels of biovigilance (urgent communication, routine notification and continuous, proactive monitoring) depending on the types of measures and actions that can be taken into consideration. It also expands on the notion of surveillance as a means to provide indicators and information on stratification of risks, and it provides examples of serious adverse events.

- Part B (Chapters 17-28) contains specific guidelines and requirements for the various tissue and cell types. Each of the chapters has been revised, updated and extended with additional information provided by experts from scientific and professional organisations, including the European Association of Tissue and Cell Banks (EATCB), the European Eye Bank Association (EEBA), the European Society for Human Reproduction and Embryology (ESHRE), the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee–ISCT & EBMT (JACIE). Relative contraindications for the acceptance of donors of these tissues and cells have been revised, considering case-by-case scenarios requiring careful risk assessment. In addition, non-exhaustive lists of examples of adverse events and reactions have been provided. Due to the increased interest in new processing methods and applications, every chapter contains information about developing applications for treatment of patients. Some other changes include:
- The inclusion of a new chapter, **Chapter 23**, dealing with umbilical cord blood progenitors, along with several related appendices (**Appendix 29. Health assessment questionnaire for cord blood donor**; **Appendix 30. Data collection for cord blood donor**).
- Dedicated chapters expand the guidance on pancreatic islets (**Chapter 24**), hepatocytes (**Chapter 25**) and adipose tissue (**Chapter 26**).
- In **Chapter 27: Medically assisted reproduction** the nomenclature has been thoroughly

revised in order to better comply with the International Glossary on Infertility and Fertility Care from 2017. As a consequence, an important change in terminology – from assisted reproductive technology (ART) to medically assisted reproduction (MAR) – has been implemented throughout the Guide. In addition, this chapter has been revised to give updated guidance on viral screening, serodiscordance and quality control, including biovigilance and definitions and benchmarking of serious adverse reactions and events. The sections on pre-implantation genetic testing and developing applications have been expanded, while the sections on reproductive screening of the male/female have been shortened.

– **Chapter 28: Fertility preservation** has been updated with new information to provide better context for this topic. New material has been added in almost all sections of the chapter, with special emphasis on procurement, processing and storage, and biovigilance. The new material includes informative illustrations on procurement of both male and female gonadal tissue and methodology aspects. The examples of adverse events and reactions, with their management and quality-control aspects, have been developed.

- Part C (Chapters 29-35) addresses novel therapeutic approaches. As the fields of donation and transplantation of tissues and cells evolve, new and more sophisticated technologies provide opportunities to make tissues and cells safer and their engraftment more effective. This section includes not only tissues and cells that are already in routine use in patients but others that are in research and development and are undergoing clinical trials. It is worth noting that, in different countries, depending on the degree of complexity of the processing or the manner in which the tissues or cells are applied to the recipient, some of these products may be regulated under different regulatory frameworks. For example, in the EU, some of the activities described in this section are considered to be manufacturing of advanced therapy medicinal products (ATMPs), and professionals involved in the processing, distribution and clinical application of such products are required to comply with the regulatory framework for medicinal products. In many non-EU European countries, this differentiation is not made at a regulatory level, or there is no regulation of the field. Even in EU member states, many tissue establishments are working with tissues or cells that are subsequently sent for manufacture as ATMPs or are manufacturing those products themselves under the 'hospital exemption' allowed by the EU Regulations on ATMPs. Some other substances described in this section may fall under yet different regulatory frameworks, such as those governing blood or food. This Guide does not attempt to provide any regulatory guidance on the processing or use of these substances and aims only to provide scientific recommendations to ensure their quality and safety. Chapters of this Guide that relate to donor selection, consent, procurement and the testing of tissues and cells apply in full to the tissues and cells donated for any clinical application, including those covered in Part C. Some of the main features in this section include:

– The new **Chapter 29. Introduction of novel processes and clinical applications** outlines key elements to be considered when developing and authorising novel processes and clinical applications; **Chapter 30: Developing cell technologies** provides a didactic overview of the field by describing the different ways in which cells can be expanded, modified or combined with scaffolds to replace damaged or diseased tissues in the recipient. This chapter also includes information about the regulatory framework governing the production and use of some of these therapies in the EU.

– **Chapter 31: Preparation of natural scaffolds** provides technical guidance to any tissue establishment in decellularisation techniques and the preparation and potential use of scaffolds.

– **Chapter 32: Somatic cells in clinical use** focuses on developing applications for several types of cells (apart from haematopoietic progenitor cells) that are rapidly becoming important tools for the treatment of patients. The chapter does not attempt to give complete guidance on the processing of these cells since the field is expanding rapidly. Instead, the specific issues in donor selection, procurement and testing (including testing for quality/specificity if applicable) are covered, and the chapter includes an overview, with references, as a table.

– The new Chapters 33: **Breast milk**, 34: **Faecal microbiota** and 35: **Blood components for topical use or injection** expand the information about other substances obtained from humans for autologous or allogeneic use, based

on the most recent scientific evidence. The regulatory status of these substances varies in most countries. However, the risks associated with their human origin and the processes applied to procure, process and preserve them are analogous to those of the rest of the tissues and cells described in this Guide. Therefore, these chapters provide a generic quality and safety framework for healthcare professionals treating patients with these substances.

- Part D includes the novel tissue and cell monographs that provide information about tissue and cell preparations and clinical applications that are precisely defined and have been shown to be safe and effective when used in patients (consolidated processing for a consolidated use). Tissue and cell monographs are complementary to other sections of the Guide and aim to be useful tools for tissue establishments and Health Authorities, providing the minimum criteria and controls necessary to ensure the quality of tissues and cells processed by tissue establishments. By reference to these tissue and cell monographs, both tissue establishments and Health Authorities will know that products that do not have a matching monograph may need more attention (more risk assessment, validation, possibly clinical studies etc.) before they can be authorised and supplied for routine use.
- Finally, Part E contains the newly developed Good Practice Guidelines (GPG) for tissue establishments that follow EU Directives, with the aim of promoting and assuring high levels of quality in the field of human tissues and cells. These guidelines consolidate the guidance already defined in EU legislation, the recommendations from the main chapters of this Guide, relevant elements derived from the detailed principles of GMP, the results of relevant EU-funded projects and expert opinion consistent with current scientific knowledge. The GPG should be seen as a complementary document for tissue establishments and inspectors/auditors, a document that describes in detail, and from a practical point of view, the key elements which should be defined and controlled for achieving comprehensive quality management in tissue establishments that are required to comply with EU legislation.

A dedicated working group, composed of well-recognised international experts nominated by member states, was convened for the preparation of this Guide. This group was chaired by Jacinto Sánchez Ibáñez (Spain) and John Armitage (European Eye Bank Association). This expert group made an exceptional contribution by sharing their expertise, reviewing the literature in their respective specialist areas and extracting and distilling knowledge from numerous international guidelines, collaborative projects and diverse publications and websites, with the aim of ensuring that all of this up-to-date information is made available and accessible to professionals and regulators. Members of the group co-ordinated preparation of the chapters and ensured access to appropriate and relevant expertise through the engagement of a number of additional experts from European countries and beyond, who co-authored and contributed to the discussions on various parts of this Guide. The final draft was submitted to open consultation, and close to a thousand comments and suggestions were received, all of which were carefully analysed by the working group. The professionals who participated in the preparation of the Guide are listed in **Appendix 33**. In addition, we should also acknowledge all those professionals who participated in the open consultation and provided extremely useful comments and suggestions.

The elaboration of this Guide was partly funded by the European Commission through a Direct Grant Agreement (contract number 2014 54 01) entitled 'Dissemination of best practices in tissues and cells donation/transplantation'. Special thanks are due to Deirdre Fehily, from DG-SANTE, who ensured that the current text remained aligned with EU directives and who made available the results from EU-funded projects. In addition, Ana Mancho Rojo, from the Executive Agency for Health and Consumers (CHAFEA), provided tremendous assistance with the financial and administrative tasks.

Several professional associations – in particular, the EATCB, EEBA, ESHRE, EBMT and JACIE – should also be thanked for sharing their experience and knowledge.

The drafting and publication of the 4th edition of the Guide was co-ordinated by Marta López Fraga (Scientific Officer in charge of the Council of Europe European Committee on Organ Transplantation [CD-P-TO]) and Mar Lomero (Scientific Assistant), with the assistance of Ahlem Sanchez, David Crowe, Isabelle Vernay and Gerard M.-F. Hill.

The entire project has been an exceptional combined effort, with extensive discussions dedicated to

the common goal of increasing the safety, efficacy and quality of donated tissues and cells for human application. The result is this 4th edition of the Guide, which constitutes a common European standard, based on the long-standing expertise and knowledge of the EDQM.

Part A. General requirements

Chapter 1: **Introduction**

We are entering a new age of medical and biotechnological progress. Medical procedures that were unimaginable a generation ago are a reality today. One aspect of the recent and rapid advances in biological and medical research is that human tissues and cells are being used increasingly in new ways. Many of these developments, such as advances in transplantation therapy or in medically assisted reproduction (MAR), have unquestionable benefits. However, using human tissues and cells in different ways also raises questions of safety, quality and efficacy, and presents new ethical dilemmas.

Tissue from one deceased donor may be transplanted into as many as 100 individuals. Some other tissues and cells can be provided only by living donors, as long as this procedure does not risk serious harm to the donor or endanger the donor's life. Transplantation of tissues and cells can range from life-saving treatments (e.g. in the treatment of catastrophic burns) to quality-of-life improvements. In addition, donated gametes and embryos may help fulfil a person's wish to have children.

Some tissues are used practically unaltered from the condition in which they were removed from the donor. Deceased donor corneas, for example, are used to restore sight, heart valves replace damaged ones and extend life, tendons and ligaments may be used for the treatment of sporting injuries or to repair degenerative defects, and skin can be employed to cover major burns or support the healing of ulcers. Other tissues, however, are processed into products that are almost unrecognisable as bodily material. Skin, for example, may be cut into conveniently sized dressings, incorporated into sprays or gels, or decellularised for use in various surgical procedures. Bone can be processed into hundreds of different products and distributed via a global medical market for use in orthopaedics (general and oncology), sports medicine, craniofacial/maxillofacial/dental surgery and neurosurgery. Cellular components of bone may be removed entirely and even the calcium may be removed to promote incorporation and tissue regeneration. Bone allografts may be precision-cut and sized, and bone can also be supplied in soft, pliable or injectable forms. If a deceased donor has consented to the use of any part of their body for the treatment of others (or their relatives have authorised this to fulfil the donor's wishes), then many tissues – including bone, heart valves, skin, corneas, ligaments, cartilage, connective and adipose tissue, glands and nerves – can be used for therapeutic purposes.

In contrast, amniotic membranes and placenta, parathyroid tissue and skull bone are donated by living persons. Additionally, femoral heads removed during an operation to replace a hip joint and heart valves from patients receiving a heart transplant are sometimes processed and 'recycled'. In addition, many types of cell can be donated (some during life and some after death) and submitted to different degrees of manipulation before application in humans. Examples include haematopoietic progenitor cells (e.g. bone marrow, peripheral blood progenitor cells, umbilical cord blood), somatic cells (e.g. peripheral blood cells, keratinocytes, chondrocytes, hepatocytes), mesenchymal stromal cells and limbal stem cells. Oocytes, spermatozoa, ovarian or testicular tissue and embryos can be used in MAR procedures to achieve pregnancy.

Human tissues and cells could also be the potential starting material for much more complex products in the future.

1.1. Scope and purpose of this Guide

This is the 4th edition of the Council of Europe *Guide to the quality and safety of tissues and cells for human application*. This Guide has two main objectives:

a. It aims to provide sound information and guidance – for all professionals involved in donation, banking, transplantation and other clinical applications of tissues and cells – to optimise the quality and minimise the risks of these complex procedures. All material of human origin carries risks of disease transmission that must be controlled by application of scrupulous criteria of donor selection and testing, and comprehensive systems to assess quality. The idea behind this Guide is to help professionals on a practical level by providing generic guidance that will help improve the rate of successful clinical application of tissues and cells.

b. This Guide includes ethical principles and guidelines to be considered for the donation and human application of tissues and cells.

The field of tissue and cell donation and banking is now highly regulated in many countries. In the European Union (EU), several directives describe the requirements and have been transposed into the national legislation of the EU member states. This Guide refers to those requirements where appropriate, but goes beyond them to describe generally accepted good practice at a technical level and includes some consideration of ethical issues. Therefore, it will be useful as a source of practical information for those working within the EU legislative framework and those working within national legal frameworks in all Council of Europe member states and beyond. It is worth noting that, in the EU, activities involving cells and tissues that are subject to substantial manipulation or are used for a different essential function in the recipient than in the donor are not governed by tissues and cells directives but fall under Regulation (EC) No. 1394/2007 (the 'ATMP Regulation'). Thus, in EU countries, this Guide is not applicable to such activities, other than considerations related to donation, procurement and testing. In any case, it is important to take into account that this Guide does not provide any guidance on how human tissues and cells are or should be regulated, so users of this Guide are advised to carefully consider the national legal requirements that apply to the activities they are undertaking.

According to the World Health Organization (WHO) aide-mémoire on the donation and transplantation of tissues and cells [1], national Health Authorities are responsible for ensuring that the donation, banking and human application of tissues and cells are promoted, regulated and monitored appropriately in the interests of patient safety and public transparency. More specifically, they are responsible for ensuring that:

a. an appropriate legislative/regulatory framework is in place;

b. national/international practice standards have been defined;

c. there is inspection/authorisation of screening, testing, procurement, processing, storage, distribution, import and export;

d. there are programmes for vigilance and surveillance of adverse outcomes;

e. there is monitoring and reporting of donation, processing, storage, distribution and import/export activity.

In this Guide, the term 'Health Authority' is used throughout to refer to a body that has been delegated the responsibility for these activities on a national or regional basis by their government. Other similar terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it. It should be noted that in some countries, the activities described in this Guide may be controlled by different Health Authorities – e.g., separate authorities may regulate MAR and the donation, banking and human application of other tissues and cells. Unless otherwise indicated, the term 'member states' applies to member states of the Council of Europe.

Human tissues and cells also play a key part in medical research. In clinical trials of new medicines, for example, vital information about the effects of the medicine on an individual can be obtained from samples of tissues or cells and other materials provided by research participants. However, tissue is also used much more widely in medical research, from early drug 'discovery' (such as using human tumour samples to discover possible targets for treatment) to later clinical development whereby samples may be used to identify which subgroups of patient populations respond best to a new medicine. Additionally, current research aims to develop artificial tissue that could alleviate the shortage of tissue available for human application. These forms of 'basic' research

using human tissue still have an ultimately therapeutic goal in mind. However, important though all these possibilities are, this Guide covers only tissues and cells used for current therapeutic purposes.

Similarly, all tissues and cells that are both procured and applied within the same medical procedure are outside the scope of this Guide.

Finally, a glossary of terms is provided in Appendix 3.

This book is the result of the collective effort and expertise gathered by experts nominated by the member states and professional associations in the field (see Appendix 33), as well as by the members and observers of the European Committee of Experts on Organ Transplantation (CD-P-TO), for which see Appendix 34.

For matters dealing with the use of organs and blood or blood products, see the *Guide to the quality and safety of organs for transplantation* and the *Guide to the preparation, use and quality assurance of blood components* [2], both published by the Council of Europe.

1.2. Brief history of the application and banking of tissues and cells of human origin

The best documented accounts of early transplants deal with skin transplantation, though the success or failure of these procedures has not been well documented. The first reliable account is that of the Indian surgeon Sushruta in the 2nd century BC, who used autografted skin transplantation for a nose reconstruction (rhinoplasty). Centuries later, the Italian surgeon Gasparo Tagliacozzi carried out successful skin autografts, but he consistently failed with allografts, offering the first suggestion of rejection several centuries before that mechanism could be understood. He attributed it to the 'force and power of individuality' in his 1596 work *De Curtorum Chirurgia per Insitionem*. Orthopaedic surgeons refer to the origin of their discipline as 1668 when Job van Meekeren reported on the grafting of bone from a dog's skull to correct a defect in a soldier's cranium. It was not until 1869 that the first completely documented fresh human-skin allograft was carried out by the Swiss surgeon Jacques Reverdin.

The first successful full-thickness corneal transplant, a keratoplastic operation, was carried out in 1905 by Eduard Zirm at Olomouc Eye Clinic in Moravia (now Czech Republic). Pioneering work in the surgical technique of transplantation was done in the early 1900s by the French surgeon Alexis Carrel, together with Charles Guthrie, who developed techniques for suturing arteries and veins. Their skilful anastomosis operations and new suturing techniques laid the groundwork for later transplant surgery, and Alexis Carrel won the 1912 Nobel Prize in Physiology or Medicine for his work in the field. Major steps in skin transplant occurred during the First World War, notably through the work of Harold Gillies in Aldershot, UK. Among his advances was the tubed pedicle graft, which maintained a fleshy connection from the donor site until the graft established its own blood supply.

Bone is the oldest tissue transplant on record and the most common tissue transplanted today. The first bone transplant recorded in modern times occurred in Scotland in 1878 when Sir William Macewen removed an infected humerus from a 12-year-old boy and replaced it with three allografts from an amputated tibia from another child with rickets. In 1907, Erich Lexer in Berlin developed a procedure to remove a whole knee joint from an amputee in one operating room and transport the 'warm' graft to an adjacent operating room for immediate transplant into the recipient. Five years later, Alexis Carrel's work predicted the storage of tissues for future transplantation, and surgeons began to use bones and developed their own 'bone banks'. These pioneers included Inclan in Cuba, Bush, Wilson and Hibbs in the USA, Hult working in Sweden, Judet in France, and Klen in what was then Czechoslovakia. Most of these early bone banks were simply refrigerators and, later, freezers, but greater sophistication was developed by bone banks in Leeds (UK), Berlin, Athens and Warsaw. When long-term freezer storage of long bones became feasible, limb-sparing surgery using this type of bone allograft to avoid amputations in the treatment of malignant skeletal tumours became popular. Burrwell (UK), Parrish and Mankin (USA) and Ottolenghi (Argentina) published their results.

The orthopaedic profession realised that, if very large segments of bone could be transplanted successfully, smaller segments could also be used. This resulted in a very large increase in the use of bone allografts. Tissue-storage methods were developed further during the 1950s by Hyatt at the US Navy Tissue Bank in Bethesda, Maryland, where they adapted methods of lyophilisation from the food preservation industry and applied the process to the preservation of bone and skin, which could then be easily stored, transported and reconstituted for use when needed. This method of preservation allowed

bone to be stored and transported easily without any electrical or mechanical requirements, and has had a profound effect on the availability and use of bone allografts. By the end of the 1990s, use of musculoskeletal tissue allografts (i.e. bone, cartilage, soft tissue) had become commonplace in many clinical areas. Similarly, the first deceased-donor eye bank was established in Odessa using eyes (packed in glass containers) sent by rail from a trauma centre in Moscow.

The first recorded cardiac valve transplantation was carried out in Toronto by Gordon Murray, who implanted an aortic allograft in the descending thoracic aorta to relieve aortic insufficiency in 1956. The first orthotopic transplantation of the aortic valve was undertaken by Donald Ross in London in 1962 and independently by Brian Barratt-Boyes in Auckland, New Zealand, a few weeks later. Pulmonary and mitral valves were first used as allografts in subsequent years, with the pulmonary autograft procedure being carried out first in 1967.

After the atomic bomb explosion in Japan that ended the Second World War, many scientists began to explore ways of protecting humans from radiation. The first experiments were done in mice and later in dogs by E.D. Thomas. As early as 1956, the idea that bone-marrow transplants might exert a therapeutic effect against malignancies was proposed by Barnes and Loutit, who observed an anti-leukaemic effect of transplanted spleen cells in experimental murine models. In 1959, the first human bone-marrow transplants gave proof of concept that infusions of bone marrow could provide haematological reconstitution in lethally irradiated patients with acute leukaemia. E.D. Thomas performed transplants in two patients with advanced acute lymphoblastic leukaemia, with a syngeneic graft after high-dose total body irradiation; the grafts were successful but the patients died a few months later of relapse. G. Mathé administered allogeneic bone marrow for the treatment of several patients who had suffered accidental exposure to irradiation, and most survived with autologous reconstitution. In 1965, Mathé was the first to describe long-term engraftment of sibling bone marrow, thereby demonstrating chimerism, tolerance and an anti-leukaemic effect. Although the transplant itself was successful, the patient eventually died of varicella with chronic graft-*versus*-host disease (GvHD). In 1970, M. Bortin reported 203 transplants carried out between 1958 and 1968, with only three patients alive at the time of the report. The major causes of death were graft failure, GvHD and relapse. After these disappointing results, few centres persisted and the number of transplants declined sharply.

Major progress came from the discovery of the human leukocyte antigen (HLA) system by J. Dausset and J.J. Van Rood. Selection of HLA-identical siblings as bone-marrow donors diminished the risk of rejection and GvHD. Using animal models, R. Storb and E.D. Thomas developed the model of total body irradiation for conditioning (in dogs) and the use of methotrexate for GvHD prevention. In mice, G. Santos showed that the use of cyclophosphamide could add immuno-suppression to the myeloablation of total body irradiation. He was also the first to use busulfan instead of total body irradiation. In 1988, the first successful cord blood stem-cell transplant was done to treat a child with Fanconi's anaemia with cells from his healthy HLA-identical sibling (related) donor. The first unrelated donor registry was established in London in 1974 by Shirley Nolan, whose son was diagnosed with Wiskott–Aldrich syndrome. After this first donor recruitment drive, the number of bone-marrow and peripheral haematopoietic progenitor cell donors increased all over the world, with more than 33 million donors now registered, including more than 750 000 cord blood donors [3].

Transplantation of pancreatic islets has been carried out in humans since 1990 [4]. However, it was not until 1999 that the first successful transplant of pancreatic islets, using the so-called Edmonton Protocol, was undertaken by James Shapiro [5]. European centres became active around the same period, but their transplant recipients had complications of type-I diabetes that could not be managed with insulin injections. The advantage of the Edmonton Protocol was that it allowed restoration of the finely tuned regulation of glucose metabolism through appropriate insulin production by transplanted islets. In 2005, the first pancreatic islet transplant from a living donor – from a 56-year-old woman to her 27-year-old diabetic daughter – resulted in transplanted cells producing insulin within minutes after transplantation.

On 25 July 1978, Louise Brown, the first *in vitro* fertilisation (IVF) baby, was born in Oldham, UK [6]. Her birth was the result of the collaborative work of Patrick Steptoe and Robert Edwards. Since then, this research area has seen major improvements in the laboratory – e.g. cryopreservation of gametes and embryos, intracytoplasmic sperm injection (ICSI) [7], pre-implantation genetic diagnosis [8] and clinical management (such as improvements to methods for ovarian stimulation and embryo culture conditions) – thereby leading to a considerable increase in the use of assisted reproductive technologies (ART). Since 1978, more than 7 million babies have been born worldwide through MAR. Data from the International Committee Monitoring Assisted Reproduc-

tive Technologies (ICMART) show that around 1.5 million ART cycles are now performed globally each year, with around 350 000 babies born as a result [9]. This number continues to rise.

1.3. Benefits and risks of human application of tissues and cells

Progress in the medical sciences has made it possible to effectively transplant human cells and tissues from one person into another. Transplantation of tissues, such as corneas, cardiovascular tissues, bone, tendons and skin, are all well-established therapeutic techniques. Cornea and musculoskeletal tissues are the most commonly transplanted, outnumbering organ transplants by more than tenfold. Although not all of these tissues are necessarily life-saving, such transplants nevertheless offer major therapeutic benefits to a wide range of patients. Indeed, demand is rapidly increasing for bone transplantation, particularly for secondary revision of hip-replacement operations, as well as for skin treatment of severely burned patients. Successful transplantation, even when not acutely life-saving, offers recipients major improvements in their quality of life.

Table 1.1. **The main differences between organ and tissue transplants**

Organs	Tissues
Usually life-saving	Usually not life-saving but life-enhancing
Donor pool is small	Donor pool is larger
Time to implantation is usually measured in hours and the organs cannot be preserved for future use	Time to implantation can be measured in days or years, depending on the tissue and the preservation method applied
Donor can supply only a small number of recipients	One donor's tissues can be transplanted into many patients, so donor-selection failures can affect many recipients
Cannot be sterilised or exposed to robust decontamination processes	Tissues and cells can often be subject to decontamination and/or sterilisation methodologies
Often the only therapeutic option	Alternative treatments usually available

The main differences between organ and tissue transplants are summarised in Table 1.1 Because of these differences, donor-selection criteria for tissue donors can often be more stringent.

Cells fall somewhere between organs and tissues in this comparison. They are intended to be life-saving and are usually transplanted on the basis of one donor to one recipient. However, they can be processed to some extent, though not sterilised, and they can be stored for extended periods. Where bone marrow is donated by an unrelated donor for a specific recipient and transplanted without freezing, the situation is very analogous to organ transplantation. In contrast, when cord blood is donated to a public bank, stored for years and possibly selected later for transplant to a matching recipient, the situation is more analogous to tissue banking.

In practice, the decision to transplant any donor-derived tissues or cells will always be based on a clinical assessment of the risk *versus* the benefit to the patient, taking any alternative potential therapies into consideration. This is because any human application of tissues and cells carries not only process-related risks, but donor-related disease-transmission risks. The factors influencing the clinical outcome are complex because there is an interaction between two different biological systems, namely, those of the donor and the recipient. Therefore, when assessing the risk of human application of tissues and cells, both donor and recipient should be considered. In both cases, the potential benefits of the transplant procedure should outweigh the risks. Transparent communication and good collaboration between Health Authorities, tissue establishments and clinicians treating patients are vitally important in any donation process.

Some of the most widely used tissues and cells, and their benefits for transplant recipients, are listed in Table 1.2.

With regard to the risks associated with the human application of tissues and cells, Article 6 of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin clearly establishes that:

> all professionals involved in organ or tissue transplantation must take all reasonable measures to minimise the risks of transmission of any disease to the recipient and to avoid any action which might affect the suitability of an organ or tissue for implantation.

Careful evaluation – of the donor's medical history, travel history, behavioural risks and history of malignancies – is necessary to keep the risk of transmission of infections or malignancies to the recipient as low as possible. These risks are covered in Chapter 4. Specific criteria regarding tissues and cells are discussed in detail in the relevant chapters of Part B of this Guide.

Only tissues and cells recovered, processed, stored and distributed within well-controlled quality management systems of donation, processing, storage and distribution are likely to function satisfactorily and to reach an acceptable level of safety. The donor-selection criteria and the conditions of processing and preservation are crucial parameters that need to be tightly controlled. Therefore, any organisation involved in these processes should implement a comprehensive quality-management system. Management commitment and support are essential for the development, implementation and monitoring of a quality system to ensure continuous improvement. All staff should understand the importance of quality and their role in achieving it consistently.

In summary, human application of tissues or cells can confer great benefit for a patient, but it is not without risk. In exceptional cases, a donation of tissues or cells that does not meet all the necessary safety or quality requirements may be used for human application for a particular patient. This may occur, for instance, where the transplant is likely to be life-saving and the alternative options for treatment of that patient carry a poor prognosis. Similarly, couples undergoing MAR treatments often use gametes that would not meet selection criteria for non-partner procedures (e.g. gametes from an infected partner,

Table 1.2. **Most widely used tissues and cells: the benefits for the transplant recipient**

Tissues and cells	Function	Benefits for the recipient
Amniotic membrane	Forms the amniotic sac, filled with amniotic fluid, which surrounds and protects the foetus; transfers oxygen and nutrients from mother to foetus.	Used in burns and wound healing (to reduce surface inflammation, scarring and pain in surgical applications), in certain types of ulcers and in oral, maxillofacial and ocular surface surgery.
Bones and cartilage	Support the body and protect vital organs.	Used to repair or stabilise the spine and other bones and cartilage damaged by degeneration, trauma, cancer or birth defects; also used in oral surgery and in filling bone cavities or other areas where bone mass has been lost.
Corneas/eyes	Cornea and sclera together form the outer coat of the eye: the cornea is transparent and lets light into the eye; the white sclera is opaque.	Indicated for visual problems caused by damage or deterioration of the front part of the ocular globe; if whole eyes are donated, the corneas can be used in transplants for corneal blindness and the sclera can be used for reconstructive and glaucoma surgery.
Gametes, reproductive tissues and embryos	Generate a new human being.	Used primarily for the treatment of infertility and to achieve pregnancy and live birth in single women and same-sex couples; can be stored to preserve fertility or even re-establish gonadal function (in the case of reproductive tissues). Assisted reproductive technologies can also be applied to avoid transmission of some genetic or infectious diseases.
Fascia	Fibrous tissue that covers muscles.	Used to repair tendons, muscle, ligaments and deformities.
Haematopoietic progenitor cells (bone marrow, peripheral blood progenitor cells and cord blood)	Haematopoiesis.	Used for the treatment of haemato-oncologic disorders, and genetic and autoimmune diseases.
Heart valves	Direct the flow of blood in the heart.	Used for patients with valve defects, especially in children.
Pancreatic islets	Contain beta cells, which are responsible for insulin production.	A transplantation method that restores an adequate mass of insulin-producing beta cells in patients with diabetes.
Pericardium	Forms protective lining around the heart.	Used for replacement of *dura mater* in the brain and for eye surgery.
Skin	Protects the body against injury, infection and dehydration.	Used for the treatment of burns patients, certain types of ulcer, abdominal wall repairs and reconstructive or plastic surgery.
Tendons	Attach muscle to bone.	For use in joint injuries.
Veins and arteries	Provide a structure for the flow of blood through the body.	Replace blood vessels that are damaged by disease, trauma or prolonged dialysis treatment. Also used in bypass surgery to re-route blood flow.

low-quality sperm, gametes with a well-known risk of transmission of a generic disease). Ultimately, patients contemplating use of any donated tissues or cells should discuss the risks and benefits of surgery/therapy with their surgeon/physician and make the decision that is best for them.

1.4. The process of donation of tissues and cells and their application in humans

Donation of tissues and cells and their application in humans continue to be fast-moving fields. Such rapid developments bring their own challenges. These challenges include: control of all crucial technical activities and services (procurement, transportation, processing, preservation, quality control, storage) that enable tissues and cells to be removed from one person and transferred to another body, reimbursement of expenses and service charges, safeguards from exploitation or misuse (e.g. formal requirements for consent from the potential donor before procurement of tissues or cells) and the complex chain of intermediaries (people and institutions) in the process of donation and human application.

The process of donation of tissues or cells from a deceased donor is, in many respects, quite different from the process in living donors; but, in all cases, a complex network of interactions underlies the many ways in which human material may be provided by one person for the benefit of others. Some of these complex links, using the example of a deceased donor, are summarised in Figure 1.1.

We can conceptualise the entire process in terms of organisation and workflows. In the case of donation after death, transplantation can take place only if trained professionals are available to talk to the next of kin of the deceased potential donor, if there is the necessary infrastructure to procure tissues within a given timeframe and process them, if transport services exist to transport tissues appropriately and if surgeons are available to carry out tissue transplantation into the recipient. Similarly, living donation is possible only if professionals recruit and evaluate potential donors, and adequately trained personnel carry out the processes that will generate the medical products used to treat patients.

Tissue establishments play a central role in modern medicine by providing material for treatment and research. Tissue banking and cell banking are increasingly interconnected as part of the complicated networks that now connect the sources and recipients of donated bodily material, and the many intermediaries involved in processing the material to facilitate its use by clinicians.

Centralised management of tissue and cell donations could be the ideal scenario. However, tissues and cells can be provided from public organisations and private companies. Co-operation between establishments that store tissues and cells may be relatively limited. National and international efforts have focused on good practice for tissue establishments without usually providing a mechanism for comprehensive, nationwide sharing of donated material. In the meantime, an industry based on the supply of human tissue and cells has evolved worldwide, with multiple providers competing in a market driven by, among other things, biotechnology companies, pharmaceutical companies and private clinics. Thus, the flows involved between the original source or donor of the material, the amount of processing of the material involved and the commercial nature of some of those transactions are becoming ever more complex.

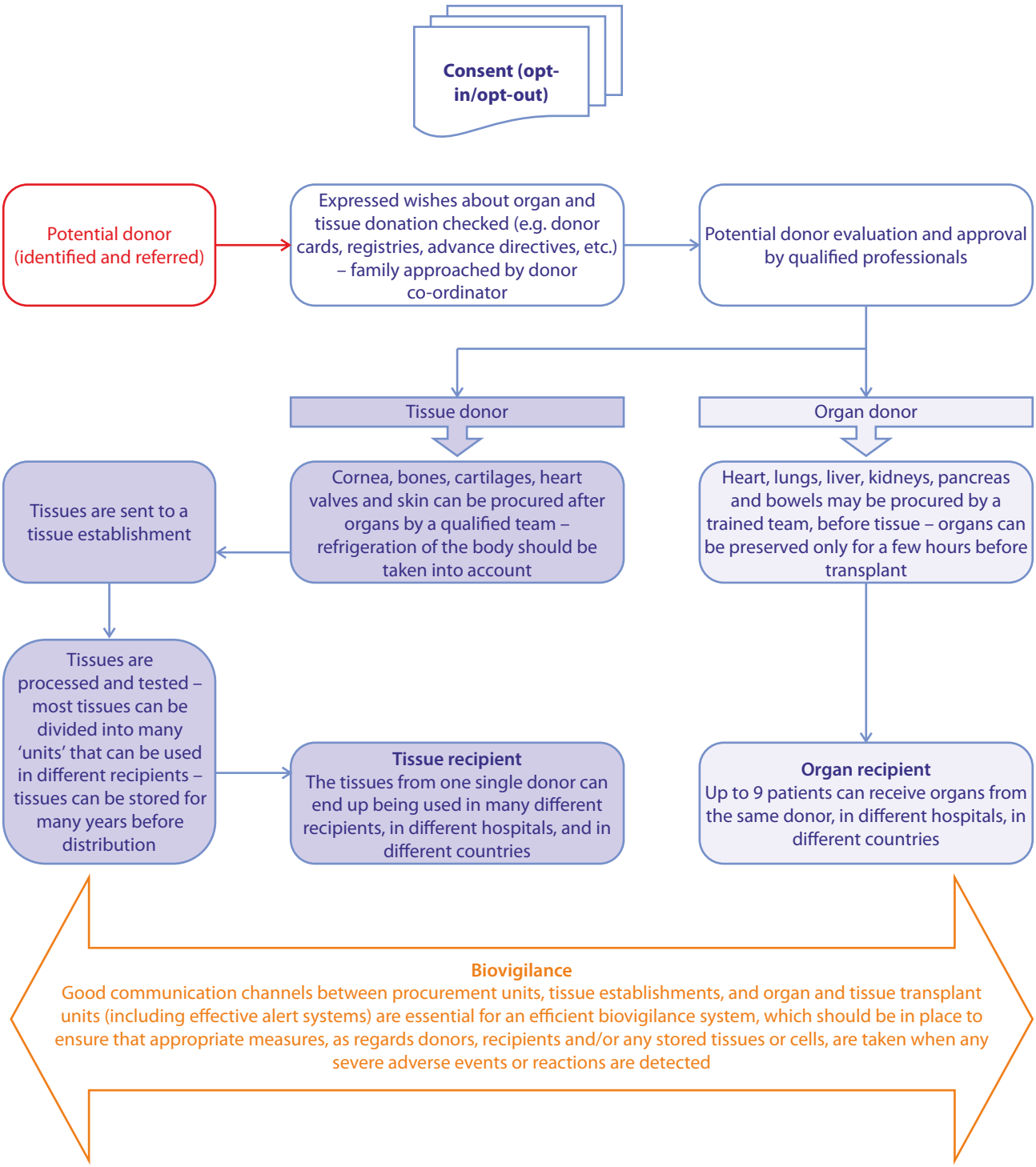
It is important to emphasise how consideration of policy surrounding donation must now take into account these complex flows and multiple intermediaries [10]. Awareness is needed of the central part that must be played by organisations and organisational structures in the donation and subsequent use of bodily material. Everyone involved needs to understand how the process includes, for example, the creation of professional roles such as 'donor co-ordinators', the extent to which they are expected to maximise opportunities for donation, how these professionals approach the next of kin of potential donors and form relationships with them, how well one part of the system links with another and where responsibility is seen to rest, and the way professionals in different fields interact and co-operate with one another. Awareness of this also points to added complexities in the form of legal agreements, liabilities and obligations that may arise where donated material is transformed, banked or otherwise handled as a commodity by successive intermediaries.

The increasing possibilities in using many forms of bodily material to benefit others in medical treatment has brought about increased pressure in member states to meet demand. There is a continual need to recruit new tissue and cell donors to maintain an adequate supply. Shortages of supply may affect particular subgroups of the population more than others because of the need to match material according to immunological criteria or age. 'Demand' for material is inherently variable; as scientific devel-

opments make more treatments possible, the demand for that treatment is likely to increase, whereas the development of alternatives may lead to reduced demand. Public expectations of what medical science can achieve may serve to put further pressure on demand.

Talking in terms of 'supply' and 'demand' may resonate with the experience of many professionals and patients (potential recipients), who are only too aware of the impact of any shortage in supply. This feature is exacerbated in situations in which the requirement for a high degree of matching or phenotypical similarity between donor and recipient calls for recruitment from ethnic minorities and international collaboration. However, at the same time, it may imply a lack of consideration of the human nature of the source of the material. It is important to emphasise when using these impersonal terms that we are talking about people and people's lives.

Figure 1.1. **Complex links between donors and recipients in the context of donation after death**



1.5. Tissue banks, tissue establishments and biobanks

A 'tissue bank' is a term commonly used to describe an establishment that collects and stores human tissues or cells for either medical research or human application.

Increased use of tissues and cells for human application and for research calls for terminology that will distinguish between establishments that collect and store tissues and cells for one of these purposes or the other. In Europe, the terms currently in use are 'tissue establishment' (for clinical applications) and 'biobank' (for research applications).

The term 'tissue establishment' became widely used in Europe following publication of the EU Tissues and Cells Directive 2004/23/EC, which defined it as:

> a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells for human application are undertaken. It may also be responsible for procurement or testing of tissues and cells.

In the field of MAR, the term 'tissue establishment' refers to the laboratories in MAR centres or clinics as well as banks of gametes. These centres or clinics often also include clinical units in which the patients are treated. In the context of this Guide, the term 'tissue establishment' will be used and refer to all these banks, units, centres and clinics. The directive does not cover research using human tissues and cells, so tissue establishments are concerned only with tissues and cells intended for human application. Similarly, the directive does not cover the clinical application and practices undertaken in the clinical units of MAR centres.

In the USA, the American Association of Tissue Banks (AATB) uses the term tissue bank for:

> an entity that provides or engages in one or more services involving tissue from living or deceased individuals for transplantation purposes. These services include assessing donor suitability, recovery, processing, storage, labeling, and distribution of tissue. [11]

The term 'biobank' is widely used for repositories storing human biological samples for use in research. Presently, there is not an internationally agreed definition of a biobank, but the term is generally used for organised collections of human biological material (blood, tissues, cells, other body fluids, DNA, RNA, etc.) and associated information stored for one or more research purposes. In its glossary, the Organisation for Economic Co-operation and Development (OECD) defines a biobank as 'a collection of biological material and the associated data and information stored in an organised system, for a population or a large subset of a population' [12]. Several other definitions, as used in EU legislation or guidelines, are available on the website of the EU-funded project PRIVILEGED (Privacy in Law, Ethics and Genetic Data) [13].

In the USA, the term 'biorepository' is preferred to 'biobank'. For example, according to the glossary of the National Cancer Institute, a biorepository is:

> a facility that collects, catalogues, and stores samples of biological material, such as urine, blood, tissue, cells, DNA, RNA, and protein, from humans, animals, or plants for laboratory research. If the samples are from people, medical information may also be stored along with a written consent to use the samples in laboratory studies. [14]

The biobanking field is continually evolving, and tissue establishments may become interested in collecting samples for research purposes, so the terminology should also be refined to reflect these changes in the future.

In this Guide, it has been agreed to use the term 'tissue establishment' and its definition in accordance with Directive 2004/23/EC.

1.6. Quality and safety

High-quality, safe and efficacious procedures are essential for donors and recipients alike. The long-term outcomes of tissue and cell donation and human application should be assessed for the living donor, as well as the recipient, to document benefit and harm.

The level of safety, efficacy and quality of human tissues and cells for human application as health products of an exceptional nature must be maintained and continually optimised. This strategy requires implementation of quality systems (see Chapter 2) that include traceability (see Chapter 15) and vigilance (see Chapter 16), with adverse events and reactions reported both nationally and for imported/exported human products.

Optimising the outcome of the human application of tissues and cells entails a rules-based process that encompasses clinical interventions and *ex vivo* procedures from donor selection through to long-term follow-up. Under the general supervision of Health Authorities, transplant and MAR programmes should monitor donors and recipients to ensure that they receive appropriate care, including

information about long-term risks and benefits. Evaluation of information on long-term risks and benefits is essential to the consent process and for adequately balancing the interests of donors and recipients. The benefits to both must outweigh the risks associated with donation and human application. Donors should not be permitted to donate in clinically hopeless situations.

Locally organised donation, transplantation and MAR programmes should store details of their activity and follow-up data in national and/or international registries. All deviations from accepted procedures that could increase the risk to recipients or living donors (as well as any untoward consequences of donation or human application) should be reported to, and analysed by, the responsible Health Authorities.

Transplantation of human material that does not involve long-term medical care of the recipient may not require active, long-term follow-up, though traceability should be ensured for the anticipated lifetimes of donor and recipient. Internationally agreed means of coding to identify tissues and cells used in transplantation are essential for full traceability (see Chapter 14).

In the specific case of MAR, traceability should include the outcome of the pregnancy as well as the health of the donor, recipient and newborn. It is of the utmost importance to put the welfare of donors (especially with respect to non-partner oocyte donors) in a central position in determining what constitutes acceptable practice. This requirement might entail additional effort in the context of cross-border reproductive care. All gamete donors should be recorded in national registers, and all centres should participate in the collection of national and international data. MAR centres and Health Authorities should collect data on a systematic basis to follow up the long-term health effects of MAR activity, including the health of the donor, recipient and newborn. Good-quality evidence on these effects is essential for appropriate concern to be given to the welfare of oocyte donors in future policies. In addition, there should be a limit to the number of times a woman may donate, and a minimum interval between donations should be established. Ultimately, the welfare of oocyte donors should underpin any consideration about donation.

1.7. Ethical issues

Human tissues and cells can be derived only from the body of a person – hence the ethical challenges associated with their use. The range of tissues and cells described in this Guide makes explicit the very different circumstances under which a person can donate. The person providing the material may be living or deceased, the material may be used almost immediately or stored for long periods of time, and the material may be used unprocessed or heavily processed. Whatever the case, handling and disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and for the human body.

Ethical standards of all aspects of tissue and cell donation and transplantation have to conform to the Oviedo Convention on Human Rights and Biomedicine (1997) [15] and the Additional Protocol on transplantation of organs and tissues of human origin (2002) [16]. Other important guidelines to observe from an ethical viewpoint are Committee of Ministers Resolution (1978) 29 on harmonisation of legislation of member states relating to removal, grafting and transplantation of human substances [17], the WHO Guiding Principles on human cell, tissue and organ transplantation [18], the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [19] and the Barcelona Principles on the use of human donated tissue for ocular transplantation, research and future technologies from the Global Alliance of Eye Bank Associations (GAEBA) [20].

Tissues donated for transplantation after death are governed by the same ethical principles as organs since they enter a common pool to be used according to need and their use cannot be directed to a particular individual. Cells such as those from the bone marrow can be donated by a living person and directed for transplantation to another specific person. Similarly, gametes may be donated for use within the couple but may also be donated to unrelated recipients for altruistic reasons.

For tissue donation from deceased individuals, the 'dead-donor rule' (which states that patients must be declared dead before removal of any vital organs or tissues for transplantation) must be strictly respected [21].

1.7.1. Consent

The Oviedo Convention states that an intervention in the health field may be carried out only after the person concerned has given free and informed consent to it. This person must make a free choice in the absence of any undue influence and must be given appropriate information beforehand as to the intended use and nature of the intervention as well as its consequences and risks. The person concerned may freely withdraw consent at any time.

Together with the Declaration of Istanbul, a joint initiative of the International Society for Nephrology (ISN) and The Transplantation Society (TTS), the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin expands these provisions further for the specific case of donation and transplantation. These provisions are explained further in detail in Chapter 3. Specific cases related to consent in MAR procedures are outlined in Chapter 27.

Tissues must not be removed from the body of a deceased person unless that person has been certified dead in accordance with the national law and consent or authorisation has been obtained. The removal must not be carried out if the deceased person had objected to it.

Finally, it is crucial to emphasise the importance of consent in creating and maintaining the trust of the general public in health professionals and the healthcare system as a whole. 'Medical mistrust', or mistrust of the healthcare system, is one of the reasons why people are reluctant to donate bodily material. This may be associated with concerns about consent in that the terms of the consent may be abused (for example, by using the donated material in a manner which is not in accordance with consent) and that additional material may be taken without explicit consent. Values such as honesty and trust are central in both the professional and personal relationships when donation of bodily material takes place. Therefore, it is of vital importance that the limits of the consent are clearly established, made explicit and scrupulously respected.

The recipient – and, if appropriate, the person or official body providing authorisation for the human application – must be given suitable information beforehand on the purpose and nature of the procedure, its consequences and risks, and the alternatives to the intervention.

In summary, all donation and transplantation programmes are dependent upon the goodwill and voluntary donation of relevant material from donors to continue their activity. It is, therefore, important that public confidence is maintained by standards of good practice. By engaging donor trust and commitment when obtaining consent, healthcare professionals will reduce the risk of nefarious trading and potential physical harm from the use of transplantable tissue for human application.

1.7.2. Conflicts of interest

To avoid any potential conflict of interests, physicians determining the death of a potential donor should not be directly involved in tissue or cell procurement from the donor or subsequent transplantation procedures, and nor should they be responsible for the care of any intended recipient of such tissues or cells.

Health Authorities will set out the legal standards for determining that death has occurred and specify how the criteria and process for determining death will be formulated and applied.

It is of the utmost importance that patients undergoing ART treatment should be counselled appropriately for their conditions and given realistic estimates of the prospects of success of their treatment, based on their age and specific medical circumstances. Similarly, the welfare of potential donors (especially oocyte donors) should be central in determining what constitutes acceptable practice. Gamete scarcity or financial profit should never influence the decision to accept a donor into a programme. Also, financial incentives must not be used to encourage donations because they will render women more likely to consider repeat donations or to continue donating despite potential risks to their health. Furthermore, financial incentives may lead donors to not disclose all the information necessary for a complete and adequate donor selection, thus compromising the quality and safety of the donated oocytes.

1.7.3. Financial aspects of donation and human application of tissues and cells

Discussions around how best to increase the supply of human tissues and cells often focus on questions of donor motivation: specifically, how individuals may best be encouraged to donate different forms of bodily material. Nevertheless, it is essential to recall the Oviedo Convention which, in Article 21, clearly states that the human body and its parts must not, as such, give rise to financial gain. The Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors [22] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation. This notion is reiterated in the additional protocol to that Convention, which also clearly states in its Article 21 that the human body and its parts must not, as such, give rise to financial gain or comparable advantage. The aforementioned provision does not prevent payments that do not con-

stitute a financial gain or a comparable advantage, in particular:

a. compensation of living donors for loss of earnings and any other justifiable expenses caused by the removal or by the related medical examinations;

b. payment of a justifiable fee for legitimate medical or related technical services rendered in connection with transplantation;

c. compensation in cases of undue damage resulting from the removal of tissues or cells from living persons.

In the donation of any tissue or cell, removal of barriers to donation must not render a decision to donate non-altruistic. Initiatives that reduce the barriers to donation should only facilitate individuals to carry out an action that they were already inclined to take by concern for the welfare of the recipient. In this sense, the Nuffield Council on Bioethics suggests distinguishing between two types of intervention, both of which aim to increase donation by changing its costs and benefits [23]. The first type is 'altruist-focused interventions', which typically involve removal of various disincentives to act and, in doing so, remove countervailing concerns that may hinder potential donors from acting on their altruistic motivations. For the purpose of this Guide, we will call these interventions 'compensation'. The second type is 'non-altruist-focused interventions', which are targeted at potential donors who have no strong motivation to help others through donation of their bodily material and who, therefore, if they are to donate, need to be provided with different reasons for action, perhaps in the form of payment or 'incentive' going well beyond the reimbursement of expenses. These incentives are particularly worrisome in the case of gamete donors (especially oocyte donors), where they may change the donor's perception of the relative risks and benefits of a donation that is not free of potential health hazards and psychological consequences. In addition, gamete donation for treatment purposes presents further ethical implications because it involves the potential generation of a new human being.

In summary, voluntary unpaid donation, long promoted as the only ethical basis for donation of bodily material, should continue to have a central role in the donation process of any type of tissue or cell. Compensation to donors should cover only justifiable expenses and loss of income, and should not act as a direct or indirect incentive or inducement.

Physicians and other health professionals should not engage in transplantation procedures, and health insurers and other payers should not cover such procedures, if the tissues or cells concerned have been obtained through exploitation or coercion of, or payment to, the donor or the next of kin of a deceased donor.

Promotion of altruistic unpaid donation of human tissues or cells by means of advertisement or public appeal may be undertaken in accordance with domestic regulations. However, advertising the need for availability of tissues or cells with a view to offering or seeking financial gain or comparable advantage for the donor, or their next of kin where the individual is deceased, should be prohibited. Brokering that involves payment to such individuals or to third parties should also be prohibited.

Tissue establishments storing and supplying human tissues and cells have developed largely in response to the increasing demand for supplies of human tissues and cells for therapy and research. However, professional bodies should ensure that their guidelines reflect their members' responsibilities in the acquisition and supply of human tissue. Tissue establishments should operate on a non-profit basis. Tissues and cells should be supplied on an operational cost basis and no payment should ever exceed the justifiable fee for the services rendered; in other words, tissue establishments can claim a reasonable amount for certain expenses but should not quote an unfair amount greater than the actual cost in order to make profits. When calculating the operational costs of a tissue establishment, which may have a variety of funding sources, consideration should be given to the long-term sustainability of the tissue establishment. In order to do this, it is important to analyse the clinical need (for the different types of tissue that the tissue establishment will be processing and distributing) and to efficiently manage the tissue establishment's 'value chain', which includes the costs of procurement, processing, storage, distribution, personnel, transport, infrastructure and administration, and the need to incorporate state-of-the-art processes and equipment, among others.

The allocation of tissues and cells should be guided by clinical criteria and ethical norms, not financial or other considerations. Allocation rules, defined by appropriately constituted committees, should be equitable, based on clinical need, externally justified and transparent.

1.7.4. Equitable access to transplantation or to medically assisted reproduction

Healthcare in general is a human right because it secures and protects people's access to the normal

range of opportunities and because it allows people to thrive. Given the importance of health for the general well-being of a person, every person, regardless of his/her income or financial means, should have access to a decent minimum of healthcare.

Requests (the demand) for human tissues and cells may often exceed what is available (the supply). Significant practical and ethical questions of efficiency and fairness arise in deciding how to distribute these limited resources. Article 3 of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin states that transplantation systems must exist to provide equity in access to transplantation services for patients. Except in the case of direct donations, tissues and cells must be allocated among patients only in conformity with transparent, objective and duly justified rules according to medical criteria. The persons or official bodies responsible for the allocation decision must be designated within this framework.

With regard to access to MAR, infertility treatment covers a broad range of 'causes' (e.g. age-related decline in fertility, male factors, blocked Fallopian tubes) and applications that cannot necessarily all be fitted into the same framework. The pivotal point in this discussion seems to be whether the desire for a child should be considered a fundamental need or a personal wish. Current regulatory frameworks in MAR are heterogeneous and, in some countries, still under development. The debate on ethical and social issues (including access to ART for social indications, anonymity of gamete donors, genetic selection of donors, compensation for donation, posthumous reproduction or surrogacy) is ongoing. There is, however, general agreement that reproductive cloning must be forbidden.

Inclusion of infertility treatment in the basic healthcare tier is dependent upon the general level of welfare in society. Cultural, educational and religious backgrounds may also affect the availability of these therapies. Nevertheless, given the rightful claims of other types of healthcare and other fundamental needs in society (as well a limited availability of non-partner gametes and embryos), access cannot be expected to be unlimited. Ultimately, access to MAR should be considered in a structured way to include efficiency, safety and equity to avoid discrimination [24]. Some countries have opted to give public access to a fixed number of cycles/treatments for everyone, even if this means that those who need more treatment have to pay for it themselves.

Cross-border reproductive care refers to a widespread phenomenon whereby patients seeking MAR treatment cross international borders to obtain reproductive treatment outside their country of residence. The reasons for travelling vary between countries, but the most common reason is access to certain treatments or techniques not legally allowed in the country of origin (e.g. non-partner donation treatment, pre-implantation genetic testing of embryos) or if a particular group is excluded from treatment (e.g. same-sex couples, single women, women above a certain age). There may be other limitations to access at home (e.g. long waiting lists). Further reasons for travelling may be better quality of care and less expensive treatment [25, 26].

The ideal situation is fair access to fertility treatment in the home country for all patients. This ideal should be promoted at all levels [26]. However, if for some reason treatment is not possible in the country of residence or not available, cross-border reproductive care may provide a solution for patients. Furthermore, it is in accordance with the principle of freedom of movement of patients within Europe [27]. However, crossing borders may also lead to increased shortage of scarce resources in the visited country and to the detriment of local citizens. Health Authorities may want to introduce a system for fair allocation of scarce resources that takes into account local needs, such as a maximum number (or percentage) of treatments provided to foreign patients. In addition, cross-border reproductive care should always follow the same strict quality and safety criteria that govern domestic treatments, including appropriate traceability and biovigilance measures that cover both recipients and their children born as a result of the MAR treatment.

1.7.5. Equity in donation

Individual motivation and choice is only one part of the donation picture; the central role of organisations, organisational procedures and professionals in facilitating donation should not be underestimated, nor indeed the importance of trust in these systems. An example of such organisational aspects is that, whenever a person dies in circumstances where donation is a possibility, this should be raised with their family.

The role of the state with respect to donation should be understood as one of stewardship: that is, actively promoting measures that will improve general health (thereby reducing the demand for some forms of bodily material) and facilitating donation. Such a stewardship role should extend to taking action to remove inequalities that affect disadvantaged groups or individuals with respect to donation.

Equity in donation refers to the absence of systematic disparities in the burden of donation between social groups who have different levels of underlying social advantage or disadvantage (i.e. different positions in a social hierarchy). Inequities in donation would, in a systematic manner, put groups of people who are already socially disadvantaged (e.g. by virtue of being poor, female and/or members of a particular racial, ethnic or religious group) at further disadvantage with respect to their health.

As discussed above, introduction of financial incentives for donation in the field of MAR renders certain social groups (and especially women) particularly susceptible to disparities based on social and economic status.

With respect to cross-border reproductive care, safeguards must be in place to guarantee that all donors, regardless their origin, receive similar care and follow-up. To prevent abuse of donors coming from abroad, the use of intermediate agencies – which may lead to violations of the rules of good clinical practice and, in the worst-case scenario, to trafficking – should be avoided. Post-donation care must be provided to the best possible standards at home or abroad.

1.7.6. Anonymity

The identity of the donor and recipient should (except in the case of donation between persons having a close personal relationship) be maintained in strict confidentiality. Such precautions will prevent abuse and protect the families of donors and recipients from feelings of anxiety associated with emotional involvement, obligation to return favours or guilt.

In the specific case of MAR, different regulations are applied in different member states with regard to the anonymity of non-partner donors. Debate has focused around the donor's right to anonymity, the welfare of the resulting offspring and his/her right to family life, and the effect of removal of donor anonymity on the supply of gametes for treatment. Presently, some countries require that donors always remain anonymous, whereas other countries require that their identity might be known only in exceptionally urgent medical situations. Other countries allow the possibility of the offspring gaining access to non-identifying information about the donor (e.g. hair colour, ethnicity). Other countries even allow the offspring to contact donors after the offspring has reached a certain age. Hybrid models exist in some countries. A common standard seems to be that donors do not have the right to information about children generated from their gametes (unless the child chooses and is legally allowed to obtain information about the donor).

1.7.7. Transparency

The organisation and execution of activities based on donation and human application, as well as their clinical results, must be transparent and open to scrutiny, while ensuring that the personal anonymity and privacy of donors and recipients are protected (if relevant).

Transparency can be achieved by maintaining public access to regularly updated comprehensive data on processes; in particular allocation, transplant activities and outcomes for both recipients and living donors, as well as data on organisation, budgets and funding. Such transparency is not inconsistent with shielding (from public access) information that could identify individual donors or recipients, while still respecting the requirement of traceability. The objective of the system should be not only to maximise the availability of data for scholarly study and governmental supervision to allow determination of clinical outcomes and efficacy of treatments but also to identify risks (and facilitate their mitigation) to minimise harm to donors and recipients.

1.8. Recommendations and regulations in the field

1.8.1. Council of Europe

The Council of Europe, based in Strasbourg (France), is an international organisation that promotes co-operation between all European countries in the areas of human rights, democracy, rule of law, culture and public health. After the 3rd Conference of European Health Ministers on the Ethical, Organisational and Legislative Aspects of Organ Transplantation [28], held in Paris in 1987, the Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation (SP-CTO) was created. This committee consisted of experts in different aspects of transplantation: immunologists, surgeons and physicians, as well as co-ordinators and representatives from organ-sharing and organ-procurement organisations. In 2007, the secretariat responsible for activities related to organs, tissues and cells was transferred to the European Directorate for the Quality of Medicines & HealthCare (EDQM) of the Council of Europe [29], and the newly appointed CD-P-TO took over as the steering committee [30].

Today, the CD-P-TO is composed of internationally recognised experts from Council of Europe member states, observer countries, the European Commission and the WHO, as well as representatives from the Committee on Bioethics of the Council of Europe (DH-BIO) and several professional societies and non-governmental organisations. It actively promotes the non-commercialisation of organ donation, the fight against organ trafficking, the development of ethical, quality and safety standards in the field of organs, tissues and cells, and the transfer of knowledge and expertise between member states and organisations.

Within the framework principle of sharing knowledge through international co-operation, the Council of Europe has established widely recognised recommendations and resolutions in the field of transplantation, covering the ethical, social, scientific and training aspects of the donation and transplantation of organs, tissues and cells [31]. Whereas agreements and conventions are binding on the states that ratify them, resolutions and recommendations are policy statements to governments that propose a common course of action to be followed.

The Council of Europe Convention for the Protection of Human Rights and Fundamental Freedoms (European Treaty Series, No. 5) [32] is an international treaty to protect human rights and fundamental freedoms in Europe. It was drafted in 1950 by the then newly formed Council of Europe and came into force on 3 September 1953.

The European Agreement on the Exchange of Therapeutic Substances of Human Origin (European Treaty Series, No. 26) [33], signed in Paris on 15 December 1958, aims to provide mutual assistance with respect to the supply of therapeutic substances of human origin.

The European Agreement on the Exchange of Tissue-Typing Reagents (European Treaty Series, No. 84) [34], signed in Strasbourg on 17 September 1974, lays the groundwork for development of mutual assistance in the supply of tissue-typing reagents and the establishment of joint rules between signatory parties. The signatory parties undertake to make reagents available to other parties who are in need of them, by the most direct route, subject to the condition that no profit is made on them, that they must be used solely for medical and scientific purposes and are free of import duties. The Additional Protocol (European Treaty Series, No. 89) [35], which was opened for signature on 24 June 1976 and came into force on 23 April 1977, provides for the accession of the European Community to this agreement.

The Oviedo Convention – the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (European Treaty Series, No. 164) [15], which was opened for signature on 4 April 1997 and came into force on 1 December 1999 – is the first legally binding international text designed to preserve human dignity, fundamental rights and freedoms, through a series of principles against the misuse of biological and medical applications. The Convention is inspired by the principle of the primacy of human beings over the sole interest of science or society. It lays down a series of principles applying to medical practice as well as biomedical research, organ transplantation and genetics. The Convention includes the principle of consent, non-discrimination on the basis of genetic characteristics, and protection of private life and access to information. The Convention specifically prohibits any financial gain from the body and its parts, as such. The Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors [22] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation.

The Oviedo Convention was extended by an Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin (European Treaty Series, No. 186) [16], which was opened for signature on 24 January 2002 in Strasbourg and came into force on 1 May 2006. This additional protocol aims to protect the dignity and identity of everyone and to guarantee, without discrimination, respect for his/her integrity and other rights and fundamental freedoms with regard to the transplantation of organs and tissues of human origin, thereby establishing principles for the protection of donors and recipients. However, the additional protocol does not apply to gametes and embryos.

The Council of Europe Convention on Action against Trafficking in Human Beings (European Treaty Series, No. 197) [36], which was opened for signature in Warsaw on 16 May 2005 and came into force on 1 February 2008, alongside its Explanatory Report, addresses the trafficking of human beings for the purpose of organ removal.

The Council of Europe/United Nations joint study on Trafficking in organs, tissues and cells and trafficking in human beings for the purpose of the removal of organs [37], presented at the United Nations headquarters in New York on 13 October 2009, focuses on trafficking in organs, tissues and cells for the purpose of transplantation. The joint

study made it evident that existing criminal-law instruments dealing exclusively with trafficking in human beings (including for the purpose of organ removal) left loopholes that allowed several unethical transplant-related activities to persist. This is why the Council of Europe decided to undertake the task of drafting a new international legally binding instrument against trafficking in human organs.

The Council of Europe Convention against Trafficking in Human Organs (European Treaty Series, No. 216) [36], with its Explanatory Report [36], adopted by the Committee of Ministers on 9 July 2014, identifies distinct activities that constitute 'trafficking in human organs'. The central concept is 'the illicit removal of organs', which consists of removal without the free, informed and specific consent of a living donor; removal from a deceased donor other than as authorised under domestic law; removal when, in exchange, a living donor (or a third party) has been offered or received a financial gain or comparable advantage; or removal from a deceased donor when a third party has been offered or received a financial gain or comparable advantage.

Other major resolutions and recommendations in the field of tissues and cells include:

- Resolution (78) 29 of the Committee of Ministers on Harmonisation of legislations of member states relating to removal, grafting and transplantation of human substances [17];
- Recommendation No. R (94) 1 of the Committee of Ministers to member states on human tissue banks [38];
- Recommendation No. R (98) 2 of the Committee of Ministers to member states on provision of haematopoietic progenitor cells [39];
- Recommendation Rec (2004) 8 of the Committee of Ministers to member states on autologous cord blood banks [40];
- Recommendation Rec (2006) 4 of the Committee of Ministers to member states on research on biological materials of human origin [41].

Monitoring of practices in member states has become an evident need for the sake of transparency and international benchmarking. Keeping this goal in mind, since 1996 the EDQM/Council of Europe has published *Newsletter Transplant* [42], which is co-ordinated by the Organización Nacional de Trasplantes (ONT) in Spain. This publication summarises comprehensive data (provided by national focal points designated by governments) on donation and transplantation activities, management of waiting lists, organ-donation refusals and authorised centres for transplantation activities. *Newsletter Transplant* provides information from ≈ 70 countries, including Council of Europe member states, observer countries and observer networks (e.g. Iberoamerican Donation and Network Council on Organ Donation and Transplantation, Mediterranean Network). The *Newsletter Transplant* database is connected with other international projects on data collection (e.g. WHO Global Observatory on Organ Donation and Transplantation, Eurocet database) to avoid duplication of efforts. *Newsletter Transplant* has evolved into a unique official source of information that continues to inspire policies and strategic plans worldwide.

The Council of Europe also produces other guidelines, including this *Guide to the quality and safety of tissues and cells for human application*, the *Guide to the quality and safety of organs for transplantation* and the *Guide to the preparation, use and quality assurance of blood components*.

1.8.2. World Health Organization

In 1987, the 40th World Health Assembly, concerned about the trade for profit in human organs, initiated preparation of the first WHO Guiding Principles on transplantation, endorsed by the Assembly in 1991 in resolution WHA 44.25 [43]. These Guiding Principles have greatly influenced professional codes and practices, as well as legislation, around the world for almost two decades. After a consultation that took several years, on 21 May 2010 the 63rd World Health Assembly adopted resolution WHA 63.22 [44], which endorsed the updated WHO Guiding Principles on human cell, tissue and organ transplantation [18] and called on WHO member states to implement these Guiding Principles, promote voluntary and unremunerated donation, oppose trafficking and promote transparent and equitable allocation. It also urged its members to strengthen oversight, to collect and publish activity data, including adverse events and reactions, and to implement globally standardised coding. These WHO guidelines are intended to provide an orderly, ethical and acceptable framework for the acquisition and transplantation of human cells, tissues and organs for therapeutic purposes.

The World Health Assembly adopted resolution WHA 57.18 [45] in 2004, which urged WHO member states 'to take measures to protect the poorest and vulnerable groups from transplant tourism and the sale of tissues and organs, including attention to the wider problem of international trafficking in human tissues and organs'. Subsequently, the Declaration

of Istanbul on Organ Trafficking and Transplant Tourism [19] was adopted in 2008, as an initiative of The Transplantation Society (TTS) and the International Society for Nephrology (ISN). In 2018 it was updated to incorporate revised definitions and briefer and more comprehensive principles to provide up-to-date guidance and practical advice for health professionals, policy makers, and law-enforcement authorities. The declaration emphasises that organ trafficking and transplant tourism should be prohibited because they violate the principles of equity, justice and respect for human dignity, targeting impoverished and otherwise vulnerable donors and inexorably leading to inequity and injustice.

United Nations Resolution 71/322, adopted by the WHO General Assembly on 8 September 2017, aims at strengthening and promoting effective measures and international co-operation on organ donation and transplantation to prevent and combat trafficking in persons for the purpose of organ removal and trafficking in human organs [46].

Robust bi-directional donor–recipient traceability is a prerequisite to achieving effective vigilance and surveillance worldwide. For this reason, Resolution WHA 63.22 [44] also urged WHO member states to collaborate in collecting data (including adverse events and reactions) in addition to implementation of globally consistent coding systems. The Notify project was a specific follow-up action that was led by the WHO to promote the sharing of information on adverse incidents for improving safety and efficacy [47].

As a result of resolutions WHA 57.18 and WHA 63.22 (which requested that global data on the practice, safety, quality, efficacy and epidemiology of transplantations be collected in the WHO member states that have transplantation programmes), an international watchdog on transplantation was set up as a collaborative initiative between the Spanish ONT and WHO, and was termed the Global Observatory on Donation and Transplantation [48]. The universal availability of these data is recognised as a prerequisite for global improvements in demonstrating transparency, equity and compliance, and for monitoring systems in countries. In addition, the data provided also help to give an overview of the legal and organisational aspects in very different settings and countries, which enables the regulating bodies to monitor transplantation activities.

The WHO has also published two aide-mémoires specifically on the donation and transplantation of tissues and cells [1, 49].

In recent years, the WHO has been promoting use of the term 'medical products of human origin' (MPHO). This category includes blood, organs, tissues, bone marrow, cord blood, reproductive cells and milk derived from humans for therapeutic use. Use of these MPHO, obtained from living and deceased donors, entails practical, scientific and ethical considerations.

1.8.3. European Union

1.8.3.1. *EU tissues and cells legislation*

Article 168 of the Treaty on the Functioning of the European Union [50] (previously Article 152 of the Treaty of Amsterdam) gives the EU a mandate to establish high quality and safety standards for substances of human origin, such as blood, organs, tissues and cells.

Acknowledging that the human application of tissues and cells is an expanding medical field that offers important opportunities for the treatment of disease, the EU aims for a common approach to the regulation of tissues and cells across Europe.

The EU tissue and cells directives have created a benchmark for the standards that must be met if carrying out any activity involving tissues and cells for human applications, including gametes, embryos and germinal tissue. The directives also require that systems be put in place to ensure that all the tissues and cells used in human applications are traceable from donors to recipients and vice versa.

Directive 2004/23/EC [51] of the European Parliament and of the Council of 31 March 2004 applies to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells intended for human use (including reproductive cells used in ART procedures). The directive introduced obligations on EU member states' authorities, from supervision of human tissue and cell procurement and authorising and inspecting tissue establishments, to ensuring traceability and vigilance and maintaining a publicly accessible register of national tissue establishments. It also lays down rules on donor selection and evaluation (e.g. principles governing tissue and cell donation, consent, data confidentiality) and quality and safety of tissues and cells (e.g. quality management, tissue and cell reception, processing and storage conditions).

Commission Directive 2006/17/EC [52] established specific technical requirements for each step in the human tissue and cell preparation process, in particular the requirements for procurement of human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for donors, tissue and/or cell donation, the procurement and reception procedures at tissue establishments and the

requirements for direct distribution to the recipient of specific tissues and cells. Directive 2006/17/EC was amended in 2012 by Commission Directive 2012/39/EU with regard to certain technical requirements for the testing of human tissues and cells [53].

Commission Directive 2006/86/EC [54] includes traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

In 2015, two new Commission directives were adopted, one an implementing directive on the procedures for verifying equivalent standards of quality and safety of imported tissues and cells (Directive 2015/566) [55] and a second one amending Directive 2006/86/EC, providing detailed requirements on the coding of human tissues and cells (Directive 2015/565) [56].

Quality and safety standards for human organs intended for transplantation are laid down in Directive 2010/53/EU and Commission Implementing Directive 2012/25/EU. These detail the standards and procedures for information exchange between EU member states regarding human organs intended for transplantation [57, 58].

The EU directives dictate that EU member states must encourage voluntary and unpaid donations of tissues and cells and must endeavour to ensure that the procurement of tissues and cells is carried out on a non-profit basis. Promotion and publicity activities in support of the donation of human tissues and cells with a view to offering or seeking financial gain or comparable advantage are not allowed. The EU directives also provide clear mandates for the consent of donors and the anonymity of all data collected, and instruct EU member states to adopt measures to ensure data security and prevent unauthorised modifications to files and records.

These directives do not cover research using human tissues and cells (e.g. *in vitro* research or research using animal models) and do not interfere with the decisions of EU member states on the use or non-use of any specific type of human cell, including embryonic stem cells. Similarly, these directives do not interfere with provisions of member states defining the legal term 'person' or 'individual'.

The European Commission has supported EU member states in their efforts to implement EU directives on tissues and cells by providing funding for several projects under the Programme of Community Action in the Field of Health [59]:

- EQSTB (European Quality System for Tissue Banking) focused on four main work packages: (i) identification of the key requirements for tissue banking; (ii) development of a registry to support exchange of tissues; (iii) provision of training programmes, both online and face-to-face, to fulfil the needs of tissue establishment professionals, and (iv) development of an audit model and audit guide for tissue establishments, with recommendations for tissue establishments and guidance for auditors.
- Eustite (European Standards and Training in the Inspection of Tissue Establishments) [60] developed guidance and training courses for EU competent authorities on the inspection of tissue establishments and on vigilance procedures for tissues and cells used in transplantation and in assisted reproduction. The guidance document served as a basis for the guidelines on implementation of inspection and control measures in the field of human tissues and cells included in Commission Decision 2010/453/EU of 3 August 2010.
- Poseidon (Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation Organisation and Networking in unrelated haematopoietic stem-cell transplantation in Europe) provided recommendations for improvements in the safety of unrelated haematopoietic progenitor cell transplantation, for the optimisation of human stem-cell donation policy, and for promoting equal access to this therapy throughout the EU.
- Eurocet [61] is a platform that was funded initially by the European Commission but is now maintained by the Italian National Transplant Centre. It collects and publishes annual activity data on donation, processing and human applications of tissues and cells. However, the Tissue Establishment Registry has been temporarily suspended in order to avoid confusion with the official EU Tissue Establishment Compendium.
- EuroGTP (European Good Tissue Practices) [62] developed a guide to good tissue practices and personnel training guidelines for tissue establishments on the recovery, processing and preservation of tissues, to ensure that all tissue establishments guarantee the highest level of quality and safety of tissues for human application. EuroGTP has provided a crucial basis for much of the technical content of this Guide. A strong collaboration between the European Association of Tissue and Cell Banks (EATCB), which will update and maintain the GTPs as their own standards, and the Council of Europe will be maintained to ensure consist-

ency and development in the light of the most up-to-date scientific knowledge.

- The project SoHO V&S (Vigilance and Surveillance of Substances of Human Origin) [63] addressed the harmonisation of terminology and documentation relating to adverse events and reactions. It aimed to find a consensus on how information should be exchanged between EU member states, the European Commission and third countries to enhance efficient management of incidents involving cross-border distribution of tissues and cells. The project drafted important guidance documents for the EU competent authorities, on the detection and investigation of suspected illegal and/or fraudulent activity related to tissues and cells, the communication and investigation of serious adverse events and reactions associated with human tissues and cells, and vigilance and surveillance in the field of assisted reproductive technologies. The project also prepared a guidance document for healthcare professionals on vigilance and surveillance of human tissues and cells. It also provided a training model for competent authorities in the investigation and management of vigilance and surveillance of tissues and cells.
- The joint action ARTHIQS (Good Practice on Donation, Collection, Testing, Processing, Storage and Distribution of Gametes for Assisted Reproductive Technologies and Haematopoietic Stem Cells for Transplantation) [64], launched in 2014, was a three-year project to build institutional and inspection guidelines for assisted reproductive technologies as well as guidelines related to the set-up and regulation of haematopoietic stem-cell donor follow-up registries and banking of cord blood.
- The joint action VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation) [65], launched in 2015, aimed at promoting and facilitating the harmonisation of inspection, authorisation and vigilance systems for blood transfusion and tissues and cells for human application.
- The project EuroGTP-II (Good Tissue Practices for demonstrating safety and quality through recipient follow-up) [66], launched in 2016, aimed at developing technical guidance to assess the quality and safety of novel tissue and cell therapies and demonstrating their efficacy based on recipients' outcomes.
- The joint action European Cornea and Cell Transplant Registry (ECCTR), launched in 2016, aimed to develop a common assessment methodology, based on the three existing European corneal transplant registries in the Netherlands, Sweden and the UK, and establish a web-based European registry to assess and verify the safety, quality and efficacy of ocular tissue transplantation [67].
- The EU-funded project TRANSPOSE (TRANSfusion and transplantation: PrOtection and SElection of donors) [68] was launched in 2017 and aims at harmonising European donor selection and protection policies, while maintaining adequate health and safety protection of the recipient.
- The joint action GAPP (Facilitating the Authorisation of Preparation Process for blood, tissues and cells) [69] was launched in 2018 and aims at facilitating the development of a common and optimal approach to assess and authorise preparation processes in blood and tissue establishments, adapting requirements as prescribed by Article 29 of Directive 2002/98/EC and Article 28 of Directive 2004/23/EC.

These projects have strengthened collaboration among Health Authorities, and between these Health Authorities and the professional associations in the area of tissues and cells for human application, allowing continuous input from field practice into the regulatory framework.

1.8.3.2. *Other relevant EU legislation*

Cells and tissues that are substantially manipulated or used for a different essential function can be classified as a gene therapy medicinal product, a somatic cell therapy medicinal product or a tissue-engineered product.

Activities involving such cells and/or tissues have to comply with Regulation (EC) No. 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products (the 'ATMP Regulation') [70, 71] (see Chapter 30 for additional information). This Guide is not applicable to such activities, other than aspects related to donation, procurement and testing of the cells/tissues. Clinical trials of ATMPs must comply with the EU framework on clinical trials (Regulation (EU) No. 536/2014 and, until it applies, Directive 2001/20/EC) [72]. Donation, procurement and testing of the tissues and/or cells must comply with the quality and safety standards laid down in Directive 2004/23/EC and its implementing directives. In 2017, two new regulations on

medical devices were adopted and entered into force. Regulation (EU) 2017/745 of the European Parliament and of the Council, on medical devices, amends Directive 2001/83/EC, Regulation (EC) No. 178/2002 and Regulation (EC) No. 1223/2009 and repeals Council Directives 90/385/EEC and 93/42/EEC. The revised requirements apply to medical devices combined with tissues and cells and to medical devices incorporating non-viable derivatives of human tissues or cells, in particular human collagen. Regulation (EU) 2017/746 of the European Parliament and of the Council on *in vitro* diagnostic medical devices repeals Directive 98/79/EC and Commission Decision 2010/227/EU [73].

Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and the free movement of such data [74] must be applied when processing personal data (e.g. data related to donors and recipients).

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Chapter 2: Quality management, risk management and validation

2.1. Quality management

2.1.1. Introduction

This chapter outlines the general principles of a quality management system (QMS) that should be applied at all stages, from identification of a potential donor through processing and storage of the tissues or cells to the final preparation for application to the patient. Quality of tissues and cells is achieved through compliance with requirements at four levels:

a. The legal framework that provides the overall context in which the donation, procurement, testing, processing, storage, distribution and import/export activities for tissues and cells are performed;

b. The QMS, which is a tool to ensure that tissues and cells consistently comply with technical and legal requirements;

c. The technical requirements specific to each type of tissue or cell, which ensure quality, safety and efficacy, as detailed in Part B of this Guide;

d. The authorisations in place for the specific activities, from specific competent authorities.

A tissue establishment must implement a QMS that covers the scope of all of its activities. The following non-exhaustive list of standards and legal instruments includes tools to support a tissue establishment in the construction of a robust and efficient programme:

- Good Practice Guidelines for Tissue Establishments (see Part E) that follow the EU directives. The guidelines are based on a QMS approach. They form the basis of good practice in all tissue establishments and should be used in preparation for both inspection and continuous improvement.
- The International Organization for Standardization (ISO) requirements, as addressed in the ISO 9000 QMS family of standards. ISO standards have been developed to assist organisations of all types and sizes to implement and operate effective QMS. ISO 9001 on QMS requirements is particularly relevant to tissue and cell processes.
- Good Tissue Practices for European tissue banks were developed by the EU-funded project EuroGTP, which aimed to agree harmonised practices and techniques across Europe and to increase the know-how and level of competence of tissue establishment personnel. Much of the guidance developed in that project has been incorporated in the chapters of this Guide.
- The EU Guidelines for Good Manufacturing Practices (GMP) [1] provide specific guidance for the preparation of medicinal products. However, much of their content is also relevant for the procurement, processing, storage and distribution of tissues and cells. Wherever (in the EU) products containing tissues or cells are classified as advanced therapy medicinal

products (ATMPs), then the full requirements of GMP must be applied [2].

- Directive 2004/23/EC – which sets the standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells – and its associated technical directives provide key elements to be included in a tissue establishment QMS; these requirements are legally binding in EU member states.
- FACT–JACIE International Standards for cellular therapy product collection, processing and administration, published by the Foundation for the Accreditation of Cellular Therapy and the Joint Accreditation Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation.
- NetCord-FACT International Standards for cord blood collection, processing and release for administration.
- European Society of Human Reproduction and Embryology (ESHRE) Guidelines for good practice in IVF laboratories [3].
- World Marrow Donor Association International Standards for Unrelated Haematopoietic Stem Cell Donor Registries.

2.2. Applying a quality management system in donation and banking of tissues and cells

Quality is the responsibility of all personnel involved in the process of providing tissues and cells for clinical application. A systematic approach to quality management must be implemented and maintained throughout the entire process. A good system addresses quality management under the following headings:

a. Personnel and organisation;
b. Premises;
c. Equipment and materials;
d. Outsourced activities management (contractual arrangements);
e. Documentation;
f. Quality control;
g. Quarantine and release;
h. Qualification and validation;
i. Traceability;
j. Complaints;
k. Investigation and reporting of deviations, adverse events and reactions;
l. Recall;
m. Self-assessment, internal and external audit;
n. Quality risk management;
o. Fiscal and continuity planning;
p. Tools for continuous quality improvement.

2.3. Personnel and organisation

There must be sufficient, suitably qualified personnel to carry out all tasks in compliance with quality and safety requirements. Tasks and responsibilities must be clearly defined, understood and documented. All personnel should have clear, documented and up-to-date job descriptions. There should be an organisational chart that describes the structure of the organisation with clear delineation of responsibilities and lines of reporting.

2.3.1. Key personnel

Key personnel in each organisation involved in the process (from the initial donor-selection stage to the final delivery of tissues and cells) should include an identified person who is responsible for all activities carried out in their organisation, along with a designated person who takes over this responsibility in their absence. For those countries that are members of the EU, the Responsible Person (RP) in a tissue establishment must meet qualification criteria defined in Directive 2004/23/EC. Each relevant organisation must also have an identified medical specialist/adviser who may or may not be the RP. The processing and quality-control functions should be independent to ensure the effective and reliable evaluation of processes.

The responsibilities of key personnel who manage the quality system should include (but are not limited to):

- ensuring training is completed,
- ensuring validation is completed,
- checking maintenance of premises and equipment,
- approving specifications and test methods,
- evaluating batch manufacturing records,
- plant hygiene,
- approval and monitoring of suppliers,
- document control,
- retention of records,
- compliance of all other personnel with establishment requirements.

Tissue and cell processing should be carried out by appropriately qualified personnel.

2.3.2. Training

Personnel must receive initial and continued training appropriate to the duties assigned to them. Criteria should be defined and satisfied before declaring personnel qualified for a specific task or processing tissue and or cells. Training methods must be documented and training records maintained. The effectiveness of training programmes should be monitored by regular assessment of the competence of personnel. Personnel should also be trained in quality principles relevant to their duties and in the broad ethical and regulatory framework in which they work. When applicable, personnel should have relevant knowledge of microbiology and hygiene, and should be constantly aware that microbial contamination of themselves, donors, recipients and tissues and cells should be avoided. The training programme should include mid- to long-term training plans, be adequately resourced and target all the personnel that might be involved in any activities within the scope of this Guide, irrespective of whether the activity is routine or occasional.

2.3.3. Safety issues for healthcare personnel working with tissues and cells for human application

Personnel carrying out tissue and cell procurement and processing activities are exposed to a risk of infection to a similar degree as operating theatre personnel. In some cases, donors will not have been fully tested at the time of procurement or initial processing and, even where they have been tested, a residual risk of infection by untested agents remains. There may also be occasions when a donation is still required to be processed following receipt of positive test results, increasing the risk to the healthcare personnel, for example when an autologous donation is assessed as being the most appropriate treatment methodology. Standard universal precautions and suitable personal protective equipment (PPE) must be applied to protect personnel from these risks. Documented procedures should be in place describing the actions to be taken if an individual is directly exposed to the blood or tissues of a donor or their donation (e.g. needle-stick injury). These procedures might include: accelerated and extended testing of the donor, rapid testing of the staff member, and prophylaxis for the transmissible agent(s) where appropriate.

2.3.4. Safety issues for tissues or cells handled by personnel with bacterial or viral infections

Personnel involved in procurement and processing of tissues and cells might also pose a risk to the quality and safety of the tissues and cells if they themselves are infected with a transmissible agent. Organisations should have documented policies describing the requirements for health screening of personnel and for individuals to inform the organisation, in a confidential manner, if they have accidentally exposed tissues or cells to risk of contamination.

2.4. Premises

Premises must be designed, located, constructed, adapted and maintained to suit the operations to be undertaken. Their layout and design must aim to minimise the risk of errors and permit operations to proceed in an orderly sequence. Their layout must also allow effective cleaning and maintenance to avoid contamination and cross-contamination.

Suitable premises should be available for confidential interviewing of living donors or the families or friends of deceased donors.

Facilities in which tissues or cells are procured must meet appropriate grades of air quality and cleanliness. The appropriate standard of cleanliness will depend on the type of tissues or cells being procured, the degree of exposure of the tissues or cells during the procurement process, and the decontamination or sterilisation processes that will subsequently be applied to the tissues or cells during processing. Most operating theatres are now environmentally monitored and have controlled air systems that make them suitable for the procurement of tissues that are not subsequently sterilised. Other types of facility, such as mortuaries, may also be adequate for the procurement of certain types of tissues, but they should be assessed for suitability on a case-by-case basis. Further guidance on facilities for tissue and cell procurement is given in Chapters 6, 7 and 8, and in Part B of this Guide.

Processing facilities should be dedicated to this activity and should be designed, classified, qualified, validated and monitored to ensure that air quality is appropriate for the process being carried out. An international standard should be followed in full to achieve the appropriate air quality (e.g. rules governing medicinal products in the EU, Volume 4: EU guidelines to good manufacturing practice, or ISO 8573-1, ISO 14644 and ISO 14698). For tissue establishments in the EU, the zone in which the tissues

or cells are exposed to the air must be equivalent to Grade A, with a surrounding environment of at least Grade D (GMP classification), unless specifically defined criteria for exemption from this requirement are met; the latter applies notably in the field of assisted reproductive technology (ART). Some national requirements are more stringent, requiring Grade B and C backgrounds for certain processes or tissue or cell types. Processing and storage facilities should be cleaned according to a schedule and procedure that has been validated to achieve the required level of cleanliness, and all cleaning procedures should be documented.

More specific guidance on requirements for processing facilities is given in Chapter 8 and in Part B of this Guide.

Storage conditions for tissues and cells must be controlled and monitored. If certain conditions are critical to maintenance of the required properties of tissues or cells, appropriate alarms must be in place to indicate if conditions are approaching, or fall outside, predefined limits. Standard operating procedures (SOPs) should define the actions to be taken in response to alarms. Storage requirements apply equally to interim storage of tissues and cells before transport to a processing facility. Further guidance on requirements for storage is given in Chapter 9 and in Part B of this Guide.

Premises should include adequate dedicated areas that allow the 'first in, first out' – or, when applicable, the 'first expired, first out' – principle for critical consumables and reagents to be respected. In this context, 'critical' means those consumables and reagents that come in contact with the tissues or cells or influence the critical quality/safety attributes of the tissues and cells directly (e.g. an additive) or indirectly (e.g. donor testing kits). These areas should allow for adequate (physical or electronic) segregation of those materials in quarantine from those released for use. They should be temperature-mapped and monitored when required.

2.5. Equipment and materials

2.5.1. Equipment

A list or register of equipment that might influence the quality or safety of the tissues or cells should be maintained. All equipment on this list must be designed, qualified, validated and maintained to suit its intended purpose and all such equipment must minimise any hazard to donors, recipients, operators or the quality and safety of the tissues and cells. The validation plan should be designed through a risk-assessment exercise and should indicate when and how critical pieces of equipment should be validated and re-validated as necessary (see §2.16) [4]. Equipment should be selected that permits effective cleaning. Maintenance, monitoring and cleaning must also be carried out according to a schedule and documented in equipment logbooks.

Trending and analyses of calibration and monitoring results (e.g. via statistical process control) should be a continuous process. Intervals of calibration and monitoring should be determined for each item of equipment to achieve and maintain a desired level of accuracy and quality. The calibration status of all equipment that requires calibration must be readily available.

To ensure appropriate performance of a system or equipment, a monitoring plan must be developed and implemented. The plan should take into account the criticality of the system or equipment, and should outline monitoring, user notification and mechanisms for problem resolution. If an unusual event is observed, personnel should follow the standard response described in the monitoring plan. The standard response should involve notifying affected personnel and, if possible, initiation of a resolution response to the problem and risk assessment of the affected tissues or cells. Depending on the severity of the problem and the criticality of the system or piece of equipment, a back-up plan may need to be implemented to keep the process or system operating.

All equipment with a critical measurement function must be calibrated according to a planned schedule. Calibration is a procedure that confirms, under defined conditions, the relationship between values obtained from an instrument or system and those obtained using an appropriate certified standard. Calibration addresses measurement uncertainty. 'Measurement uncertainty' refers to the closeness of agreement between a measured quantity value and the true quantity value of what is being measured. 'Measurement precision' refers to the closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Hence, if the measured value is close to the true value, the measuring system has high accuracy and if the spread of the values is small when measurements are repeated, the measuring system has high precision. The acceptable tolerance should be set according to the critical quality attributes of a tissue/cell and these tolerance limits of the same equipment may have different needs depending on the tissues/cells subject to the process.

In practice, each piece of critical measuring equipment must be traceable. There must be an unbroken chain of calibration back to a recognised standard. Hence, the equipment is compared against a standard; the standard is compared against a higher standard; and the chain is documented through calibration certificates. If calibration is carried out by a third party, a copy of the calibration certificate for the specific measuring device used must be provided together with the calibration report. There must be an SOP that provides specific requirements for the calibration of each measuring device, such as defining the frequency of calibration, the number of measurement repeats, and the expectations and interpretation of obtained results which define acceptable limits. If the limits of measurement uncertainty are not met, there must be provisions for remedial action to re-establish conformity with these limits. These remedial activities must be documented. If calibration activities provide evidence that tissues or cells were processed and released for use when critical measurement equipment was not measuring accurately or precisely, risk assessment should be applied to decide on appropriate corrective or preventive actions regarding the fate of the tissues and cells.

A periodic review process should be established to ensure that the documentation for system or equipment is complete, current and accurate. If deviations or problems are found, actions should be identified, prioritised and planned.

2.5.2. Materials, consumables and reagents

A controlled list should be constructed of all materials and consumables that come into contact with the tissues or cells or that influence the quality or safety of the tissues or cells. Detailed specifications for these critical reagents and consumables must be documented. Only materials from qualified suppliers that meet the documented specifications should be used. When indicated, manufacturers should provide a certificate of compliance for every lot/batch of materials supplied. Batch acceptance testing or checking of each delivery of materials should be carried out and documented before release for use in tissue or cell procurement or processing. Equipment and materials should conform to international standards and EU and national licensing arrangements, where these exist.

Inventory records must be kept for traceability and to prevent use of materials after their expiry date. Each batch of critical reagents or consumables must be traceable to the respective procurement or processing session of tissues and cells in which they were used.

Apparent deviations in the quality and/or performance of equipment and materials must be investigated and documented promptly. Outcomes of these investigations should be reported in a timely manner to the RP, who should consider and approve the corrective and preventive actions to be implemented. For relevant deviations, a notice should be sent to the manufacturer and, where appropriate, reported to the Health Authority.

Further guidance on reagents and materials used in tissue and cell processing is provided in Chapter 8.

2.6. Outsourced activities management (contractual arrangements)

Where steps influencing the quality or safety of tissues or cells (i.e. critical steps) are carried out by a third party, there must be a contract or service-level agreement in place that describes the roles and responsibilities of all parties for maintaining the quality chain and the quality requirements for the service provided. Agreements should allow for on-site audits of contracted third parties to confirm their compliance with expectations. An example of an expectation is that if a supplier changes specifications for equipment or reagents provided to a tissue establishment, or they provide a substitute for an ordered item, they must first ensure that these changes are acceptable to the tissue establishment.

In EU member states, tissue establishments must establish written agreements with a third party each time an external activity takes place that influences the quality and safety of tissues and cells processed in co-operation with a third party. They must keep a complete list of these agreements and make them available at the request of Competent Authorities.

Agreements must be dated, reviewed and renewed on a regular basis. Written agreements should be in place for at least the following service suppliers:

a. testing laboratories (including donor, tissue and environmental testing);
b. procurement teams that are independent from a tissue establishment;
c. transport companies;
d. suppliers of critical equipment, consumables and reagents;

e. suppliers of services such as tissue and cell storage, processing or sterilisation;
f. suppliers of information technology (IT) services.

Once tissues and cells have been distributed for clinical application, they usually leave the QMS of the tissue or cell facility. However, appropriate control of transport and storage conditions, appropriate handling and preparation before use, and full traceability must all be maintained. Maintenance of quality and traceability is usually achieved by providing users with clear and detailed written instructions. The establishment should implement appropriate measures, such as instructions for use and traceability record labels, which are provided to the clinical user in order to ensure product quality and traceability down to the recipient. Some national standards require the organisation responsible for human application of tissues and cells (ORHA) to provide the supplying tissue establishment with details of the patient to whom the tissues or cells were clinically applied. However, this is not necessary for partner donation treatment in an ART centre or autologous haematopoietic progenitor cell transplantation where all steps (including clinical application) are carried out in the same facility.

Distribution direct to the patient for use without supervision of a health professional (e.g. serum eye drops) requires particular attention to instructions for storage and use, and should be carried out only if it is the only available option. Direct distribution of sperm to individuals for use without supervision of a health professional must be avoided for quality and safety reasons.

2.7. Documentation

Documentation must enable all steps and all data relating to the quality and safety of the tissues and cells to be checked and traced, from the donor to the recipient and vice versa (see Chapter 15: Traceability). In ART, traceability also involves follow-up of the outcome from these treatments, including the children (see Chapter 27). Written documentation ensures that work is standardised and prevents errors that may result from oral communication. Where oral communication is necessary for critical information exchange, audio recordings may be useful. Donor documentation in general, and donor-referral records in particular, must be subject to the same controls.

Quality-assurance (QA) document control is the process used in the management, co-ordination, control, delivery or support of an item required for QA purposes. QA document control is an essential part of the QA system of good tissue and cell practice. It allows authorised persons to approve, review and update documents; make changes and identify revision status; control document distribution; and prevent use of obsolete documents, facilitating proper archiving.

Documentation must be version-controlled and include at least the following items:

a. a quality manual;
b. specifications for materials and reagents;
c. approved SOPs for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;
d. identification and analysis of risks and a risk-mitigation plan;
e. records of the performance of operations, including processing records;
f. records of complaints, audits and non-compliances;
g. training and competency records of personnel;
h. qualitative and quantitative specifications for tissues and cells;
i. key quality indicators for tissues and cells.

Documents, including SOPs and forms, must be approved by appropriate and authorised persons and be part of a document-control system that ensures that only the current version of the document is in use. The system for distribution of controlled documents must ensure that all relevant personnel have access to the correct version.

A documented system for change control should be in place that controls changes to premises, equipment, processes, personnel and any item that may impact the quality and safety of the tissues and cells. This change-control system should link the rationale for change with the approval/rejection of the proposed change, criticality of the change with respect to the quality and safety of the tissues and cells; impact of the change on the tissue establishment as a whole, validation requirements of the proposed change and associated training requirements.

Records must be legible and indelible. Handwritten records should be avoided. Any alterations made to a record must be dated and signed or in the case of digital records an audit trail of alterations must be recorded. Documentation must be retained according to national requirements. Processing records must be maintained for all critical steps, and they must be dated and signed by the personnel responsible for carrying out the activity. All quality-control tests and checks must be documented. Any

deviations from the standard documented procedures must be recorded and reviewed, and corrective action must be documented.

The QMS must define the period of time for which documents will be retained. In the EU, records that are critical for the safety and quality of tissues and cells, including quality-system documentation and raw data, must be retained for 10 years and traceability documentation for 30 years after use or expiry of the tissues and cells.

Data can be stored on paper, electronically or on microfilm. An establishment responsible for using personal data has to follow strict rules on data protection. They must make sure the information is:

a. used fairly, lawfully and transparently,
b. used for specified, explicit purposes,
c. used in a way that is adequate, relevant and limited to only what is necessary,
d. accurate and, where necessary, kept up to date,
e. kept for no longer than is necessary,
f. handled in a way that ensures appropriate security, including protection against unlawful or unauthorised processing, access, loss, destruction or damage.

Information which is deemed more sensitive may require additional protection. This includes data on:

- race,
- genetics,
- biometrics (where used for identification),
- health.

International and national regulations on data protection have to be adhered to. See Chapter 13, Computerised systems, for further information on data protection. Personnel should have access only to those categories of data for which they are authorised.

Quality specifications should be prepared for each type of tissue and cell graft; these should be the basis for quality-control testing and product release.

2.8. Quality control

'Quality control' refers to those activities, such as verification steps, sampling and testing, which are used to ensure that materials, processes and the final product meet the required specifications. Internal quality control in a testing laboratory includes use of positive, weakly positive or negative control samples as appropriate. External quality assessment (sometimes called 'proficiency testing') involves analysis of unknown samples and evaluation of the results by a third party. Quality control of critical functions can be undertaken using audit techniques that include a sampling plan.

Detailed guidance on microbiological testing is provided in Chapter 10. Guidance on specific quality-control tests for specific types of tissues and cells is provided in Part B of this Guide.

2.9. Quarantine and release

All tissues and cells must be stored with an unambiguous quarantine status until all quality-control tests and checks have been conducted and the results reviewed by the individual responsible for release. Release of tissues and cells may be conducted in two steps. The first step confirms compliance of the donor with defined acceptance criteria (which is usually carried out by clinical personnel). The second step confirms compliance of the tissues or cells themselves, their characteristics, processing and storage, with those criteria defined in the product specification. The latter is usually carried out by quality-assurance personnel. The concept of 'quarantine and release' is not applicable to partner donation in ART and to some types of autologous or direct donation (see §12.5 for guidance on exceptional release). Tissues and cells that cannot be categorised as 'released' during storage must be stored with an unambiguous quarantine status.

For further information, see Chapter 9: Storage and release'.

2.10. Change control

Change-control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a product of the desired quality consistent with the approved specifications. Change control should be carried out prior to the implementation of a revised/new process. Written procedures should be in place to describe the actions to be taken if a change is proposed to any starting material, final product specification, equipment, environment (or site), method of production or testing, or any other change that may affect the quality of tissues or cells or the reproducibility of the process. All such changes should be requested, documented and accepted formally. The likely impact of the change in facilities, systems and equipment on the final product should be evaluated (including a risk analysis). The need for, and the extent of, any re-validation should be determined.

The training programme should be re-assessed for any critical change in environment, equipment or processes. Training records (including plans and training plans) must ensure that training needs are identified, planned, delivered and documented appropriately by taking into account any changes to systems and equipment.

Some changes may require notification to, or licence amendment from, a national regulatory authority.

2.11. Traceability

Full traceability – both of donations from donor to recipient and of all materials, reagents and equipment that come into contact with tissues and cells – is fundamental to recipient safety. Detailed guidance is provided in Chapter 15.

2.12. Complaints

All complaints must be documented, carefully investigated and managed in a timely manner. The complaints procedure should take into consideration complaints from:

a. living donors or the families of deceased donors;
b. personnel;
c. third-party health professionals;
d. clinical users, including those in another jurisdiction;
e. patients.

A mechanism for categorising, tracking and trending complaints should be in place and should be readily available for audit. Categorisation of complaints should in any case lead to the assessment of whether the complaint is justified and is related to a potential non-compliance. Any potential non-compliance should then be investigated thoroughly, including root cause analysis and identification of corrective measures (see §2.13, §2.18 for details).

2.13. Investigation and reporting of deviations, adverse events and adverse reactions

A deviation (which could be understood as non-conformity) might result in an adverse reaction in a living donor or in a recipient and must, therefore, be linked to the vigilance reporting system. There must be an SOP in place that defines how the organisation manages deviations and this SOP must include a log of all the instances of deviations that are investigated, including detailed documentation of the investigation, root cause analysis and corrective/preventive actions taken. A categorisation of deviations, depending on how critical they are to the quality and safety of tissues and cells, is a useful tool for prioritising corrective actions.

Procedures should be in place to identify appropriate corrective and preventive actions to be taken and to inform the relevant authorities as appropriate. Reporting of errors and incidents in a non-punitive context should be encouraged to help achieve improvements in practice. Tracking and trending of deviations should be carried out to identify common failures and identify areas for concern.

Serious adverse events and serious adverse reactions should be reported through a vigilance system. For detailed guidance on vigilance of tissues and cells, see Chapter 16. If products containing tissues or cells are classified in the EU as ATMPs, adverse occurrences should be reported either through pharmacovigilance systems for process events or through biovigilance systems for donor reactions.

2.14. Recall

An effective written procedure must be in place for recalling defective tissues or cells or those suspected of not meeting required quality or safety requirements. This written procedure must encompass the need to agree and document any corrective and preventive actions that might be necessary, remembering that other tissues or cells procured from the same donor might be affected. Therefore, a recall procedure could affect more recipients than initially presumed. If other organs, tissues or cells from the same donor were used, transplant teams should be promptly informed. The actions should be communicated to the end user, where appropriate. Further guidance on recall is provided in Chapter 16.

2.15. Self-assessment, internal audit and external audit

Auditing is an essential tool for ensuring compliance with the quality system and for supporting continuous quality improvement.

Internal audits should be scheduled and conducted in an independent way by designated, trained and competent persons. Internal audits are normally carried out by the organisation's quality assurance personnel, or can be done by a mandated person from

outside (quality assurance personnel from another TE) with the help of one internal person.

External audits are undertaken by independent bodies (often designated as approved/competent authorities or ISO certifying bodies) and are required for certification, accreditation and licensing purposes. External audits provide an opportunity for critical review by experts unfamiliar with the systems in place locally. They can provide an excellent opportunity for systems improvement.

All audits should be documented and recorded. Clear procedures are required to ensure that the agreed corrective and preventive actions are undertaken appropriately. These actions and the evaluation of their effectiveness should be recorded.

2.16. Qualification and validation

2.16.1. General principles

Validation is the part of the QMS concerned with proving that all critical aspects of the establishment's operations are sufficiently under control to provide continual assurance that tissues and cells will remain safe for patients and fit for purpose. The critical aspects subject to validation include:

a. the facilities and equipment used in procurement, processing, storage, testing and distribution, and any equipment and software used to manage their operation and data;
b. materials and reagents used which come into contact with cells and tissues;
c. labelling and tracking materials, equipment and software;
d. operational staff and the written procedures that they use to instruct their work;
e. process stages from procurement to distribution where there is a risk of a detrimental effect on the quality and safety characteristics of tissues and cells if not performed correctly;
f. analytical test methods used to assess and confirm the safety and quality of donors, donations, tissues and cells;
g. other auxiliary processes such as the transport and cleaning processes.

Validation is a highly technical activity requiring a good understanding of the risks associated with critical processes conducted by the tissue establishment and the potential risks and impact of materials and equipment used in these processes. Small establishments performing very simple, minimal manipulation of a limited range of tissues and cells in accordance with published methods, or following long-established practices using the same materials and equipment, may rely on ongoing quality control and periodic reviews to confirm that the method has the intended outcome. Such small establishments should still document their validation policy, explaining their approach on the basis of risk.

The risks increase significantly with the introduction of more complex processes, a wider range of tissue and cells handled, computerised systems, expansion of facilities and significant growth in workforce. In these circumstances it becomes more important to take the formal approach to validation as described in this section to ensure that the establishment's processes remain safe for donors and patients.

Validation is usually split into two components, qualification and process or test-method validation. The term 'qualification' is applied to each part of the process and to individual items including cleanroom facilities, equipment, computer systems, materials and operators. Such items should be qualified before they are first used in a process and then re-qualified at predetermined intervals, or when significant changes are made. Each individual item should be qualified separately to demonstrate consistent performance.

Process (or test-method) validation should only be performed once all the items used have been qualified. Process validation should be performed before a new process or method is used routinely and, where required by local legislation, before any associated tissue or cell product is released for human application (prospective validation). It is possible to conduct process validation during the processing of tissues and cells intended for subsequent release for human application (concurrent validation). Where establishments have not validated any of their processes or methods because they have been in routine use without change for many years, they may use existing data and information as a basis for validation (retrospective validation). Any process or method changes should be assessed for impact and risk in accordance with quality risk-management principles (see §2.16) and re-validation should be considered where there is unacceptable risk.

The objective of validation is to challenge the critical aspects of items through a series of controlled tests representative of the conditions under which they are expected to operate, to demonstrate that they achieve predefined acceptance criteria for quality and safety. The challenge should include the normal variation of possible conditions expected, but also more extreme conditions where there are

high risks, to provide a safety margin, for example with sterilisation processes. The test methods to be used and the acceptance criteria should be documented and approved by the establishment management before qualification or process validation commences. This document is commonly called the validation plan. The validation should be performed by trained and competent persons. The results of the validation should be compared with the acceptance criteria and any deviation from the plan should be recorded during the validation and documented in summary form with a conclusion. This document is commonly called the validation report. Following validation, the acceptance or rejection of the item or process by designated establishment management should be documented.

It should be clear through documentation and/or status labelling which processes and items have been validated and which are in the process of validation. Where anything is not in a fully validated state, there must be controls to prevent its use.

2.16.2. Validation planning

The validation policy should consider a process design phase where deep knowledge of the process is achieved. In this stage, the critical quality attributes of the tissues/cells are identified and the subsequent critical process parameters are identified. According to the critical process parameters affecting the critical quality attributes, a process control strategy should be developed.

During the validation phase itself, the process control strategy is implemented, and all the elements involved, such as equipment, utilities, suppliers and transport, are qualified before proceeding with the process validation.

All validation must be carefully planned in advance. Validation planning requires technical expertise in the processes involved and items used in the processes as well as expertise in any applicable regulations and technical and quality standards. It should therefore involve a validation team of relevant operational, quality, regulatory and medical experts in the establishment. Where necessary, for example the validation of new cleanroom facilities, external experts may be employed to advise.

Effective validation is not possible unless the establishment management is completely clear what its specific technical and quality requirements are. Establishments should use quality risk-management procedures to help determine their expectations for the processes and items used, which must address any significant risks to donors, recipients or quality of products, and any risks of regulatory non-compliance. Consideration should be given to possible modes of failure and the need to detect failure. Each establishment should document their expectations in the form of specifications. For processing stages there should be product specifications, for test methods there should be test specifications and for items used in processes, there should be user-requirement specifications (URS). For bespoke items of equipment and facilities, the manufacturer or supplier will need to interpret the URS and write a design specification to instruct their engineers, who will then write associated detailed technical specifications for the construction. For off-the-shelf items, the URS is used to assess suitability and to inform purchase decisions.

The validation team will need to develop validation plans that prove that the relevant specifications will be met under all likely conditions, with expected margins of safety where necessary. Appropriate tests and associated acceptance criteria should be established. This requires knowledge of the critical operational parameters and the expected variation in those parameters. Such parameters may include operating temperatures, exposure times, air flows, bioburden, location and product characteristics. Statistical methods will often be needed to demonstrate consistent performance with the necessary level of confidence. Means for detecting failures and alarms will need to be tested. Where relevant standards such as ISO and the *European Pharmacopoeia* provide detailed validation methods, these should be included in the validation plan.

If it is not possible to complete any procedures strictly in compliance with the plan, then there should be a review by the validation team, who should decide whether to repeat all or part of the validation or to allow a deviation for the modified procedure. Such decisions must be recorded in the validation records and report. Where establishments employ an independent quality manager or a designated RP in accordance with EU legislation, then that person should supervise all validation activity and be responsible for approving or rejecting the outcome of validation.

2.16.3. Documentation

As with all elements of the quality system, the policy and process for planning, executing and recording validation must be documented in written procedures. This documentation may be assembled into a validation master plan (VMP). The VMP will typically include the following:

- validation policy;

- organisational structure of validation activities;
- summary of facilities, systems, equipment and processes to be validated;
- documentation format;
- planning and scheduling;
- change control;
- reference to existing documents.

2.16.4. Qualification of operators

Operators are qualified as part of the training programme (see §2.3.2). There should be a documented training specification and plan for each operator, identifying how they are to be trained and listing the desired outcomes (acceptance criteria) from the training. The capability of individual operators to meet the desired outcomes should be assessed through observation and tests before they are approved as qualified to carry out procedures unsupervised. Particular attention is given to qualification of operators' aseptic techniques working in Grade A environments with Grade B background. The usual approach under such circumstances is to conduct simulated processes using culture medium or broth in place of, or added to, tissues or cells.

Before written procedures (SOPs and Work Instructions) are approved as part of the document-control system (see §2.7) they should be read and qualified by an experienced operator to confirm that they are clear, understandable, accurate and practical.

2.16.5. Qualification of materials and suppliers

Detailed User Requirements Specifications (URS) should be available for materials (see §2.5.2 and §2.16). Before introducing a new material into a process it must be qualified. This will involve confirmation that it meets the URS through examination of the material and of test data either from the manufacturer (Certificate of Analysis), a third party or in-house testing. Where the URS requires, or where there may be significant variation that might affect the outcome from the process in which the material is to be used, there may be a need for process qualification of the material. Process qualification may be carried out on a scaled-down version of the process or at full scale and may be run in parallel with the existing material to demonstrate a comparable outcome.

Suppliers and manufacturers of materials, equipment and contract services should be qualified before any purchase is made. The purpose is to ensure that they can be relied upon to continue supplying the goods and services that meet the URS. This relies upon compliance with quality specifications and operation of an acceptable quality system. This should be confirmed through audit (see §2.15) of their operations and quality system. It is possible to conduct the audit through a questionnaire supported by copies of relevant certification from a recognised independent body or regulatory authority. Otherwise an on-site audit should be performed. The information gathered through the audit should be formally documented and assessed to determine whether the supplier may be deemed qualified for the established requirements of the auditing organisation.

2.16.6. Qualification of facilities and equipment

Facilities and equipment should be qualified and processes must be validated before use and when any significant change is implemented.

Facilities and equipment should be qualified following the four steps shown in Figure 2.1. Each step should be completed, and verification obtained that acceptance criteria have been met, before proceeding to the next step.

2.16.6.1. *Design qualification*

The first element of the validation of new facilities, systems or equipment can be considered 'design qualification' (DQ). This validation involves demonstration and documentation of the compliance of the design with good practice (i.e. the design is suitable for the intended purpose). DQ is not required for off-the-shelf equipment and systems as these have already been designed and built for specific uses. Only new facilities and equipment being designed or built specifically for the establishment require DQ, which should be complete before fabrication of equipment, systems or facilities starts.

Figure 2.1. **Steps in qualification of facilities and equipment**

2.16.6.2. *Installation qualification*

Installation qualification (IQ) should be carried out on new or modified facilities, systems and equipment once installed on site. IQ should include (but is not limited to) the following:

a. installations of equipment, piping, services and instrumentation, which are checked to current engineering drawings and specifications;
b. collection and collation of the operating and working instructions as well as the maintenance requirements of the supplier;
c. calibration requirements, including verification of the uncertainty of measurement for any measuring equipment;
d. verification of construction materials.

IQ for new facilities and more complex equipment may be performed by the supplier, but the establishment should verify that agreed acceptance criteria have been met. An example of a cleanroom qualification document is available in Appendix 4.

2.16.6.3. *Operational qualification*

Operational qualification (OQ) should follow IQ. OQ should include (but is not limited to) the following:

a. tests that have been developed from knowledge of processes, systems and equipment;
b. tests to include a condition or a set of conditions encompassing upper and lower operating limits (sometimes referred to as 'worst-case' conditions).
c. Completion of a successful OQ should allow calibration, operating and cleaning procedures, operator training and preventive maintenance requirements to be finalised. It should permit a formal 'release' of the facilities, systems and equipment.

2.16.6.4. *Performance qualification*

Performance qualification (PQ) should follow successful completion of IQ and OQ. PQ should include (but is not limited to) the following:

a. tests, using production materials, qualified substitutes or simulated products, which have been developed from knowledge of the process and the facilities, systems or equipment;
b. tests to include a condition or set of conditions encompassing upper and lower operating limits.

The number of tests carried out should achieve reproducibility of the process, to the grade of warranty that the process is required to demonstrate. The more knowledge there is of the process, the less testing during PQ may be needed.

2.16.7. Qualification of software

Computer and automated systems controlled by bespoke and off-the-shelf software are extensively used by tissue and cell establishments to help manage procurement, processing, testing and distribution activities and data. The design of, and changes to, software can have a significant impact on the quality and safety of tissues and cells and the integrity of critical data. It is therefore essential to maintain effective version control over software in use and to qualify new software and requalify existing software when changes are made. Establishments should be aware of the current version of software operating their relevant computer and automated systems at all times and should not allow upgrades of existing software by system suppliers without their approval.

Software requirements should be included in the appropriate URS for the system they control. For bespoke software, the URS will be used by software system engineers to produce a detailed technical specification to be used by a programmer to write new or revised code for the system. For an off-the-shelf system the URS will be used to assess candidate software and to inform the local IT staff how to configure the software. New and revised versions of software should be qualified in operation by users (user acceptance testing or UAT) before being put into service. At the very least, this testing should include verification of critical settings encoded in the software, for example when there is a version update to software for running an apheresis machine or testing system. However, usually the software will be tested in its operational state through process-simulation tests or parallel running as part of the system (equipment) qualification. It is important during user acceptance testing to verify that existing functionality continues to operate as expected (regression testing) as well as testing new functionality.

For additional information, see Chapter 13.

2.16.8. Test-method validation

The approach to test-method validation will depend on whether the test is quantitative or qualitative. In all cases the validation plan should take into account the variety of sample types and analytes to be tested, as there may be substances present that interfere. For quantitative assays, the acceptance criteria should consider accuracy, reproducibility, linearity, limits of detection and required range of measure-

ment. The uncertainty of measurement should be established and quoted with subsequent results. For qualitative tests, then specificity and sensitivity are the key criteria. It may be considered unnecessary to perform in-house method validation if test systems and kits certified as compliant with the EU *In vitro* Diagnostic Medical Device Directive are used along with qualified equipment in accordance with the manufacturer's instructions. However, in-house verification studies should be done to demonstrate that the performance of the kit or test system, as used in the establishment, meets the expected specification. If using *Pharmacopoeia* methods, e.g. for sterility testing, the methods should be verified in accordance with the method monograph.

2.16.9. Process validation

Process risk-assessment methods should be used to identify what processing stages require validation. Most processing of tissues and cells involves the removal, exclusion or reduction of unwanted or undesirable substances, while maintaining the functionality of the required tissue or cells. As a minimum, validation will focus on demonstrating that the desired characteristics are achieved in performing consecutive processes (usually three) and confirming that the purity specifications have been reproducibly met in all cases. Process validation may also include *in vitro* and *in vivo* tests of functionality, where there is a risk that this may be affected. However, because of the inherent variability of human cells and tissues, establishments should consider supplementing prospective or concurrent validation with an ongoing process-verification programme of quality-control testing before release and of quality monitoring.

The processes for removing undesirable substances and in particular potentially pathogenic micro-organisms should be validated with a safety margin, or 'worse case' scenario. This will usually involve spiking the material with a larger-than-normal level of the undesirable substance and demonstrating its effective removal, or reduction to safe levels, by the process. In the case of micro-organisms, strains that are known to be resistant to antimicrobial treatment, e.g. spore-forming, heat-resistant bacteria, may be used for spiking and to validate sterilisation processes, at sufficient levels to demonstrate at least a 6-log reduction. For safety-critical process validation it is recommended that published guideline methods are consulted where available.

More specific guidance on approaches to validation is given in other sections of this Guide. Some examples of qualification are given in Appendix 4 and Appendix 5, and examples of validation are in Appendix 6 and Appendix 7.

2.17. Risk management

The procurement, testing, processing, storage and distribution of tissues and cells should be subjected to comprehensive risk assessment to allow identification of those steps where most of the quality-system controls are required and where validation of procedures is necessary. A 'process flow' diagram listing all relevant steps, processes, reagents, tests and equipment can form the basis for the assessment exercise. Risk assessment should include an estimation of the severity of any identified hazard (source of harm) and an estimation of the probability that the hazard will result in harm. Probability should be based on evidence and experience whenever possible.

Risk-mitigation strategies should be developed to protect the tissues and cells, the donor and recipient, personnel and the process itself, as well as other processes being undertaken in proximity to it. The degree of control within the quality system should be related to the degree of risk associated with each step in the process.

Risk assessment should refer to current scientific knowledge, should involve appropriate technical expertise and should be related to the protection of the patient. The level of effort, standardisation and documentation of the risk-control process should be aligned with the estimated risk level.

Risk assessment should be repeated and documented whenever a critical process is changed as part of a change-control process. Actions to mitigate any significant new risks, including validation, should be completed before any change is implemented.

Risk assessment is also an essential tool for making important decisions, particularly when departures from standard procedures or their standards and specifications are under consideration. Examples would include:

a. selection of a donor where full compliance with the normal criteria has not been met, but where the donation has a particular clinical value and the potential risk can be mitigated sufficiently to justify the deviation from standard procedures;

b. exceptional release of non-complying tissues or cells on the basis that the potential benefits for the recipient and the lack of availability of alternatives outweigh the potential risks;

c. retention or removal of tissues and cells in storage that had been historically released according to former criteria, when new, more

sensitive procedures or tests have been implemented that imply an additional level of safety or quality and new, more stringent criteria for release;

d. eligibility determination where certain test results are reactive, for example, where EU Directive 2006/17/EC Annex 2 requires further investigations with a risk assessment when antibody to hepatitis B core antigen (anti-HBc) is positive and hepatitis B surface antigen (HBsAg) is negative or where a donor is reactive for a *Treponema*-specific test (see Chapter 5 for further guidance on donor testing);

e. prioritisation of a list of corrective actions following an audit or inspection, or prioritisation of quality improvements in general.

The approach to risk assessment should be systematic and should be documented. The most commonly applied risk-assessment methods are Hazard Analysis and Critical Control Points (HACCP), Failure Mode and Effects Analysis (FMEA) and Failure Mode, Effects and Criticality Analysis (FMECA).

2.17.1. Hazard Analysis and Critical Control Points

HACCP was developed in the 1950s in the food industry but is now widely used for many manufacturing processes, including biological control. HACCP is also recognised by ISO 14644 as a formal system for risk assessment. HACCP is a system that requires that potential hazards are identified and controlled at specific points in a process. HACCP has seven principles:

1. Conduct a Hazard Analysis (HA).
2. Identify the Critical Control Points (CCP).
3. Establish the critical limits.
4. Monitor CCP.
5. Establish corrective actions.
6. Verification.
7. Record keeping.

The World Health Organization published a paper in 2003 providing more details on using HACCP as a tool within a pharmaceutical environment, details which can be further adapted and interpreted for use within a tissue establishment [5].

The *Quality Assurance Journal* has also published papers on the use of HACCP as a tool within a QMS [6].

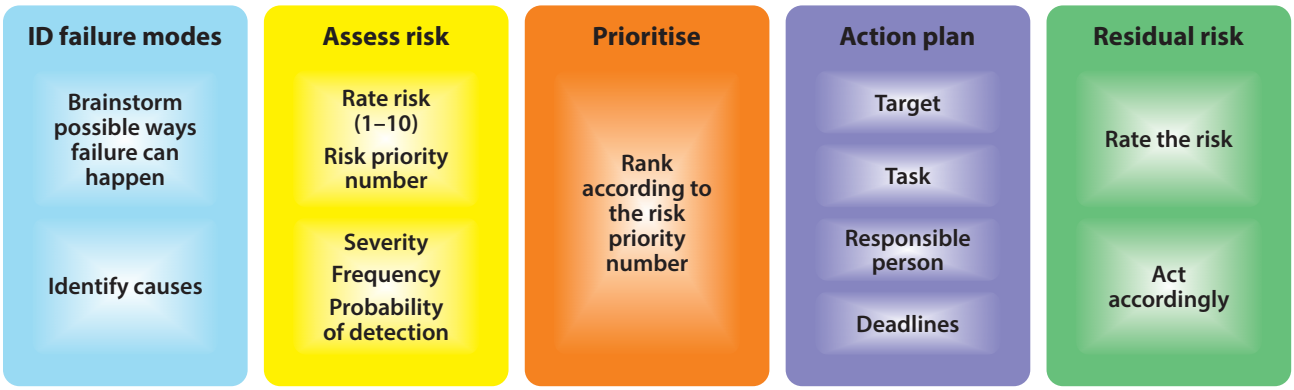
2.17.2. Failure Mode, Effects and Criticality Analysis

FMECA is an extension of Failure Mode and Effects Analysis (FMEA) that includes a factor for detectability, taking into consideration those hazards that are more easily detected and represent a lower overall risk. FMECA allows the estimation of a risk priority number (RPN) for the ranking of identified risks [7]. The estimated level of risk should take into account the benefits, possible alternatives and costs associated with reducing risk further. An organisation should have a clear policy regarding risk acceptance (see Figure 2.2).

Undertaking risk assessment at various stages helps to define requirements and alternatives, aids the process of supplier selection and helps to determine the scope and extent of validation. The methodology has been used for tissue and cell banking for specific process steps [8] and for reviews of an entire process [9].

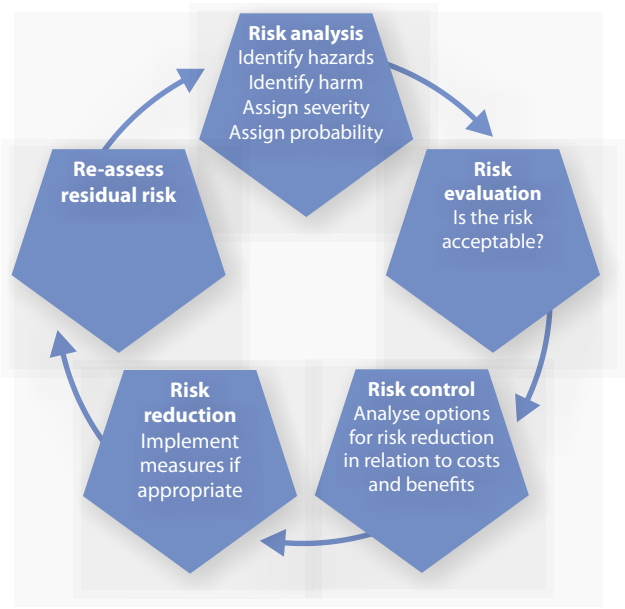
Risk assessment is not a once-only process but a cyclical one (Figure 2.3). Risk assessment should be followed by risk avoidance and reduction (if possible) and continuous re-evaluation of residual risk.

Figure 2.2. **Failure mode effects and criticality analysis (FMECA)**



Guidance on quality risk management is provided in Part III Q9 of the Rules governing medicinal products in the EU, Volume 4: EU Guidelines for good manufacturing practice for medicinal products for human and veterinary use [1], in which several well-established risk-assessment methodologies are listed. Inclusion of this new section in GMP guidance reflects the current thinking that risk management should be an integral part of quality management.

Figure 2.3. **Cycle of risk assessment**



2.18. Root Cause Analysis

Root Cause Analysis (RCA) is a tool used to understand the true cause of why an event has occurred. Through investigation and getting to the root cause, you are able to identify corrective and preventive actions (CAPA) which will prevent any occurrence of the event in the future.

RCA has five purposes:

a. Establish the facts and events that led to the event;
b. Identify what went well;
c. Determine what went wrong;
d. Establish the root cause;
e. Identify CAPA.

When carrying out RCA there are five main factors which need to be considered:

- Plant – what was used, was it calibrated, was it maintained etc?
- People – who was involved, were they trained, competent and capable?
- Procedure – is there an SOP, was it followed, is it correct, has it been validated?
- Premises – location, department, environment etc?
- Product – how many times has this occurred, number of products affected, damage, defects?

When performing RCA, there are additional tools that can be used, such as fishbone diagrams, why whys, flowcharts and timelines. Examples of these have been included as appendices 8, 9 and 10.

2.19. Continuity planning

General quality-management responsibilities include budgetary/fiscal oversight and contingency planning to ensure that essential services for patients are not interrupted. Each organisation in the chain – from donation to distribution and biovigilance of tissues and cells – should have a continuity plan in place that details how procurement services, donated tissues and cells and all associated documents will be maintained in the event that activities must temporarily be suspended or permanently ceased. Usually this plan will include a mutual agreement (a service-level agreement or contract) with another organisation for the transfer of tissues or cells, documentation and services in these circumstances.

2.20. References

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Related material

• Appendix 4. Example of cleanroom qualification
• Appendix 5. Example of incubator qualification
• Appendix 6. Example of process validation – tissue transportation
• Appendix 7. Example of method validation – oocyte vitrification
• Appendix 8. Example of root cause analysis: why, why? – receipt of tissue
• Appendix 9. Example of root cause analysis: fishbone diagram – receipt of tissue
• Appendix 10. Example of root cause analysis: fishbone diagram – medically assisted reproduction

Chapter 3: Recruitment of potential donors, identification and consent

3.1. Introduction

Human tissues and cells may be used in transplantation therapy or for medically assisted reproduction (MAR). They can be obtained from deceased or living donors, as long as the procedure does not compromise the respect due to deceased persons nor endanger the life or health of living donors. Transplantation of tissues and cells can range from life-saving treatments (e.g. serious burns victims, general sepsis due to prosthesis infection, haematological malignancy) to quality-of-life improvements (sight or motion restoration). In addition, donated gametes and embryos may help fulfil a person's wish to have children. Human tissues and cells are also starting material for advanced therapy medicinal products (ATMPs).

In order to ensure the safety and success of any transplantation or human application programme, potential unrelated living donors need to be recruited and potential deceased donors need to be identified and referred. In any case, screening must be performed to exclude any contraindications to donation and, in the case of a living donor, to exclude any medical situation that could potentially harm the donor.

Successful donation programmes should at least include [1]:

a. adequate public-awareness strategies, promoting not only organ donation but also tissue and cell donation;

b. effective systems to facilitate the recruitment of living donors in an ethical manner, ensuring their safety and well-being, and the identification and referral of all potential deceased tissue donors to the appropriate organisation (e.g. routine medical chart reviews in every case of in-hospital death);

c. adequate training of professionals involved in the recruitment, or in identification and referral.

Once potential donors are recruited or identified and referred, informed consent is required before donation can take place. Consent is obtained from the donor themselves if alive or from their legal representatives if juvenile or incapable, or for deceased donors either from the donor before death (e.g. donor registries, donor card, advanced directives) or from their relatives; see Appendix 11 and following. The way in which consent is obtained depends on the type of donor, the specific circumstances and the different legal systems for consent. Although the term 'consent' will be used throughout the chapter, the Guide recognises that in some countries, the term 'authorisation' rather than 'consent' is used to enable lawful recovery of tissues and cells.

This chapter describes the process, requirements and key elements at the beginning of the donation pathway for both living and deceased donors.

3.2. Living donors

Some tissues and cells can only be obtained from living donors. This is true for haematopoietic progenitor cells (HPC); for oocytes, spermatozoa, ovarian or testicular tissue and embryos, used in MAR procedures; and for mesenchymal stromal cells and some somatic cells, such as keratinocytes and chondrocytes.

Some tissues can be collected as surgical residues (e.g. placenta, femoral heads removed during surgery to replace a hip joint, heart valves from patients receiving a heart transplant, veins from stripping). In some cases, tissues from a patient must be processed and stored for their own treatment in the future (e.g. skull bone obtained from a decompression craniotomy, parathyroid tissue to be reimplanted in case of insufficiency after its removal during the thyroidectomy, oocytes as part of specific programmes to preserve fertility in oncological female patients).

Depending on how the tissues or cells will be used, their clinical application can be described as:

a. autologous: when the tissues or cells procured from a patient are used for the patient's own treatment;

b. allogeneic: when the tissues or cells donated by one person are used for the treatment of another person. In this case, the material can be donated for:

i. an intended recipient, who can be related or unrelated to the donor (e.g. HPC);

ii. an undirected recipient, unrelated to the donor (e.g. amnion).

In the case of MAR, depending on the origin of the gametes and embryos, the donation can be classified as:

iii. partner donation (donation between a couple who declare that they have an intimate physical relationship);

iv. non-partner donation.

The selection of a living donor must be based on a risk–benefit analysis for both donor and the recipient. In the case of surgical residues, there is no risk to the donor derived from the donation itself.

In some instances, donation may occur years after the initial selection and registration (e.g. in HPC donation, when potential donors are included in a registry and the donation only takes place if a matching recipient needs it).

3.2.1. Donor recruitment

Donor recruitment is a continuous process. It begins with increasing public awareness by educating society, at local and national levels, about the benefits of the clinical applications for different tissues and cells. Recruitment drives can focus on altruism, solidarity and social engagement, based on the principles of voluntary unpaid donation.

One way to increase awareness is to explain the benefits for the recipients of donated tissues and cells. Success stories describing patients' experiences and testimonials of family members may drive people to consider whether tissue or cells donation is right for them. However, public campaigns should aim to increase the number of undirected donations and registration on living donor registries. Publicising and/or advertising the need of donated tissues or cells for a given patient should be discouraged (in some countries such activities are forbidden/regulated by law).

The most common instances of donation from living donors where donor recruitment plays an essential role are the donation of HPC and of gametes and embryos for MAR procedures.

3.2.1.1. *Recruitment of haematopoietic progenitor cell donors*

Donation of HPC must be voluntary and unpaid, and informed consent must be obtained in the preliminary steps. Successful HPC transplantation depends on matching the donor and recipient for human leukocyte antigens (HLA). If a patient needs an HPC transplant, their siblings and close relatives should be screened to determine if their HLA types are compatible. If the donor is under the age of 18, specific consideration is needed (see Chapter 22).

For patients without a matching family donor, there is a possibility that an HLA-compatible voluntary donor can be found. Therefore, it is of crucial importance that volunteer donors are recruited and registered on donor registries around the world, particularly from diverse ethnic communities. Donor registries and cord blood banks are linked internationally, in a global database operated by the World Marrow Donor Association (WMDA), using tissue-typing to establish rapid identification of potential donors.

When a person volunteers to be an HPC donor, including expectant mothers, an initial evaluation is performed. Suitable donors are HLA-typed and their details are placed on a donor registry. Potential donors are requested to inform the registry in case of any change to their health status that could contraindicate donation or the possible use of the cells, as in the case of umbilical cord blood (UCB). They

will only be further contacted if they are identified as the best possible match for a patient. In this case they will be requested to attend a donation centre for a full explanation of the procedures by the clinical staff and a thorough medical examination and blood tests to detect any medical contraindications to donation.

Further information on donor registries and recruitment of HPC donors can be found in Chapter 22 and Chapter 23.

3.2.1.2. *Recruitment of donors in medically assisted reproduction*

With the development of MAR and changes in lifestyles, a growing demand for gamete and embryo donors has emerged. These developments in most countries also reflect changing social values and government financial support. This growth has not always been accompanied by similarly swift development of laws and regulations in the field. Gradually, countries have set legal provisions and/or guidelines based on their own historical, cultural, religious and social traditions and their political and economic situations. Consequently, there are wide variations in the techniques available and permitted by law in each country, and the types of reproductive cell that can be donated. Whatever the situation, national laws must be respected and donors recruited accordingly.

As mentioned previously, gamete and embryo donation can be classified as partner or non-partner donation depending on the link with the donor. This chapter will focus on the recruitment of non-partner donors.

The practice of gamete and embryo donation is complex, and multiple ethical rules, legal restrictions, medical facts and social and psychological consequences intermingle. Things are further complicated by such factors as:

a. the reasons why a person donates, whether for the treatment of others or for a scientific purpose;
b. what kind of reproductive cells are donated: donation of oocytes or sperm or embryos commits the donor to a different investment in the medical interventions required and the risk of harm;
c. the level of anonymity, whether a known donor, identifiable or anonymous;
d. the level of compensation schemes for donors;
e. how often a donor can donate and how many offspring may derive from a single donor.

The scarcity of donor gametes has stimulated discussion about acceptable systems of recruitment, especially since it has become evident that different clinics and countries are using different approaches to recruit donors, with various modes of compensation for donors (e.g. compensation of proven expenses, compensation through lump sums, oocyte-sharing schemes). However, it is essential to emphasise that the donation of reproductive material should strictly follow the same principles of being voluntary and unpaid as for other donations of tissues or cells. Any type of reward, benefit or incentive may be a threat to voluntariness and compromise the degree of confidence that can be placed on medical interview (see Chapter 27).

The donor-recruitment activity, whether performed by a public health system or by private clinics (where allowed by national legislation), must be authorised by the Health Authority.

As with any living donors, it is essential that gamete donors are entered into registries, not as a database of potential donors, such as for HPC, but to guarantee traceability and transparency of practice (see Chapter 15). In addition, registries allow adequate follow-up of the donors, including the collection of data on serious adverse events or adverse reactions in donors, recipients and in the resulting offspring (see Chapter 16).

Further information on recruitment of gamete and embryo donors can be found in Chapter 27.

3.2.2. Consent for living donation

The donation process differs depending on whether a person donates tissues and cells for the benefit of others, and accepts risks and inconvenience that they would not otherwise face, or donates while undergoing a medical intervention for their own benefit. Donation implies an altruistic act and, to some extent, a trade-off between individual well-being and societal utility. As a consequence, there is potential for the abuse and exploitation of individual donors. Obtaining individual consent, in any situation of donation, either collection of surgical residues or tissues and cells procurement, is crucial to assure that donation conforms to professional ethical standards and the individual's own goals and values.

Donation of tissues or cells must only be carried out after the person concerned has given free, informed and specific consent, either in written form or orally before an official body.

Consent should be recorded and/or documented in the donor/patient's record. Informed consent must be discussed with the donor or their legal representative in a language and with terms

that they can understand. If translation is needed, it is recommended that neither family nor friends serve as interpreters or translators. The record should mention that the prospective donor has understood – and, where appropriate, their legal representatives or their relatives have understood – the information given, had the opportunity to ask questions, received satisfactory answers and confirmed their position on donation. Some examples of forms to obtain consent for MAR may be found in Appendix 11 and following.

Recruitment of persons not able to consent should never be done through public registries. In addition, in some countries, specific regulations restrict donation in these circumstances (e.g. some countries do not allow procurement of peripheral blood progenitor cells from minors and/or administration of growth factors).

In accordance with Article 14 of the Additional Protocol to the Oviedo Convention, no tissue or cell procurement may be carried out on a person who does not have the capacity to consent. Exceptionally, and under the protective conditions prescribed by law, the removal of HPC for allogeneic transplantation from a person who does not have the capacity to consent may be authorised provided the following conditions are met:

a. there is no compatible donor available who has the capacity to consent;
b. the recipient is a brother or sister of the donor;
c. the donation has the potential to be life-saving for the recipient;
d. the authorisation of their representative or an authority or a person or body provided for by law has been given specifically and in writing and with the approval of the competent body;
e. the potential donor concerned does not object.

More recently, in the context of haplo-identical HPC transplantation, these conditions have been extended to include other first- or second-degree relatives (not only brother or sister) when no other donor is available and all the other conditions are fulfilled.

Potential donors must beforehand be given appropriate information as to the type(s) of tissues or cells to be procured, the purpose and nature of the intervention, its consequences, possible side-effects and risks, whether the tissues or cells will be processed and stored, and the purpose or use to which the tissues or cells will be put. All relevant information should be given because consent must be specific and cannot be assumed for uses of tissues or cells about which the donor has not been informed. Donors must be given all the information needed to ensure that they understand all potential purposes and benefits for the recipient before they give consent. Full understanding of the consent is particularly important when practices may be controversial (e.g. the use of gametes or embryos for research purposes). Similarly, some donors may not wish to donate tissues or cells to a commercial organisation where, for example, their donated tissues or cells may be used as starting material for developing therapies in a commercial setting.

Potential donors should have an advocate, who must not be involved in the treatment of the recipient. Information about potential risks for the donor and risks and benefit for the recipient must be clearly understood before consent is given. Where relevant, they must also be informed that the results of the qualification of tissues and cells for transplantation and the transplant itself are uncertain because they depend on many factors, including the recipient's situation. The donor should also be provided with information about psychological support in case the transplant does not lead to survival or cure of the recipient.

The scope and duration of the consent must be stated explicitly. When the tissues and cells are to be stored, the consent should include information about the storage time and the policy once the period has expired. In the specific case of gametes and embryos, some countries have regulations on the duration of storage.

The interview for consent should be conducted in a suitable environment. The interviewer should have received specific training for this purpose to be able to consider the donor's needs and to answer questions about donation and transplantation processes.

Potential donors, or their legal representatives, or their relatives, should be informed that tests will be performed to evaluate the possible existence of any transmissible diseases that would be a risk to the recipient, and the action to be taken in the case of a positive result. They should be then informed and receive adequate counselling. All results of the medical evaluation should be kept available to the donor. If a potential donor is found to be unsuitable, the reasons and the results of the medical assessment should be discussed with them and advice should be given on recommended actions regarding their health. If the findings do not exclude the potential donor but may have implications for the recipient (e.g. an HPC potential donor carrier of a haemoglobinopathy), the transplant centre must also be informed.

In the case of HPC donors, consent must be obtained at several stages: before HLA typing, before donor testing and before the conditioning regimen on the recipient has begun. The interview must include information about donation procedures, their risks

and side-effects, the procurement method, and the need to administer growth factors when peripheral HPC is being procured. In the case of cord blood donation, consent is usually obtained months before the delivery and should at least be obtained before the mother goes into labour to avoid interfering during the delivery.

Table 3.1. **Key parties in tissue-donation programmes and the challenges and opportunities they may pose**

	Opportunities	Weaknesses/Obstacles
Public	• Learn about donation and the benefit of transplantation to recipients; • Promote solidarity and altruism; • Express wishes to family and friends in relation to donation.	• Lack of knowledge about donation; • Unwillingness to face death; • Fear that an expressed desire to become a donor may interfere with medical care; • Lack of trust in the fairness of the donation and transplantation system.
Donor family	• Honour the wishes of the loved one; • Make some sense out of the death of a loved one; • Find comfort in knowing that donated tissues of the loved one helped save or improve lives; • Gain some control after the death of a loved one; • Know that a part of the family member 'lives on'; • Recognition and gratitude from society and recipients.	• Grief; • Lack of understanding or knowledge about donation; • Fear of disfigurement; • Not knowing the wishes of the loved one; • Lack of trust in the medical profession or fairness of the donation system.
Hospital staff	• Support wishes of the donor and his/her family; • Increase availability of tissues for patients in need; • Contribute to public awareness of donation and transplantation.	• Discomfort or lack of knowledge about donation; • Lack of trust in the donation and transplantation system; • Not acknowledging the value of tissues; • Increased workload; • Lack of acknowledgement from those involved in procurement; • Lack of resources.
Transplant co-ordinator	• Support wishes of donor and their family; • Maximise donation opportunities – tissue donation may benefit as many as 100 patients per donor; • Increase availability of tissue for patients in need; • Contribute to public and professional awareness of donation and transplantation.	• Concern that tissue consent will have an impact on consent to organ donation; • Lack of knowledge about tissue donation and its benefits; • Increased workload.
Coroner	• Mutually beneficial for coroner and procurement team/tissue establishment, who may share findings, tissue/blood samples and test results to help investigations; • Support wishes of the donor and their family; • Increase availability of tissues for patients in need.	• Lack of knowledge about donation; • Concern over potential impact on death investigation.
Funeral home director	• Extend support to bereaved families; • Raise awareness by including donation information in funeral homes and obituaries; • Allow procurement of certain tissues to occur in funeral homes; • Contribute to the conditioning of the body; • Increase availability of tissues for patients in need.	• Lack of knowledge about donation; • Fear of difficulty in preparing body for funeral services; • Increased time and costs; • Delay in funeral services.
Retirement and nursing homes	• Support wishes of the donor and his/her family; • Raise awareness by including donation information; • Allow procurement of certain tissues to occur on their premises; • Increase availability of tissues for patients in need.	• Lack of knowledge about donation; • Lack of trust in the medical profession and in the donation and transplantation system; • Many of the residents may be affected with conditions that contraindicate donation.

Documentation must be made available to the cell or tissue establishments that receive the procured material for processing and storage to confirm that detailed consent has been duly given (see Chapter 2).

Donors must be informed that they may withdraw consent at any time. In case of HPC donors, they should be informed of the possible consequences for the recipient if they withdraw their consent once the conditioning regimen on the recipient has already begun. Ultimately, this situation cannot be used to coerce the donor and their final decision must be respected.

In the case of autologous donation, the patient must be informed about options and the balance of risks and benefits of the procedure. The consent must specify what would happen if the clinical application could not be performed for any reason (e.g. contamination of the transplant or if the patient's condition contraindicated application). In such instances, the consent should indicate whether the tissues or cells could be used for the treatment of others, for research or must be disposed of.

Consent should be obtained from the patient before procurement; but, in some circumstances, this may not be possible (e.g. emergency craniotomies) and the decision to procure and, if necessary, to process the tissues or cells should be taken by the medical team.

In the case of planned surgical procedures where it may be possible to donate residues, consent should be obtained before procurement. As is the case for all tissue or cell donors, the donor should be informed beforehand about tests to determine the suitability of the transplant, and consent should include information about the processing, storage and intended use of the donated material. The consent must also specify the fates of tissues or cells if their clinical application cannot be performed for any reason.

Donors, or their legal representatives, or their relatives, should be aware of the importance to recipient safety of providing the medical staff with information about any medical condition of the donor that may occur after donation.

They should be informed about, and give consent for, the use of personal data in computerised data processing.

3.3. Deceased donors

Tissues donated by a single donor can be used to treat as many as 100 recipients. Tissues donated by deceased donors typically include the following tissue types:

a. musculoskeletal: bones, tendons, ligaments of the upper and lower extremities, menisci, fascia, cartilage;
b. cardiovascular: heart valves, blood vessels, pericardium;
c. skin: split thickness (typically, only the epidermis) and full thickness (epidermis and dermis);
d. ocular: corneas, sclera;
e. specific cell types from certain tissues (e.g. limbal stem cells) or from certain organs (pancreatic islets, hepatocytes).

In order to guarantee the success of any donation programme from deceased tissue donors it is essential that hospitals (and other centres where potential tissue donors may be found) have a system to identify potential donors, to check the presence of donor's consent or lack of donor's objection expressed by donor before their death or to obtain consent from family members or relatives of potential donor and refer donors to the appropriate procurement agencies or tissue establishments.

Communication with bereaved family members or relatives will require clear and sensitive procedures or protocols with consent obtained by appropriately trained specialists in donation (see Appendix 14).

3.3.1. Identification and referral of deceased tissue donors

Tissue donation depends on commitment and the development of strong working relationships between tissue establishments, procurement organisations and hospitals and other centres where potential tissue donors may be found, such as medical examiners' offices, mortuaries, coroners' offices, forensic institutes, funeral homes, emergency medical services, and nursing or retirement homes.

It is recommended that any potential donors considered for organ donation are also referred for potential tissue donation. Furthermore, it is recommended that all deaths (typically hospital but also community deaths) should be routinely referred to a donor co-ordinator, procurement organisation or tissue establishment, regardless of the age of the patient, the cause of death or the known wishes of the donor or their family to become a tissue donor. Routine referral of all potential donors with no known medical contraindication gives every individual the opportunity to donate and allows for the standardisation of donor-selection criteria.

In every deceased donation programme, it is essential to identify all of the parties who may be involved, in order to co-ordinate and facilitate the process and maximise the opportunities for successful procurement. The key parties are summarised in Table 3.1.

Before procurement, checking the donor's identity is an essential prerequisite.

A proper investigation needs to gather as much information as possible about the donor, using medical records, interviews with medical staff who treated the donor (attending physician, general practitioner, nurse) and relevant information provided by the donor's relatives or legal representatives, family physician or other persons who have information about the donor's behavioural and medical history. The donor's medical suitability is assessed in accordance with the selection criteria, and after the evaluation of risk factors such as sexual behaviour, travel and exposure to sources of infection. These criteria may vary, depending on the type of tissue to be procured for human application (see Chapter 4 for general criteria and Part B for tissue-specific criteria).

In the event that a health facility does not have the means to manage a potential tissue donor or is not licensed/authorised for tissue procurement by their Health Authority, arrangements should be made, where possible, for transfer of the potential donor to a suitable hospital or procurement centre.

3.3.2. Consent for deceased donation

Before procurement of tissues from a deceased person can take place, consent to donation must be obtained and recorded in order to ensure that their wishes are fulfilled. In some cases, the deceased person may have expressed their wishes while alive through a donor registry, donor card or advanced directive. In other cases, it is members of the family of the deceased person who decide if donation was in accordance with the person's wishes, values and beliefs, and whether the deceased had expressed an objection to donation during their lifetime and give consent accordingly.

It is important to emphasise that consent must be specific. Therefore, donors' relatives or legal representatives must be given all the information needed to ensure that they understand all potential uses, including processing and storage, before they give consent. The specificity of consent is particularly important when the donated tissues and cells may not be used for transplantation: for example, when the donated tissues may be sent to a biobank for research or used as starting material for advanced therapies (ATMP) by commercial organisations (see Chapter 1).

3.3.2.1. *Legal consent systems*

Consent for the donation of organs and tissues from deceased donors is subject to national legislation and regulation in each country.

There are two legal consent systems for expressing consent to donation. Opting-in is a system in which consent to donation has to be obtained explicitly from the donor during their lifetime or from an authorised individual (usually the next of kin). Opting-out is a system in which consent to donation is presumed, no objection to donation has been registered by an individual during their lifetime or is known to have existed, or consent is inferred by the donor's family who could testify the donor position. In practice, variations exist within both systems, and the relatives may play a prominent role in the decision.

An example of an opt-in system that allows the donors themselves, or their relatives after their death, to give consent is the Human Tissue Act 2004, which applies to England and Northern Ireland. According to this legislation, consent for removal of tissue from deceased donors must be given by the person when they were alive or, after their death, by their legally authorised representative or a 'person in a qualifying relationship to the deceased'. The Act prescribes a hierarchy of qualifying relationships (ranked from highest to lowest: spouse or partner, parent or child, brother or sister, grandparent or grandchild, niece or nephew, stepfather or stepmother, half-brother or half-sister, long-standing friend) and states that consent should be obtained, where possible, from the person ranked highest in the hierarchy.

The opt-out system was introduced to help meet the shortfall in organs and tissues available for transplantation. However, in most programmes, if the family is against the idea of the donation proceeding, tissue procurement will not proceed; this is called a 'soft' opt-out system.

Even if the legislation is based on a presumed consent or opt-out system, the family and/or other persons who knew the donor well must be engaged in the donation process to provide information on medical and behavioural risks about the potential donor to ensure the safety of donated material.

Table 3.2 gives an overview of national consent systems in Europe. The information is reproduced from a survey conducted by the European Commission and was up to date in August 2014 (Directive 2010/53/EU Implementation Survey). Of the 36 answering countries, it appears that the majority

(21 countries) have an 'opt-out' system, 12 countries have an 'opt-in' system. Three countries have a mixed system either combining elements of both 'opt-in' and 'opt-out' or, as in the United Kingdom, with an 'opt-in' system in three of the four UK administrations (England, Scotland and Northern Ireland), and an 'opt-out' system introduced in Wales from December 2015.

Table 3.2. **Legal provisions in European Union countries (and Norway) for consent to/authorisation of organ donation from deceased persons**

Country	National consent system	Donor registry	Non-donor registry
Austria	opting-out		×
Belgium	opting-out		×
Bosnia and Herzegovina	opting-out		
Bulgaria	opting-out		×
Croatia	opting-out		×
Cyprus	opting-in	×	
Czech Republic	opting-out		×
Denmark	opting-in	×	×
Estonia	opting-out	×	×
Finland	opting-out	NA	NA
France	opting-out		×
Germany	opting-in		
Greece	opting-out		×
Hungary	opting-out		×
Iceland	opting-in	NA	NA
Ireland	opting-in	NA	NA
Italy	opting-out	×	×
Latvia	opting-out	×	×
Lithuania	opting-in	×	
Luxembourg	opting-out	NA	NA
Malta	opting-out	×	
Montenegro	opting-in		
Netherlands	opting-in	×	×
North Macedonia	opting-in	NA	NA
Norway	opting-out	NA	NA
Poland	opting-out		×
Portugal	opting-out		×
Romania	opting-in	×	
San Marino	opting-out	NA	NA
Serbia	opting-in	×	
Slovakia	opting-out		×
Slovenia	mixed system	×	×
Spain	opting-out	×	×
Sweden	mixed system	×	×
Turkey	opting-in	×	
United Kingdom	mixed system (opting-out in Wales)	×	×

×: system in use; NA: data not available

Different countries have different procedures to help people express their wishes regarding organ and tissue donation [2]. In each country, national legislation (or, if this is lacking, operational policies) should make clear what evidence (i.e. written or oral) is valid in their country to confirm consent or objection to organ and tissue donation.

Among the means to express wishes regarding organ and tissue donation after death are donor cards and donor registries. Individuals who have donor cards are also often simultaneously recorded in the national donor registry. Consent to donation recorded on a donor card or in a registry may contain detailed information, e.g. consent or not to various types of donation (donation of specific organs or tissues). In some countries, those who apply for a passport or driving licence have to state whether or not they are willing to donate organs, tissues and cells after death. Advance directives ('living wills') may also enable individuals to state prospectively under which medical conditions they do not want to receive life-sustaining therapy and allow documentation of people's wishes related to donating organs and tissues after death.

All national systems should enable individuals to modify or withdraw their consent or objection at any time. This ensures that the most recent information about an individual's wishes is recorded in some way and is available at all times should an enquiry be received from a physician or a donor co-ordinator involved in the donation process.

3.3.2.2. *Establishing consent in other circumstances*

In countries with no legal framework for consent to donation, or where a potential donor is not able to express their donation preference, for example a minor, the decision is, as a rule, left to the family of the potential donor, based on the assumption that the family would respect and represent the potential donor's wishes. Alternatively, consent may pass to those who are the nominated legal representatives of the potential donor, according to the laws of the country.

In some circumstances (e.g. when death occurs in suspicious circumstances or as a result of an illicit act), authorisation to proceed must be given by a coroner, a judge or a family court to avoid the procurement interfering with an investigation, even if the family has consented to donation.

In other circumstances, when a person was to become a donor (expressed wish or absence of refusal/objection), if no relatives can be found or contacted, national procedures and regulations should enable organ and tissue procurement where possible, but only if sufficient medical, social and behavioural information to support safe donation and transplantation can be obtained by other means (e.g. from family physician or friends). If this level of information cannot be reached, donation should not be considered, as it does not guarantee the safety of the recipient.

3.3.3. Approaching the families of potential tissue donors

Grief and mourning are crucial processes to help cope with the death of a loved one. When approaching a bereaved family to seek consent or information on the position of the deceased regarding donation, the many aspects of acute reactions to grief following a death should be understood in order to deal with the circumstances sensitively and in an understanding way. Donation professionals should learn to navigate the environment of acute grief to obtain a decision about donation that is best for the family. By applying their skills and experience, donation professionals can support the family in their mourning and provide appropriate help. The physician or donor co-ordinator who is in charge of approaching the family for donation should have accurate knowledge of the purpose and needs in tissue transplants.

3.3.3.1. *Conversations with the family*

Conversations with a family about organ donation do not generally differ from conversations related to tissue donation. Therefore, it is best to perform interviews about the donation of organs and tissues in a single interview session with the family, allowing them time to reach a decision with which they are comfortable.

It is frequently impractical to discuss donation with a large number of family members, and it is recommended that participating family members should be limited to the lawful next of kin and/or those who are key to making the decision, taking into account the legal framework in place and cultural or religious practices. This should be explained to the other family members so that they do not feel excluded.

When there are social, cultural or language barriers or difficulties, the support (if there are no other possible helpers) of interpreters or friends of the potential donor who have a greater level of integration or similar religious sympathies may be beneficial for the family. These persons should be previously informed about the donation, so that they can support the family and champion a favourable

attitude towards donation; they should not be limited to making a simple translation.

The conversation should be planned, carried out at the right time, in the right place and by trained people. Proper preparation for the conversation reduces the likelihood of errors and the need for improvisation [3, 4, 5, 6]. The discussion should take place in an environment that helps facilitate the conversation, perhaps located close to the place where their loved one died, to give family members the opportunity to say goodbye. It is important to provide the family with a quiet room, where they can speak freely. It is also advisable to have resources that meet the minimum needs (e.g. telephone, handkerchiefs, water, food).

In certain circumstances, relatives of potential tissue donors may be interviewed over the telephone. Before the interview, the co-ordination staff should be very careful and verify whether the interlocutor has been informed of the death of their relative. It may happen that the hospital staff did not reach them before. Such interviews need to ensure that the conversation takes place when the relative is in a private space and preferably not in an unfamiliar environment, such as a hospital. Telephone conversations can make it more difficult to offer reassurance and support to a family since there is little opportunity to demonstrate a personal touch, which can increase the emotional distance. However, trained personnel should be able to find appropriate words, and respect silent moments, to provide support under these circumstances.

The physician or donor co-ordinator who is conducting the conversation with the relatives should respect their grief. This type of conversation requires interpersonal skills, sensitivity and empathy. In situations when there is a huge pressure on medical staff, conversation with families can become difficult, rushed or insensitive.

3.3.3.2. *Family objections*

Conversations about tissue donation aim to fulfil the will of the deceased donor and to obtain family consent or support for donation. Regardless of the legal position, conversations must aim to achieve a decision that is acceptable and accepted by the relatives. Agreement to donation must not be coerced or conditional, nor should it be achieved under pressure or by offering any financial inducements or other material benefit.

It is difficult to proceed with donation when a family is strongly against it, even if there is evidence that their deceased family member wished to be a donor. The family has the right to express their opinion about donation, and clinicians need to make a balanced decision to continue with the procurement without the support of the family and risk damaging the emotional health of the relatives, incurring possible bad publicity and a loss of public confidence in the donation programme, or accept that it is not feasible to follow the wishes of the deceased and abandon the donation process.

It might be helpful to use the following when discussing refusal with the family:

- If the family claims that the deceased patient did not agree to donation or had changed their mind, explore the basis on which the family gives such a statement.
- When the family does not know anything about the attitude of the deceased to donation, discuss whether the deceased helped people generally, e.g. as a blood donor or giver to charity, and how donation could help many people to benefit from a transplant.
- The experience of interviews with families suggests that some difficulties and possible opposition may occur in procurement of tissues from 'visible places' like skin, bone and, in particular, eyes when family members fear disfigurement of the body. In these situations, reassure them that the deceased's body will be fully respected. If necessary, some technical aspects of procurement should be explained, for example the use of specific surgical incisions and sutures or suitable prostheses or artificial eyes or bones. Reassurance should be given that they should not notice anything if they see the deceased person after procurement, albeit there can be rare problems such as bruising or bleeding, and they should be made aware of such possibilities.
- In the case of religious concerns, offer a consultation with a religious leader or representative.
- Give special attention to cases of dissatisfaction with the healthcare provided, record the complaints but explain that the issue of donation should be kept separate.
- Identify the persons involved in the refusal to donate and their role within the family, and attempt to communicate with them separately to understand and try to address their concerns.
- Identify whether a disagreement to donation by individual family members is based on conflicts between family members which come to light when a person has died. In this case, try to separate the conflict from the issue of tissue donation.

3.3.3.3. *Informing the family*

There is often debate about the amount of information a donor family should receive, how much information is enough and when does it become too much. One opinion is that the family should be given only the information they request; another is that the family must be told absolutely everything: which tissues will be donated, a description of the procurement process including reconstruction, potential uses of the tissue in both clinical practice and research, the method of discard, the potential need for follow-up if some test results are found positive, etc. The first approach has the limitation of not taking into consideration the fact that the family may not know much about donation and will therefore not know what to ask. The latter could cause harm to the family and could risk converting an interest in donation into a refusal. The solution to this dilemma should be found by the donation professionals on a case-by-case basis.

All questions posed by the potential donor family should be answered and, beyond that, professionals should apply their own judgement to decide how much information the family would want or need to obtain. While providing the information, they may observe agitation, frustration or irritability in a family member; this may signal unwanted stressful information and suggest reconsideration of how much information is needed or wanted. Ultimately, the amount of information made available to donor families in order to obtain consent should vary with the type of consent being provided and should be increased if the potential application of the donated material is controversial. The interview in any case should be prepared in advance with systematic content, built with the interviewer's owns words. Global and generic sentences may be helpful to raise the main subjects, which the family can investigate further with questions if needed.

It is helpful to ensure that, following donation, the family receives the appropriate care they need. In many countries hospitals have dedicated bereavement teams to provide psychological support, access to social services, administrative support or religious counselling. The clinical team should establish whether there are any specific religious or spiritual requirements of the family and whether the family wishes to retain keepsakes such as locks of hair or handprints. Finally, establish whether the family wishes to assist with the final preparation of the body following donation, such as washing or dressing in certain items of clothing.

3.4. Conclusions

The continuing development of transplantation medicine gives hope to many patients in need. However, the need for tissues and cells for clinical application cannot be met only by autografts or surgically discarded tissues. The ability of a tissue establishment to meet patients' needs requires multiple efforts to increase tissue and cells donation activity. These efforts involve organisational measures, the development of proactive donor recruitment and identification programmes, and the engagement of many parties, including the general public, hospital staff, coroners, procurement organisations and tissue establishments. By establishing strong links with and co-ordination between all these parties, and by adequately training personnel to acquire the necessary medical expertise and key social and emotional skills, tissue establishments can ensure the success of the tissue and cell donation programmes.

Since tissues and cells come from a human being, either living or deceased, it is necessary to ensure that donors have the autonomy to decide freely about matters that are essentially their own choice. Obtaining informed consent is relatively straightforward when donors are alive. It is essential, however, that they fully understand the risks and consequences of the donation procedure and the final use that will be given to their donated material. In the case of deceased donors, it is less clear how respect for autonomy applies but, ultimately, it is crucial that the wishes and best interest of the potential donor are scrupulously respected. This chapter has aimed to offer practical guidance for obtaining consent in all possible contexts.

3.5. References

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Related material

- Appendix 11. Example of consent form: female (NHS, UK)
- Appendix 12. Example of consent form: female (CNPMA, Portugal)
- Appendix 13. Example of consent form: male (NHS, UK)
- Appendix 14. Medical and social history questionnaire (NHS, UK)

Chapter 4: **Donor evaluation**

4.1. Introduction

Since the development of tissue and cell transplantation as clinical treatments the key objectives of all parties involved in the process have been to ensure product safety and quality as well as donor safety [1, 2]. The rationale for donor screening is twofold: firstly, to minimise the risk of transmitting disease to a recipient and, secondly, to exclude any tissues or cells whose quality may be adversely affected by a characteristic, including medical or other conditions, of the donor. When dealing with living donors, an important part of the evaluation should be to assess whether the procurement process itself could be harmful to the donor. It should also be considered whether it is necessary to have arrangements in place for long-term follow-up of living donors after procurement. Special consideration is required for paediatric donors. These aspects of living donation are detailed in Chapter 3: Recruitment of potential donors, identification and consent; Chapter 22: Haematopoietic progenitor cells from bone marrow and peripheral blood; Chapter 27: Medically assisted reproduction; and Chapter 28: Fertility preservation.

There are two main donor types, with different risks and benefits resulting from the donation: autologous and allogeneic. Allogeneic donors may be living or deceased. The evaluation of autologous donors is a special situation as the donor is the person being treated for a disease and the acceptance criteria should take this into consideration in decision making.

4.1.1. Donor evaluation

The two main objectives of donor evaluation are:

a. To obtain information about the donor to identify absolute and relative contraindications to human application that may pose risks for a recipient;

b. To ensure that the donation will not cause harm to a healthy living donor.

To meet objectives *a* and *b* above, the following information should be evaluated:

i. medical history (including genetic disease and a family history of disease);
ii. social history (personal and behavioural information, including travel history);
iii. physical examination;
iv. psychological examination (living HPC and non-partner MAR donors) – see Chapter 22 and Chapter 27;
v. tests for markers of transmissible disease, as detailed in Chapter 5 and Chapter 10.

During donor evaluation, confirmation of the validity of the consent and of the donor identity are essential steps (see Chapter 3). For European Union (EU) member states, the selection criteria for deceased donors (including additional exclusion criteria for deceased child donors) and living donors of tissues and cells are specified in Annex I/III of Directive 2006/17/EC. These criteria are the minimum, and individual member states can set additional criteria as necessary.

Table 4.1. **Sources of information and types of record for donor evaluation**

Sources of information	Types of record
1. interview with family and friends/close acquaintances 2. interview with attending clinician and nurse, as well as the healthcare provider 3. detailed review of the medical notes (see types of record, to the right) 4. general practitioner notes 5. physical examination findings 6. autopsy findings (for deceased donors), which must be communicated as soon as possible after procurement 7. tests for infectious markers (see Chapter 5 and Chapter 10) and other relevant test results (see *Types of record*, in the right-hand column)	1. emergency room and emergency medical transport (ambulance) records 2. admission records, progress notes, clinician's orders/notes and nursing observations 3. surgical records 4. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling) 5. discharge summary or death certificate (for deceased donors to confirm cause of death or to determine whether an autopsy is planned) 6. results of laboratory tests (microbiology, chemistry, haematology, virology, toxicology, genetic screening, pathology) 7. physical evaluation form 8. information relating to transfusions and infusions (to be used for evaluation of haemodilution) 9. radiography/magnetic resonance imaging/computed tomography
For deceased donors whose death occurred outside a healthcare facility, the records listed to the right may also be available and, if so, they should be reviewed	10. police records 11. records from the medical examiner or coroner 12. records from the extended-care facility

4.2. Assessment of potential autologous donors

4.2.1. General evaluation

The evaluation of autologous donors is based on the disease/condition being treated. The donor eligibility criteria for autologous donors may be very different from the criteria for allogeneic donors [1] because the direct benefit of transplant for their medical condition may outweigh potential risks associated with donating cells and tissues for autologous use. The clinician caring for the donor is making the decision on autologous donation/application according to guidelines and relevant scientific data. As the autologous donor is being treated for the disease/condition in question, the relevant medical history, results of laboratory tests and physical examination results are all available to the clinician. Eligibility for donation is evaluated on an individual basis, taking into consideration the possible risks and benefits.

4.2.2. General contraindications

There are no absolute contraindications for autologous donors. The potential benefits and risks should be analysed on an individual basis and a decision made by the clinician in charge (e.g. auto-transplantation of ovarian tissue carries the risk of cancer cells being present in the tissue). This should be clearly documented and communicated to the patient.

If the procured tissues or cells will be processed and/or stored, screening for the same biological testing for mandatory markers must apply as for an allogeneic living donor (see Chapter 5), although the results are not necessarily a contraindication for autologous donation. Potential or proven infectious (i.e. HIV/HBV/HCV-positive) materials collected from autologous donors should be handled in such a way that the risk of cross-contamination with tissues and cells from other donors within the tissue establishment is minimised. The risk of transmission of infection to personnel, during procurement, processing and storage of these cells, should also be considered. Written standard operating procedures (SOPs) should be present for these situations (see Chapter 7 and following).

4.3. Assessment of potential allogeneic donors

4.3.1. General evaluation

Allogeneic donors can be living donors related to the intended recipient, unrelated voluntary living donors or deceased tissue donors. The most common tissue donated by living donors is the femoral head, surgically removed during a hip-replacement procedure. Also recognised as living donation are the *post partum* collection of the amniotic membrane donated by mothers at the time of delivery and heart-valve donation from the discarded heart of a heart

transplant recipient; such a person can also be called a 'domino donor'. Minimising the risks to donors of highly matched HPC products for related recipients can be challenging and, in general, are much more difficult than minimising the risk of subjects donating matched or unmatched cells or tissues for strangers. The World Marrow Donor Association and similar organisations are active in many countries to help protect the health and safety of unrelated HPC donors [1, 2].

The medical and social history of a potential donor, either living or deceased, must be investigated for factors that increase risks of infection with transmissible diseases and any other conditions that may affect tissue quality and safety.

4.3.1.1. *Sources of information*

The information on medical and social history required for donor evaluation should be obtained directly from living donors. Additional information (where applicable) should be sought to ensure safety for the recipient, and this should be the same as the information obtained for deceased donors, as given below. The types and extent of records to be obtained should vary depending on the type of donor.

In addition, the health risks for the living donor must also be considered by a clinician not involved in the treatment of the potential recipient, to avoid conflicts of interest (except in the case of surgical residues).

Procurement of tissues from deceased donors takes place after circulatory arrest. Thus, the time available for full donor evaluation is limited. Several sources of information should be used to gather medical and social history about deceased donors (see Table 4.1).

Information obtained during this evaluation (see Table 4.1) must be included in the donor's medical evaluation record. An interview with relatives of deceased donors should be undertaken, bearing in mind that, under emotional stress, some details might be forgotten. Even when donor relatives trust the interviewer, they may neglect or not disclose this information or may not know the entire truth.

Contact with the general practitioner of the donor and reviewing, where available, hospital records for historic data or other sources of information (e.g. tumour registry/pathology reports if available) are important in supplementing and/or confirming information provided by the family. The donor medical evaluation record should be documented with details of hospital admission (if the donor died in a health facility); cause of death; medical and behavioural history, including general data such as age, gender, body weight (if necessary, e.g. to calculate haemodilution), date and time of death; and signs of obvious medical interventions, i.e. scars, skin or mucosal lesions.

Standardised questionnaires should be used for interviews to ensure that all the relevant information is obtained (see Appendix 14). The interviews should be performed, documented and signed by a suitably trained and competent authorised person to comply with national regulations. They should be held in private and carried out ideally before donation (see Chapter 3). The donation record, whether paper or electronic (see Chapter 2), must fully and accurately reflect the relevant information gained from reviewing these records and from discussions with medical or other personnel. Transferring information from records to a new document carries the risk of transcription or interpretation errors. These steps must be carried out by well-trained, competent staff.

Careful review of all the collected donor information will help ensure an accurate donor evaluation and assessment of the risks, including the identification of any potential contraindications for donation, either absolute or tissue-specific (see Part B for specific chapters). This analysis should preferably be performed before procurement; but, if this is not possible, the procured tissues and cells should be quarantined until a final decision is made by the Responsible Person (RP) of the tissue establishment. In addition, it is the responsibility of the person/team performing the procurement to document any suspicious anatomical findings observed during the procurement procedure and to obtain samples for histological examination if relevant.

The HPC donor should be pre-screened for factors that would place them at increased risk due to donation. Pre-screening might include health history questions, physical exam, blood tests and other medical evaluation. If large volumes of blood are to be collected, the donor's haemoglobin should be measured before the donation, and potential donors with low levels should be excluded from donating. If marrow is to be aspirated to manufacture bone-marrow stromal cells or a skin biopsy obtained to manufacture induced pluripotent stem cells, the donor's platelet count and coagulation measures should meet pre-defined criteria to be sure that they are not at increased risk from bleeding [1, 3]. More detailed information about HPC, including paediatric donors and non-partner MAR donors, is given in Chapter 22 and Chapter 27.

4.3.1.2. *Donor medical and social history*

4.3.1.2.1. Medical history

a. Current clinical information, e.g. diseases/diagnoses, transfusions/infusions, medication/vaccinations, and cause of death (COD) for deceased donors, should be reviewed. Haemodilution should be assessed in donors with trauma, intra-operative blood loss or ruptured aneurysms, bleeding from oesophageal varices, spleen rupture etc. If haemodilution is > 50 %, serology testing on blood samples drawn at the time of procurement may not be reliable (see Chapter 5 and Appendix 18). When haemodilution is suspected/confirmed, blood samples taken before haemodilution should be used for virology and serology testing. If pre-transfusion/infusion samples are not available, haemodiluted samples can only be accepted if the testing procedures used have been validated for such samples.

b. Previous diagnosis of disease, surgeries, vaccinations, genetic disease, chronic diseases and family history should be evaluated. For living allogeneic donation, where applicable, attention should be given in cases of family adoption or conception by donated gametes/embryos, as it may not be possible to trace the genetic family history. Thorough investigation of the previous diseases of the potential donor must be carried out. The evaluation should include any past medical history related to:

i. chronic/previous disease, e.g. chronic persistent infection, malignancy, autoimmune disease, neurological disease, genetic disease,
ii. medication,
iii. information on recent vaccinations [4]:
   - to identify recent vaccinations that indicate travel risks,
   - to identify vaccinations with live attenuated virus,
   - to help with interpretation of test results (a recent HBV vaccination is expressed as reactive/positive HBs Ag).
iv. family history, for instance if individuals are at familial risk of prion-associated diseases (have had two or more blood relatives develop a prion-associated disease, or have been informed following genetic counselling that they are at risk for public health purposes) [5], or for malignancies or connective tissue disease.

4.3.1.2.2. Social history, evaluation of behavioural and personal risk

Behavioural and personal risk (including travel history) must be evaluated as they may completely exclude a donor, or indicate that certain tissues/cells may be compromised or suggest an increased risk of infectious diseases [6].

Table 4.2. **Deceased donors: physical examination prior to donation**

Look for signs of	
Possible systemic disease	• malignancy (suspicious skin or subcutaneous lesions; see Appendix 16) • malnutrition, multiple deformities
Bacterial or viral infection	• recent receipt of a live vaccination (vaccination site infection, scabs, vaccinia) • recent receipt of a tattoo, body piercing or acupuncture where non-sterile instruments may have been used (shaved area, redness, swelling or scabbing may require further investigation to assess risk) • skin lesions such as a rash, petechiae, skin ulcers, blue/purple or grey/black lesions, shingles, scabs • oral lesions such as ulcers or thrush (not always possible to examine due to rigor mortis) • enlarged lymph node(s) • icterus, hepatomegaly
High-risk behaviour	• injected drug abuse (non-medical injection sites) • inspection of tattoos for hidden injection sites or for any additional information (e.g. some tattoos may suggest imprisonment or high-risk sexual behaviours) • genital or peri-anal skin lesions indicative of a sexually-transmitted disease (e.g. evidence of anal intercourse, herpetic lesions or ulcerative disease)
Trauma	• fractures, avulsions, lacerations or abrasions that may affect (contaminate, compromise integrity of) the tissue to be procured • internal trauma that can cause cross-contamination between cavities (e.g. injury to the bowel, penetrating or crushing injuries) • cleanliness of the body, the condition in which the body was found (this can also relate to increased risk for contamination/cross-contamination) • scars (surgical or other); if findings do not match the donor's history, further investigation may be required

It is necessary to ask about sexual behaviour (e.g. commercial sex workers, frequently changing partners regardless of their gender, men having sex with men, history of sexually transmitted diseases). The use of intravenous drugs and lifestyle should be queried.

Recent or pertinent travel history or residence abroad/overseas must be evaluated to rule out the risk of tropical or endemic infections, e.g. malaria, trypanosomiasis or Zika, as well as the subsequent risk of vertical transmissions. Emerging, non-tropical infections also exist in some European regions, e.g. West Nile virus, chikungunya virus.

Travel history helps to identify risks related to places/countries with less rigorous regulatory standards or with a high prevalence of certain infections. Information about hobbies (e.g. home, garden, animals, woodlands) should also be obtained with the same intention.

Seeking information about contact with fauna, especially bites from pets, domestic or wild animals, bats and birds, is necessary to evaluate the risk of infections.

4.3.1.3. *Physical evaluation of donors*

Physical evaluation of the donor (see Table 4.2) should be carried out before procurement and must be documented. Each donor (adult or child) must be thoroughly examined following established protocols (see Chapter 6), covering the anterior and posterior aspects of the body. Excessive weight of the donor cannot compromise the requirement to carry out a thorough assessment. The information obtained through physical examination is supplementary to the comprehensive summary of clinical data. Any findings suggestive of possible risk should be investigated.

For living donors, a complete physical examination should be undertaken to ensure the safety of donors and recipients according to the specific requirements of the particular type of tissue or cell donated. This examination should be done in the context of a clinical evaluation that includes an interview and a comprehensive physical examination, together with psychological evaluation of the potential donor. More detailed information about HPC, including paediatric donors and non-partner MAR donors, is given in Chapter 22 and Chapter 27.

For deceased donors, the physical examination should look for evidence of high-risk behaviour, or external signs of underlying medical conditions (see Table 4.2). Visual examination of the body is advisable during early, initial screening if adequate information on the condition of the body cannot reliably be obtained orally. The physical examination may include taking a picture of suspicious lesions that may indicate a risk or taking a sample for histology. Any findings that may indicate the risk of transmissible disease or unsatisfactory quality of the tissues should result in exclusion of the donor [7]. Any new information related to lesions (tumours, skin lesions, scars), diseases or treatments that becomes apparent during the physical examination must be investigated further by the professionals responsible for donor selection.

In all cases of abnormal findings, each tissue establishment should establish – following their SOPs – whether further investigations should be carried out. The limited sensitivity and specificity of physical examination for discovering pathologies must be considered in the donor risk assessment. An example of a tissue-donor physical assessment form can be found in Appendix 15.

Because the physical examination can result in rejection of a donor before procurement, or of the tissues or cells after procurement, its importance is clear [7].

4.3.1.4. *Special considerations for paediatric donors*

Child donors must be screened with as much diligence as adult donors. Physical assessment must not be overlooked or shortened simply because the donor is a child. Although risk associated with sexual activity may not seem relevant, infectious disease associated with child abuse (sexual) is possible, so examination of the genital and peri-anal regions is recommended.

Additional considerations are required for living paediatric donors. For donors under 18, or the relevant national legal age of consent, the parents or guardian normally give consent, but the minor, if possible, should assent to the procedure and the screening questions should be tailored to the age of the minor donor [1].

Special screening considerations are also required for some paediatric donors. An infant's immune system is not fully developed, so protective antibodies may not yet have been produced against infection, thereby increasing the risk of undetectable infection with serologic screening. If the child is 18 months old or younger, or has been breastfed in the 12 months before death, the birth mother should be tested and evaluated for risks associated with HIV, HBV, HCV, HTLV and syphilis, as with any other allogeneic donor (see Chapter 5). Other diseases that can be transmitted vertically from mother to foetus may also be relevant, such as malaria or Chagas disease.

In the EU, Directive 2006/17/EC stipulates that children aged under 18 months born to a mother with infection by HIV, HBV, HCV or HTLV, or who are at risk of such infection, and who have been breastfed by their mothers during the previous 12 months, cannot be considered as donors regardless of the results of analytical tests.

4.3.2. Generic contraindications for tissue and cell donation

The guidelines for excluding or including donors presenting certain risks vary between countries and regions and are determined by local disease prevalence and risk assessments. Therefore, this list of risk criteria should be regularly reviewed and modified according to local circumstances, as epidemiological changes and future developments in diagnostics occur.

Despite these limitations, donors should be considered as high-risk if one or more of the following conditions are present.

4.3.2.1. *Unknown cause of death (in deceased donors)*

If the cause of death (COD) is not known, the donation cannot be permitted, because death may have been due to a disease that could be transmitted to recipients of tissues and cells. The only exception would be in those cases where an autopsy is performed and can clarify the COD after tissue procurement. The circumstances of death and medical history for differential diagnosis contributing to death may help to exclude contraindications to donation until the certified cause of death is available.

4.3.2.2. *Infectious diseases*

Infectious agents transmissible by organs or tissues belong to one of five groups of pathogens

- Viruses by infection in the tissue of donor with or without current viraemia. Thereby DNA-viruses may persist latently in the tissues without detectable viraemia; RNA-viruses usually cause direct infection and disease;
- Bacteria by bacteraemia or colonisation/infection of organs or tissues;
- Fungi by fungaemia or colonisation/infection of organs or tissues;
- Parasites by latent or acute infection;
- Prion (see §4.3.2.2.5 and §4.3.2.2.6).

4.3.2.2.1. Active systemic infection

Donors with systemic infection that is not controlled at the time of donation (including bacterial diseases, viral, fungal, protozoan or parasitic infections, or significant local infection in the tissues and cells to be donated) should be excluded. Donors with bacterial septicaemia may be evaluated and considered for (avascular) cornea donation, but only if the corneas are stored by organ culture (see Chapter 16).

If the aetiology of an active infection cannot be established, the donor is not a suitable candidate for donation. Communication with the physician or medical staff caring for the potential donor is necessary if there is any doubt. These healthcare providers may know if there was a suspicion of sepsis or another infectious disease at the time of death, which may not have been well documented in the records.

4.3.2.2.2. Chronic persistent infection

Consider the history of bacterial and protozoic diseases that can lead to chronic persistent infections, including tuberculosis, brucellosis, leprosy, Q fever, chlamydiosis and salmonellosis. Specific attention should be paid to tick/arthropod-borne diseases such as borreliosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis. The risk of transmitting these infectious agents with specific tissues must be assessed, and negative effects for the recipient(s) excluded.

4.3.2.2.3. Proven transmissible viral infection

Donations must be screened for evidence of transmissible viral infections (see Chapter 5). Persons with clinical or laboratory evidence of (i.e. have tested positive for) HIV, HCV, HBV or HTLV-I/II infection are excluded from donation. Behavioural risks that could increase the risk of acquiring transmissible infections are discussed in section 4.3.3.1.

4.3.2.2.4. Recent history of vaccination with a live attenuated virus/bacterium

Vaccinations with live vaccines [4] may result in transmission of a vaccine-derived pathogen to the recipient. Therefore, it is imperative to determine if the donor has received live vaccines during the previous 4 weeks. Live vaccines include:

a. Viral: inhaled attenuated influenza (not injectable, inactivated influenza), varicella–zoster, rotavirus, measles, mumps, rubella, oral polio (not injectable, inactivated), oral cholera (not injectable, inactivated *Vibrio cholerae*) and yellow fever. Vaccinia for smallpox should be deferred for 8 weeks;

b. Bacterial: bacillus Calmette-Guérin (BCG), oral *Salmonella typhi* (not injectable, inactivated).

4.3.2.2.5. History of prion disease

Transmissible spongiform encephalopathies (TSE), which include Creutzfeldt–Jakob disease (CJD), Gerstmann-Stäussler-Scheinker (GSS), Kuru and fatal familial insomnia (FFI), are rare neurological degenerative diseases that are progressive and inevitably fatal. They are associated with transformation of the normal form of prion protein (PrP^{C}) into an abnormally-folded form (PrP^{Sc}).

There are four clinical forms of CJD: sporadic (sCJD), which is the most common; variant (vCJD); genetic (gCJD), and iatrogenic (iCJD). While Western blot and ELISA assays have been investigated for testing blood, retinal tissue, optic nerve, spleen and tonsillar tissue, diagnosis can currently be confirmed only by autopsy. Adherence to European Centre for Disease Prevention and Control (ECDC) recommendations is suggested and the risk of transmission should be considered as detailed in 4.3.2.2.6.

4.3.2.2.6. Risk of transmission of prion diseases

TSE transmission risk should be considered in the following cases:

a. persons diagnosed with any form of CJD, GSS or FFI;

b. any suspicion of prion-associated disease, such as rapid progressive dementia;

c. a diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has been ruled out by microscopic examination). If dementia has a primary cause (e.g. dementia of vascular origin), donation can be accepted;

d. degenerative or demyelinising disease or a disorder of unknown aetiology involving the central nervous system;

e. persons treated with hormones derived from human pituitary gland, such as growth hormone;

f. recipients of cornea, sclera and *dura mater* as well as persons who have undergone undocumented neurosurgery in which the *dura mater* may have been used;

g. persons who lived in the UK between January 1980 and December 1996 for longer than 6 months (for countries other than UK);

h. individuals who have been told that they may be at increased risk because a recipient of blood or tissues that they have donated has developed a prion-related disorder [5].

4.3.2.3. *Malignancies*

4.3.2.3.1. Haematological malignancies

Myeloid and lymphoid neoplasia and leukaemia are malignant diseases caused by dysregulated multipotent haematopoietic stem cells and should be considered as absolute contraindications to donation. Other myeloproliferative diseases (e.g. *polycythaemia vera*, essential *thrombocythaemia* and mastocytosis, myelodysplastic syndromes) may also affect the stem cells; thus, these donors require special attention, and donation of living cells is not recommended. The World Health Organization (WHO) has published classifications of myeloid neoplasms and acute leukaemia [8], and of lymphoid neoplasms [9]. Any information indicating haematological alterations that would be suggestive of any of the malignancies must be evaluated further. A recent blood test carried out before death, if available, may offer valuable information indicative of these alterations. Although an experienced haematologist will be able to provide a differential diagnosis, certain results should be individually evaluated [10], such as the examples shown in Table 4.3.

4.3.2.3.2. Non-haematological malignancies

A history of malignancy should be evaluated carefully to determine its effects on the quality and safety of tissue, because of either the presence of a tumour or the treatment given to the donor for malignancy.

Table 4.3. **Haematological malignancies that should be individually evaluated**

Altered haemoglobin	men > 18.5 g/dL women > 16.5 g/dL	should be carefully assessed for potential contraindications, such as *polycythaemia vera*
Altered haematocrit	men > 55.5 % women > 49.5 %	
Platelet count	< 50 × 10^9/L	is highly indicative of a haematological disorder
	50-100 × 10^9/L	should be carefully assessed for contraindications due to a possible haematological problem
	> 450 × 10^9/L	should be carefully assessed for contraindications such as essential thrombocytosis
Altered white blood cells	> 50 × 10^9/L	should be carefully assessed for contraindications such as chronic myeloid leukaemia

Results of donor evaluation may imply a donor risk, a recipient risk or both. An increased risk of harm to a living donor is not acceptable, even if the benefit of transplantation for the recipient is considered to outweigh the risk of transmission (for example in the case of past malignancies).

Detailed history of type, duration, course/recurrence and treatment history must be considered. Availability of screening programmes has improved early detection, which increases the treatment options and can lead to cure. For donors who had been diagnosed with a pre-malignant condition (e.g. Bowen's disease, polyposis coli or Barrett's oesophagus), further information should be sought to exclude malignancy because these patients are likely to be monitored regularly.

Some international bodies provide assessments on risk of transmission of malignancies through organ transplant for CNS (central nervous system) and non-CNS tumours [11], which can be used as a basis for determination of the risks of transmission through tissue transplant. The role of processing steps applied to tissues and cells in reducing the risk of transmission of malignancy for tissues and cells following transplantation should be considered. The effect of high-dose terminal sterilisation (25-40 KGy) and the decellularisation process removing viable cells are examples of tissue-processing steps that reduce the potential for transmission of malignant cells.

The *Guide to the quality and safety of organs for transplantation* (Chapter 9: Risk of transmission of neoplastic disease) [12] provides useful guidance on assessing the risk of malignancy transmission through organ transplantation, based on published evidence in the literature and national transplant registries. In addition to absolute contraindications (unacceptable risk), the risk of transmission is classed as minimal (< 0.1 %), low (0.1 to 1 or < 2 %), intermediate (1 or 2 % to 10 %) and high (> 10 %) for CNS and non-CNS tumours. A similar approach could be applied for tissue and cell transplantation based on literature review.

4.3.2.3.3. CNS tumours

Malignancy gradation in the CNS should be thoroughly evaluated, including a complete histological exam rather than a simple biopsy, due to possible heterogeneity of the mass. The 2016 WHO classification [13] of selected CNS tumours is shown in Appendix 17 (Table A17.1). WHO Grade I and II CNS tumours have a minimal or low risk of metastasis and would not normally contraindicate tissue donation. The Grade III and IV primary CNS tumours have either low (WHO grade III: < 2 % transmission risk) or intermediate (WHO grade IV: 2.2 % with an upper 95 % CI of 6.4 % transmission risk) risk of transmission through organ transplantation [11] (Appendix 17, Table A17.2). In CNS tumours, cerebral lymphoma and secondary intracranial lymphomas are considered absolute contraindications. Individual risk assessment is required for decision making for accepting donors with malignancies that have low and intermediate transmission risk for tissue donation.

4.3.2.3.4. Non-CNS tumours

The assessment of transmission risk for non-CNS tumours in an organ transplant setting [11] is summarised in Appendix 17, Table A17.3. Malignant neoplasms have been transmitted to immunosuppressed recipients through transplantation organs from donors with known or unknown malignancies. In an organ transplant setting, this risk needs to be considered against the perspective of the important, life-enhancing and life-saving benefits afforded by organ transplant. The increasing number of patients on waiting lists, along with the shortage of organs available for transplant, has encouraged reconsideration of the criteria for acceptance of organs from donors with a past or current history of malignancy [12].

The risk–benefit analysis is very different in a tissue-transplant setting. The tissue grafts are mostly used in elective settings and are life-enhancing (rather than life-saving). There may be other suitable bioprosthetic grafts. On the other hand, unlike organ recipients, tissue recipients usually do not require immuno-suppression. The processing steps and terminal sterilisation may vastly reduce the potential for transmission of tumour cells. The risk of transmission is generally much lower compared with organ transplant. There have been two recent case reports (2017) of donor-derived malignancy in keratolimbal allograft recipients [14, 15].

4.3.2.3.5. Carcinoma *in situ*

Carcinoma *in situ* (CIS) is an early form of cancer that is defined by the absence of invasion of tumour cells into the surrounding tissue, usually before penetration through the basement membrane. CIS is, by definition, a localised phenomenon, with no potential for metastasis unless it progresses into an invasive carcinoma. Therefore, its removal eliminates the risk of subsequent progression into a life-threatening condition. When explaining a laboratory report to a patient, most physicians will refer

to CIS as 'pre-cancer', not cancer. In the TNM classification, CIS is reported as TisNoMo (Stage 0).

Because most forms of CIS have a high probability of progression into invasive carcinoma [12], it is usually recommended that the lesion be completely removed. Therefore, CIS is usually treated in much the same way as a malignant tumour. If a donor had been successfully treated and cured after a CIS (e.g. cervical or vulval carcinoma *in situ*, some intra-ductile carcinoma of the breast, intraepithelial cancer of the prostate etc.) the donation could be suitable because CIS do not adversely affect the safety or quality of other types of tissues like *in situ* carcinoma of the cervix.

4.3.2.3.6. Specific considerations for EU member states

It must be noted that, for EU member states, the EU directives for tissues and cells general donor exclusion criteria require that donors with malignancy must be excluded from donation unless justified on the basis of a documented risk assessment approved by the responsible person (as specified below). Commission Directive 2006/17/EC states that the presence, or previous history, of malignant disease, except for primary basal cell carcinoma, carcinoma *in situ* of the uterine cervix and some primary tumours of the central nervous system that have to be evaluated according to scientific evidence, is a criterion for exclusion of donors for tissue or cells. This regulatory requirement must be considered as part of the risk assessment in decision-making.

Donors with malignant diseases can be evaluated and considered for cornea donation (see Chapter 17), except for retinoblastoma, haematological neoplasm and malignant tumours of the anterior segment of the eye. Malignant melanoma with known metastatic disease also excludes use of ocular tissue, including avascular cornea. Any vascularised ocular tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and should be evaluated as discussed above.

4.3.2.4. *Exposure to toxic substances*

In case of ingestion or exposure to a toxic substance (e.g. cyanide, lead, mercury, gold, arsenic, pesticides), the quality and safety (due to the presence of high level of substance) of some types of tissues and cells may be affected and, as a result, can cause harm to recipients. Exposure to asbestos in the past is a risk for developing of a mesothelioma. In this case a thorough risk assessment should be performed to estimate this risk.

4.3.2.5. *Tissue-specific contraindications*

Certain medical conditions can adversely affect specific tissues and cells which, if procured, processed and made available for human application, may result in unfavourable outcomes for the recipients of tissue and cells. This risk is evaluated on a case-by-case basis and for specific tissue types. For guidance on the specific contraindications for each tissue and cell type, please refer to the relevant chapters in Part B of this Guide.

4.3.3. Evaluation of personal and behavioural risk

All substances of human origin (SoHO) have the potential to transmit infections to a recipient. Behavioural risk is evaluated to inform assessment of donor suitability. Evidence-based donor selection is the first safeguard in minimising the risk of transmission while not compromising sufficiency of valuable grafts for clinical use.

The incidence and prevalence of these SoHO-related infections varies, depending on different risk factors [16, 17, 18], and the causes of *de novo* infection vary between European regions [16, 19]. The tissue establishment must consider available evidence from the epidemiological data on transmissible bloodborne infections such as HIV, HCV and HBV in the population, the performance (sensitivity and specificity) of screening tests used for detecting these infections and the residual risk of undetected infection that could be potentially transmitted to the recipient. This residual risk may be the result of one or all of a number of factors: error in the process, poor assay sensitivity, a donation collected from a donor in the infection window period. International peers adopt an interval of at least twice the window period since the last 'at-risk behaviour' for the length of deferral before donation [16].

It is recommended that a risk-assessment framework, such as the Alliance of Blood Operators model [20], is used to systematically analyse the information and document the decision, based on the acceptable level of risk tolerance. The outcome of this systematic approach would provide the basis for evidence-based donor deferral and acceptance policies for donors with high-risk behaviours and their sexual partners [21].

4.3.3.1. *Behavioural risk factors*

Potential donors should be considered at high risk if they have participated in any of the following behaviours or if they have had sexual contact with

persons who have participated in any of the following behaviours:

i. People who have injected drugs by an intravenous, intramuscular or subcutaneous route for non-medical reasons;
ii. Tattoos, ear piercings, body piercings and/or acupuncture, which are very popular in some European countries; usually they are applied by sterile methods and in many countries, there are specific approvals for those establishments. If tattoos, piercings or acupuncture were done in approved settings, the donor can be accepted without temporary deferral, but in case of doubt the associated risk should be considered similar to that of non-medical injections;
iii. Persons who have been newly diagnosed with, or have been treated for, sexually transmitted diseases (e.g. syphilis, gonorrhea, *chlamydia* or genital ulcers);
iv. Men who have had sex with men (MSM);
v. Persons who have had sex in exchange for money or drugs;
vi. Persons whose sexual behaviour, including frequent changes of sexual partner, puts them at risk of acquiring severe infectious diseases.

In countries where tissue establishments do not have access to data to perform risk assessment, a deferral period of 12 months is a safer option after cessation of the high-risk behaviour or sexual contact. This may be reduced to 3 months if supported by risk assessment, considering risks and benefits of the transplant, together with individual NAT testing and bacterial screening.

4.3.3.2. *Personal risks, exposure events [5]*

Exposure events that increase the risk of acquiring a communicable disease can occur at any time during life. They include accidents, certain medical therapies, occupations and travel to, or residence in, an area endemic for certain diseases. Here are examples of other risk factors:

a. Persons from a high-risk region for endemic disease, e.g. HIV-1 group O, human T-cell lymphotropic virus (HTLV-I). The Caribbean is, for example, high-risk for HTLV-I;
b. Exposure to someone else's blood (such as needlestick injury, human bite) when that person was known to be infected with HIV, HBV or HCV;
c. Sharing a residence with someone who has HBV or clinically-active HCV;
d. Persons regularly transfused with blood or blood products should be carefully evaluated case by case for the risk of disease transmission;
e. Patients with chronic haemodialysis;
f. People who have been in a lockup, jail, prison or juvenile correctional facility for more than 72 consecutive hours should be carefully evaluated for the risk of high-risk behaviours (see §4.3.3.1);
g. A history of travel to, origin in or visiting relatives in malaria-endemic areas; the minimum deferral period recommended for blood donors in EDQM guidance [19] for all groups of potential donors (visitor, origin/previous resident, travel-related illness, history of malaria) is 4 months if the result of a validated malaria antibody assay, performed at least 4 months after last exposure incident or resolution of symptoms, is negative.
h. A bite from an animal suspected of having rabies at any time.

4.3.3.3. *New and emerging diseases*

New and emerging diseases (including those that have spread to a new geographical area) can pose a significant challenge when screening donors for risks of communicable disease due to travel history. Professionals responsible for donor selection should be vigilant regarding surveillance of changes to the global movement of infectious-disease risks. Diseases that should be considered include: Middle East respiratory syndrome (MERS), dengue fever, yellow fever, malaria, trypanosomiasis, tuberculosis, plague, chikungunya virus, West Nile virus (WNV), severe acute respiratory syndrome-associated coronavirus (SARS-CoV), Q fever, antibiotic-resistant diseases, and HIV-1 group O, rabies, Ebola virus and Zika virus. In Europe, regular monitoring of the Rapid Communication Reports originating from the Eurosurveillance website [22] is recommended, as well as actively seeking information to assess the epidemiological status of diseases in the areas where a donor has lived or travelled [23]. Specific information about geographic distribution of infectious diseases can be obtained from the websites of the European Centre for Disease Prevention and Control (www.ecdc.europa.eu), the World Health Organization (www.who.int/ith/en) and the Centers for Disease Control and Prevention in Atlanta (the Yellow Book at wwwnc.cdc.gov/travel).

The risk of transmission of an infectious agent through procurement of tissues or cells from a donor who may have visited an affected area should be balanced against the likelihood of transmission occurring. Regional risks within an affected country can

vary. In cases of recent travel, if the donor remains well after return or after known contact with someone infected, the donor should be deferred for at least twice the length of the incubation period [17]. If the donor was infected, they can only be accepted after full recovery and when the donation is no longer infectious.

4.3.4. Relative contraindications

Below are listed the potential risks that have to be analysed on an individual basis, considering the potential harm and benefit.

a. Additional contraindications/risks to donation of tissues and cells for living donors:
   i. pregnancy (except for donors of umbilical cord blood cells and amniotic membrane, and sibling donors of haematopoietic progenitor cells);
   ii. breastfeeding;
   iii. health risks for donors themselves (e.g. specific procedure or superovulation).

b. Organ-transplant recipients:
Organ recipients receive immuno-suppressive drugs to prevent rejection, but this could make the serology testing unreliable; moreover, organ donor-selection criteria are less stringent than for tissues and cells. This risk should be assessed on a case-by-case basis, taking into account the level of immuno-suppression in combination with the possibility of tracing the medical details of the organ donor.

c. Impact of immuno-suppressive agents in the donor:
Treatment with immuno-suppressive agents can weaken the immune system and thus influence the reliability of serological tests (Chapter 5). Evaluating the effect of the immuno-suppressive agents on the haematological parameters (erythrocytes, leukocytes and thrombocytes) can be indicative for immuno-suppression. NAT testing may be helpful in such circumstances. All other medication of the donor should be always interpreted by a risk assessment for impact on the tissue, e.g. chronic use of corticosteroids can affect the quality of skin and musculoskeletal tissue (see Part B: tissue-specific chapters).

d. History of genetic disease:
A family history of genetic disease is a risk factor that should be assessed; where the occurrence of genetic disease in the family history cannot be traced/assured, this increases the risk of transmission of genetic disorders, especially in non-partner MAR (see Chapter 27), and should be regarded as an exclusion criterion (see Chapter 22 for HPC).

e. Deferred for blood donation for known reason:
If it is known that the potential donor was excluded or deferred from donating blood by a blood-collection establishment, the specific reason for the deferral must be discovered, and the eligibility of the donor is then evaluated on an individual basis. If the reason is not known, it may be safer to exclude the donor for tissue donation.

f. Xenotransplantation [24, 25, 26, 27]:
The reference to 'transplantation with xenografts' is clearly stated as an exclusion criterion for donors at §1.1.13 of Annex I in Directive 2006/17/EC. However, the absence of a formalised definition for its interpretation has previously led to some ambiguity in its application. Similar terms – such as 'xenotransplantation product', 'xenogeneic cell-based medicinal product' and others – have been used within different healthcare sectors, and the need for a uniform consensus on terminology is paramount. In recent years different Scientific and Technical Committees have established and adopted the fundamental opinion that the term 'xenotransplantation' is applicable to any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues or organs from a non-human animal source, or (b) human body fluids, cells, tissues or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs.
The scientific community continues to apply the principles of this approach, and xenotransplantation products include those which utilise living non-human animal cell, tissues or organs used for transplantation. With similar equivalence, the US Guidance for Industry has adopted the scientific opinion and further states that any biological products, drug or medical devices sourced from non-living cells, tissues or organs from non-human animals are not considered xenotransplantation products (e.g. porcine insulin and biological heart valves). The risk-management strategy for the control of infectious agents is primarily focused on the application of selective sourcing, effective collection and handling, and measures applied for elimination/inactivation or removal of agents. Where relevant, the medical device and medicinal sectors are regulated by

these standards to achieve high standards of quality and safety, and the products are thus viewed as risk-mitigated.

As a precautionary measure, a few countries have applied a broader interpretation of the exclusionary term, to also include non-viable cells or tissues of animal origin utilised in therapeutic products. Tissue establishments should apply documented systems to justify their local practices in relation to xenotransplantation products by the evaluation of scientific evidence, professional standards and national guidance.

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Related material

- Appendix 14. Medical and social history questionnaire (NHS, UK)
- Appendix 15. Physical assessment form (Dutch Transplant Foundation)
- Appendix 16. Evaluation of pigmented skin lesions
- Appendix 17. Evaluation of malignancies for risk assessment in tissue and cell donors

Chapter 5: Donor testing – markers for infectious diseases

5.1. Introduction

Use of tissues and cells for human application can result in unintentional transmission of disease. However, such events can be prevented by scrupulous evaluation of donors, including laboratory testing of each donor close to the time of donation in accordance with established good practice. The risk can be reduced substantially by appropriate donor sample testing, but adequate controls must be in place to ensure that test results are accurate and reliable. Controls include:

a. ensuring that the screening programme includes notably relevant infectious diseases (related to the habitus and travels, if any) and their appropriate individual screening target(s);
b. selecting a suitable testing laboratory;
c. ensuring that donor blood samples are labelled, handled and stored appropriately and that the time interval between collection and testing meets the sample requirements of the test kit;
d. use of appropriately validated tests for infectious diseases;
e. providing well-written standard operating procedures (SOPs) and training for all personnel involved in collection and labelling of donor samples, for sample storage and transport, and for technical staff carrying out testing and reporting results, as well as for those receiving and interpreting them.

These are vital elements of a tissue establishment's quality system, and any laboratory undertaking tissue-donor testing must ensure that the most appropriate technology for this activity is used, including selecting test kits that demonstrate high clinical and analytical sensitivity and specificity.

5.2. General concepts

Tissue establishments must ensure that donor samples from all donations of human tissues and cells are subjected to biological tests mandated by national or other applicable legislation, or by a specific situation such as travel. In EU member states, Annex II of Directive 2006/17/EC, amended by Directive 2012/39/EU, specifies mandatory laboratory tests and general testing requirements for living and deceased donors of tissues and cells, and requires that any such laboratory and its tests must be authorised by the competent authority.

SOPs that define the criteria for acceptance or rejection of tissues and cells based on those test results must be in place. The Responsible Person (RP) who will interpret test results should be knowledgeable about infectious-disease test kits, and decisions must meet the expectations in regulations or, if regulations are not prescriptive, follow professional standards of practice [1]. In EU member states the RP is defined by Article 17 of Directive 2004/23/EC.

Documented measures must be taken by tissue establishments that receive tissues or cells from another country or distribute tissue or cells to another country to ensure that the donor-testing requirements of the destination country are met. Evidence should also be available to show that any laboratory

involved in testing of donor samples has been accredited, designated, licensed and/or authorised by the appropriate authority to carry out such testing.

5.3. Quality of donor samples

Manufacturers of assays for infectious-disease testing provide specific sample requirements for which their assays have been validated. Personnel of procurement organisations and tissue establishments involved in collecting, transporting (having particular regard to packaging, temperature, duration), storing or testing donor samples must be aware of these requirements to ensure optimal assay performance. If inadequate or otherwise compromised samples are provided to the laboratory and tested, the results may not be valid, which increases the risk of donor-related transmission of infectious pathogens.

Donor-related conditions that could affect the quality of a test specimen must also be taken into account. Infectious-disease test results may be invalidated by haemodilution if the extent of any dilution is such that it may dilute any screening target present to a level below that which is detectable by the assay(s) used. Therefore in cases where haemodilution is known to have occurred, ideally pre-transfusion/infusion samples should be obtained for testing purposes. (See §5.3.2.)

Haemolysis may also affect test results. Haemolysis is the destruction of red blood cells in whole blood that discolours the plasma or serum, and it is noticeable after centrifuging the tube. Depending on the degree (severity) of haemolysis, the colour of the serum or plasma may be pink to red. This darker colour can promote a higher density reading by the optical component of test equipment, leading to a positive test result. Haemolysis may be caused by rapid collection of blood through a small-bore needle, or by improper sample storage or transport, such as allowing the tube of whole blood to freeze prior to testing. Other causes can be donor-derived and include an infection, a toxin, medication or autoimmune haemolytic anaemia, and haemolysis can occur after haemodialysis or after a haemolytic transfusion reaction.

Although serology tests must be used, detection of antibodies against pathogens can be impaired if the donor has received immuno-suppressive treatment prior to sample collection or when the donor has received blood products containing antibodies against Epstein–Barr virus, *Cytomegalovirus*, *Toxoplasma* or hepatitis B (HBs antibodies). In both cases this leads to problematic test results. In the first case, that leads to a false-negative result; in the second case, it leads to a false-positive result due to passive immunity communicated by antibodies included in the validated and authorised labile blood product. In the first situation, adding molecular screening tests (i.e. nucleic acid amplification technique/NAT) can be valuable because detection of viral nucleic acid in blood samples is generally not affected by immuno-suppressive therapy [1, 2]. The underlying condition requiring immuno-suppression will demand further assessment because the disease/condition in itself may constitute an independent reason for determining that the donor is not eligible. If any of these donor-related conditions exist, they must be documented in the donor record and evaluated by an RP before release of tissues or cells for clinical application. In the second situation, pre-transfusion/infusion samples should be used.

Additionally, false-negative results can occur in different scenarios: haemodilution, incorrect sampling or inappropriate test quality.

5.3.1. Sample collection (sample type, tubes, labelling, time limits and handling)

All personnel involved in any stage of the testing process must be fully trained. Testing must be carried out on plasma or serum of the donor according to the specification laid out by the manufacturer of the test kit. Testing must not be performed on other fluids or secretions, such as aqueous or vitreous humour, unless the assays selected have been specifically validated for use with that sample type. In the case of a neonatal donor (i.e. age ≤ 1 month), the required tests should be carried out using a blood sample from the donor's birth mother. Another important consideration is that, during the first 18 months of life, a child's immune system is only in development and protective antibodies may not yet have been produced against an infection, thereby increasing the risk of hidden infections in child donors. (See §5.5.1.)

Donor sample collection and manufacturer's test instructions must be followed with regard to:

a. the type of sample collection tube (no anti-coagulant or a specific anti-coagulant) required for the test being carried out;
b. sample storage and transport conditions post-collection, which can include centrifugation and/or separation within time limits or specimen refrigeration/freezing; and
c. testing required to be carried out within a specified timeframe post-collection.

To ensure traceability at each stage of the testing process, all donor samples must be identified with a permanently affixed label that contains information or references that link the sample and the laboratory test results to the donor (see Chapter 14 and Chapter 15). The date and time when the sample was drawn must be accurately documented. Unless a unique codification system is used, it is recommended that two donor identifiers, such as the donor's full name, date of birth, medical record number or other unique identifier, be used. In the case of a sample from a donor (deceased or living) the label or associated documentation should also include some identification of the person who collected it and a description of the site on the donor's body the sample was taken from (e.g. cephalic vein, femoral artery, subclavian artery, superior vena cava). It is good practice for the identity of all donor samples to be confirmed by a second person (from the procurement team, if possible), and this confirmation process should be documented [3]. If any donor blood samples were drawn before death, they can be validated for use, but there must be assurance that the patient identifier (i.e., appropriate labelling) used for any such specimen is confirmed as coming from the donor so mix-ups do not occur (i.e. to avoid carrying out testing for critical communicable diseases on the wrong person) [3]. Other donor-identification methods can be used, if validated, to ensure traceability [4].

Specimens of blood, serum or plasma from the same donor must not be mixed together for testing, whether collected at the same time or at a different time.

For obvious safety reasons, the collection of donor blood for infectious-disease testing must always occur as close as possible to the donation event. Personnel who collect, or otherwise obtain, donor blood samples to be used for this critical testing must consider factors that could influence sample degradation and cause false-negative or false-positive test results, e.g. time of sample collection, temporary storage conditions.

An adequate volume of whole blood must be collected, because otherwise the quantity of serum or plasma after centrifugation may not be sufficient to undertake all of the required tests for infectious diseases, or for any further investigations that may be required. The volume of blood required will be dependent upon the minimum requirements of the testing laboratories as well as the sample requirements of each test kit, and these parameters should be known and evaluated before blood collection. Other considerations could include a donor with a high haematocrit (which could necessitate collection of extra tubes) and a donor who took (or was given) anti-coagulant medication (fibrin clots may appear in the serum after centrifugation and cause a reduction in the volume of testable serum). If the volume needed is not clear to personnel collecting blood samples from a donor, filling three or four 6 mL blood tubes to their limit should be sufficient. However, in the case of a living donor, care should be taken not to collect an unnecessarily large volume of blood because an adverse clinical event could result. To avoid unintended consequences, personnel who collect donor blood samples should be familiar with the requirements of the testing laboratory, and written procedures should provide specific direction.

Proper handling of any donor blood sample after it is collected is necessary to ensure that testing protocols can meet the required specifications. For example, when a blood sample is collected in a tube containing an anti-coagulant, this liquid or powder requires that a completely filled tube be gently mixed by slowly inverting the tube 5 to 10 times immediately after collection [5].

After collection, specimen handling by personnel can include centrifugation and/or separation of the serum or plasma from red cells within specific time limits. In addition, specimen storage and/or transport conditions can involve refrigerating or freezing the plasma or serum aliquot. Specific instructions from the test kit manufacturer must be followed and can differ between tests [3]. In all cases, validated transport containers and validated shipping conditions must be used when sending donor samples to a testing laboratory.

The facility receiving any donor sample for testing should have an SOP in place to define the criteria for acceptance or rejection of the sample, based on collection, storage and transport conditions. The testing facility must document acceptance or rejection of the sample and should share this sample status in a timely manner with an RP at the procurement organisation or tissue establishment.

5.3.1.1. *Deceased donor*

In the case of a deceased donor, blood samples must have been obtained just before death or, if this was not possible, the time of sampling must be as soon as possible after death, and in any case within 24 h after death.

It is important to collect blood samples without untoward delay after death to avoid sample characteristics that could cause a non-specific test result (e.g. partial haemolysis) or that could lead to its rejec-

tion for testing (e.g. complete haemolysis). Delays in donor sampling have been shown to increase the incidence of red cell haemolysis, and other substances can appear in non-circulating blood due to growth of micro-organisms and release of enzymes (including by-products of tissue and cell death) [3].

Some studies have demonstrated the potential for blood sample collection to occur more than 24 h after death; however, validation of each infectious-disease test kit using such specimens is necessary to support an extension [6, 7, 8, 9, 10]; see also Appendix 19. Acceptance of such practice is controlled by national regulations.

5.3.1.2. *Living donor*

In the case of living donors, blood sampling should be obtained at the time of donation or, if this is not possible, within 7 days before or 7 days after donation. However, for practical reasons, collection of a sample from an allogeneic bone-marrow stem cell or peripheral blood stem-cell donor must occur within 30 days before donation (taking into account that re-testing at the time of donation will be informative), but before reaching a point-of-no-return when irreversible measures for preconditioning of the recipient have been initiated. If tissues and cells of allogeneic living donors can be stored for long periods before use, repeat sampling and testing is required after 180 days, unless specific exemption criteria are met. (See §5.5.2; see also Chapter 18 and following; Chapter 26 and following).

5.3.2. Haemodilution assessment

When possible, a donor blood sample collected before administration of any transfusions and infusions should be used for testing purposes.

If a donor has recently received transfusions of blood or blood components, or infusions of colloids or crystalloids, and has lost blood, any testing of donor blood collected post-transfusion or post-infusion may not be valid due to haemodilution or plasma dilution of the donor's blood and, thus, of any samples taken from the donor. Assessment of the extent of any haemodilution includes the use of a formula to calculate dilution of the donor's original circulating blood volume (and circulating levels of antigen and/or antibody, if present) and should be done by the physician in charge or the transplant co-ordinator. Current practice in a number of countries is to consider 50 % calculated haemodilution to be the maximum allowable with minimal risk of a false-negative test result arising because of the haemodilution.

Examples of when a haemodilution calculation may need to be carried out include:

- *ante mortem* blood sample collection: if blood, blood components and/or colloids were administered in the 48 h preceding blood sampling, or if crystalloids were infused in the hour preceding blood sampling;
- *post mortem* blood sample collection: if blood, blood components and/or colloids were administered in the 48 h preceding death (circulatory arrest), or if crystalloids were infused in the hour preceding death (circulatory arrest).

Refer to Appendix 18 for an example of a commonly used formula to assess the donor's potential haemodilution or plasma dilution that can be applied when the donor received any fluids that may lead to haemodilution. Adaptations of the algorithms may be needed for body sizes outside the normal adult range. Allowances may need to be made for a very large or a very small adult donor, or a paediatric donor. In brief, a donor's total plasma volume (TPV) and total blood volume (TBV) are estimated by calculations based on the donor's body weight, then direct comparisons are made to amounts of recent transfusions and/or infusions that were administered before circulatory arrest or before collection of the blood sample, whichever occurs first [3]:

a. estimate TPV of donor (weight in kg × 40 mL/kg; or, weight in kg ÷ 0.025);
b. estimate TBV of donor (weight in kg × 70 mL/kg; or, weight in kg ÷ 0.015);
c. calculate total blood (mL) received in the last 48 h (A);
d. calculate colloids (mL) received in last 48 h (B);
e. calculate crystalloids (mL) received in the last 1 h (C);
f. add B + C and compare to TPV (fluid volumes are compared);
g. add A + B + C and compare to TBV (mass/fluid volumes are compared);
h. does either comparison show > 50 % dilution? If not, the blood sample qualifies and can be used for testing for infectious diseases.

Although not normal practice, a tissue establishment may accept tissues and cells from a donor with plasma dilution of > 50 %, but only if each required test has been validated appropriately for use with a diluted test specimen. In such cases, to help reduce risk, additional testing should also be performed using molecular tests (i.e. NAT) for the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), and pos-

sibly for other viruses, depending on the donor's travel history, underlying disease or other factors.

The blood collected can also be diluted if the specimen is drawn in close proximity to an infusion or transfusion intravenous line, even if the donor is not actually haemodiluted or plasma-diluted. Samples should be drawn from the opposite side of the body in relation to the site of any infusion/transfusion.

Furthermore, in theory, a transfusion shortly before the donation can result in transmission of infectious agents to the donor.

5.4. Testing laboratories

To meet quality and safety requirements, all testing of infectious diseases for deceased and living donors must be carried out by laboratories that are accredited, designated, authorised and/or licensed for these activities according to the regulations set by the relevant Health Authority. Such laboratories will have the knowledge, skills, resources and competence required for testing blood samples from tissue donors, and should use appropriate algorithms to ensure that their testing procedures have maximum sensitivity without loss of specificity. They must also participate in relevant external quality assessment schemes (proficiency testing) and be subject to regular internal and external audits.

If additional biological assays are carried out, the laboratory used should be accredited and should participate in an appropriate external quality-assessment programme [5]. (See §5.5.1 and Chapter 2.)

Tissue establishments can undertake these testing protocols themselves or have a written agreement with any laboratory that carries out these tests [11]. Tissue establishments should evaluate and select a testing laboratory on the basis of its ability to generate reliable and appropriate results, and to keep relevant records. In addition the testing laboratory must comply with regulatory requirements and any other specific expectations of the tissue establishment (e.g. time-sensitive availability of test results, record retention). The tissue establishment should ensure that the laboratory is competent to perform this work and is using appropriate assays and procedures (ideally, with kit designed for donor screening rather than for diagnosis). There must be evidence that good laboratory practice is being followed and that personnel are appropriately trained and experienced in relevant testing procedures. The obligation of the laboratory to notify the tissue establishment when various deviations occur is mandatory. To ensure a consistent level of competence and performance, audits of the testing laboratory(ies) should be undertaken periodically by the tissue establishment or by qualified external experts as part of the tissue establishment's quality system.

In addition, test records at the laboratory must be retained for 10 years at least and must contain the date of receipt of the blood sample at the testing facility, a record of each test kit used to test donor blood samples (i.e. manufacturer, lot number, expiry date) and the results of donor testing, including repeat testing (if applicable). See Chapter 2 and Chapter 15.

5.5. Tests to be carried out

The donor-screening assays selected must be validated and used in accordance with current scientific knowledge. A higher test generation (e.g. 4th generation) leads in general to a shortened serological window period [12, 13, 14].

All assays used for donor testing within the EU should be Conformité Européenne (CE)-marked (see Appendix 19). Most of the major international manufacturers of donor-screening assays provide CE-marked assays and systems, and in some cases the manufacturers have undertaken validation work with samples from deceased persons. Where such data are not provided, laboratories performing this work will be expected either to have validated the assays for this purpose themselves or to use other available peer-reviewed data [15, 16].

5.5.1. Mandatory tests

Mandatory serological tests for HBV, HCV, HIV and *Treponema pallidum* are listed below; additional molecular assays can be carried out to confirm a putative infection. All assays must be carried out in serum and/or plasma samples of the donor according to the manufacturer's instructions [3]. Those tests, depending upon the laws of the relevant country, must/should be performed in individual samples, not in pooled samples.

a. Human immunodeficiency virus type 1 and 2: A combination assay (4th generation) including detection of anti-HIV-1/2 antibodies plus HIV-1 p24 antigen is strongly recommended. If a 3rd generation test is used, an HIV-1 RNA test (qualitative or quantitative) has to be performed additionally to exclude an HIV-1 infection. It is recommended that the sensitivity limit for the HIV-1 RNA assay should be ≤ 50 IU/mL. Samples with confirmed non-negative (i.e., weak reactive or borderline) serological screening results can be re-tested in duplicate using the same assay. If the results are negative

the donated tissues can be released. Reactive samples can be re-tested using an alternative certified serological assay of equal or greater sensitivity. Donations that are non-reactive in that assay and negative for HIV-1 RNA can be considered suitable for clinical use. If an HIV-1 RNA test is performed and the result is reactive, independently of the serology result, the donations cannot be considered suitable for clinical use.

b. Hepatitis B virus:
HBV surface antigen (HBsAg) and total antibodies to HBV core antigen (anti-HBc). HBsAg must be negative. If anti-HBc is 'reactive', an additional determination of a highly sensitive HBV-DNA method must be performed (currently between 4 and 30 IU/mL detection limit; it is recommended to use the most sensitive test); but haemodilution may influence the limit of detection. If anti-HBc is positive and HBsAg and HBV-NAT is negative, the donated tissues can be released. HBV-DNA positivity reflects potential infectivity and leads to a discard of the donated tissues.

c. Hepatitis C virus:
The screening for an HCV infection is based on detection of anti-HCV antibodies. But combined assays like the assays for HIV are not commercially available at present, and the performance of HCV-antigen assays is not yet sufficient to exclude an early infection. Presence of anti-HCV may indicate an acute, chronic or past infection. Furthermore, the pre-seroconversion window phase takes several weeks; thus, an HCV-RNA assay is strongly recommended to exclude active HCV infection. It is recommended that the sensitivity limit for the HCV-RNA assay should be ≤ 50 IU/mL. An anti-HCV-positive and RNA-negative result, which is indicative of a non-specific reaction or a past infection (confirmed from the donor's medical history, i.e. type and duration of HCV treatment and serology), needs to be confirmed by immunoblot analysis. If the result of the HCV immunoblot is negative (anti-HCV false-positive result) the donated tissues can be released. In the case of an HCV immunoblot positive result (confirmed positive anti-HCV result), only with the evidence for successful HCV treatment under medical supervision and a negative HCV-RNA test, the donated tissues can be released as well.

d. *Treponema pallidum*:
In the serological diagnosis of syphilis, a treponemal screening test should be used, e.g. *T. pallidum* haemagglutination (TPHA) test, the *T. pallidum* particle agglutination (TPPA) test, Treponemal enzyme immuno-assays (EIA) or chemiluminescence immuno-assays (CLIA). If the screening test is reactive, the results should be confirmed by means of a second treponemal test based on a different analytical method (see Appendix 20). Alternatively, an approved non-treponemal screening assay may be used to screen donors. Reactive samples can be re-tested using a treponemal-specific assay. Donations that are non-reactive in that assay can be considered suitable for clinical use.

Testing for HTLV-I antibodies must be performed for donors living in high-prevalence areas. Likewise, testing is necessary if the donor themselves, the donor's parents or the sexual partners originate from such areas [16, 17]. Reactive screening results need to be confirmed by immunoblot analysis and/or specific NAT.

In principle, if a child donor is 18 months old or younger, or has been breastfed in the 12 months before death, the birth mother should be evaluated for risks associated with HIV, HBV, HCV and HTLV. In the case of a neonatal donor (i.e. age ≤ 1 month), the required tests should be carried out using both, a blood sample from the donor's birth mother and one from the newborn. IgG antibodies in the newborn blood sample are likely to be maternal; therefore, testing for anti-HIV, anti-HCV and anti-HBc is not suggested in the newborn. However, if a maternal infection is suspected, additional testing for HBs antigen and HIV-1 RNA, HBV-DNA and HCV-RNA in the newborn sample makes sense, to exclude an congenital or postnatal infection. In the very rare case of a treated HIV infection in the mother, testing for presence of HIV-1 in the newborn must be carried out on viral cDNA.

In addition, special screening considerations are applicable to other paediatric donors and additional testing for communicable diseases (e.g. Zika virus infection, malaria or Chagas disease) may be indicated. Special attention needs to be paid to putative infections while maternal antibodies may still be detectable (up to the first 18 months of life).

5.5.2. Additional tests

It is well recognised that NAT assays for HIV, HBV and HCV reduce the risk of inadvertent disease transmission due to the substantial decrease in

window period when compared with routine serological tests [13, 14].

Because NAT assays are more sensitive, serious consideration should be given to also carrying out NAT tests for HIV, HBV and HCV. Considerations that support the use of NAT assays for each donor screening include the following:

- The medical and behavioural history obtained from a proxy for a deceased donor can be less reliable than collecting this information from a living donor.
- There is a risk that a recent exposure to HIV, HBV and HCV (several days prior to death) might not be detected by serological (antibody) assays due to insufficient amount of antibodies against the specific virus.
- If the donation includes multiple tissue types and it results in a large number of tissue grafts to be made available for many recipients, the potential risk is increased if the viral tests selected cannot detect early infection in a donor.
- The molecular methods used for the screening have to meet the requirements of each individual pathogen. Haemodilution (see §5.3.2) especially has to be scoped. In case of acute or untreated chronic infections, viral loads are usually in a range where haemodilution may not cause false-negative results. But, if low-level viraemia is expected, for instance in occult HBV infections, the polymerase chain reaction (PCR) method must be as sensitive as possible (currently ≤ 20 IU/mL). Tissue from a donor with suspected occult HBV infection and haemodilution might not be considered to be safe.
- Molecular assays from deceased donors should be performed in individual samples (see current legislation of each country), not in pooled samples. Some of these NAT assays are combination tests that can detect HIV, HCV, and HBV from a single blood specimen in one run, thus improving the feasibility of routine NAT in donor screening. (In the case of samples from living donors, the pooling could be accepted if the national requirements for the comparable NAT testing of blood donors are fulfilled.)

Any relation to high-prevalence areas for specific infections/diseases must be considered carefully. Scientific evidence for risk factors for certain diseases are provided by the European Centre for Disease Prevention and Control [16, 17]. ECDC regularly publishes risk assessments and maps that can be helpful, notably for emerging diseases.

Additional testing that may be considered (depending on the donor's history and/or the characteristics of the tissues or cells donated) includes:

- ABO (ABo) group;
- RhD (D antigen);
- human leukocyte antigen (HLA);
- antibodies to *Cytomegalovirus*, Epstein–Barr virus and *Toxoplasma gondii* might be relevant for donor-recipient risk stratification;
- Hepatitis E virus RNA (i.e. NAT).

Depending on factors like individual travel history and specific current or past clinical abnormalities of the donor as well as the epidemiological situation, the decision can be made to carry out other optional tests, which can include screening for tropical infections such as malaria, trypanosomiasis, viral infections with West Nile virus, Zika virus, etc. The need to perform such assays, or others, must be examined on a case-by-case basis. In the case of paediatric donors, such infections must be reviewed for their impact for mother-to-child transmission.

Results of blood cultures can be very useful tools to aid in the determination of bacteraemia in a donor of tissues and/or cells (see Chapter 10).

5.5.3. Re-tests of samples from living donors (allogeneic use)

Repeat sampling and serology testing is required after 180 days, unless any of the following specific exemption criteria are met:

- if samples from a living donor undergo serology testing and are also tested by molecular tests (i.e. NAT) for HIV, HBV and HCV, which is recommended – because NAT can increase sensitivity in the detection of recently acquired infections, molecular testing of all donors using this technology is highly recommended as standard practice;
- if the tissue/cells come from a living donor and have been processed using an inactivation step that has been validated for the virus(es) concerned;
- if the tissue/cells come from a living donor and will not be stored longer than 180 days prior to use.

Test-kit assays for infectious-disease markers are typically optimised for testing a sample from a living donor. For living donors, initial infectious-

disease testing is carried out at the time of donation or, when this is not possible, within 7 days of the donation. In the case of bone marrow and peripheral blood stem-cell collection, blood samples must be drawn for testing < 30 days before donation. Minimum testing requirements are the same as for deceased donors, but there are additional considerations because the donor is available for more testing. For example, more tests could be indicated because there may be unique risks of infectious disease pertinent to a profoundly immuno-suppressed recipient of bone marrow or of similar types of haematopoietic allograft [18].

For testing individuals involved in medically assisted reproduction (MAR), see Chapter 27.

5.5.4. Testing of autologous samples

For autologous donors, if the removed tissues or cells are stored or cultured, they must undergo the same serological tests as for allogeneic donors before they can be transplanted back into the donor. If an autologous donor's blood sample has not been appropriately tested or if a test is indicative for a relevant infectious disease, this will not necessarily prevent the tissues or cells, or any product derived from them, from being stored, processed and re-implanted in the autologous donor; but this is only true if appropriate storage can provide isolation/segregation to ensure there is:

- no risk of cross-contamination with stored allografts;
- no risk of contamination with adventitious agents;
- avoidance of mix-ups due to misidentification (see Chapter 14 and Chapter 15).

SOPs based on risk analyses must be in place to define the criteria for acceptance and rejection for contaminated autologous tissues and cells, or if the autologous donor has not been tested for infectious diseases (see Chapter 2).

5.6. Reporting and documentation of test results

Tissues and cells must be held in 'quarantine' until such time as requirements relating to donor testing have been completed. With this in mind, donor infectious-disease testing should be carried out and reported without delay. Reporting methods must be used that link the donor's unique identifier to the test results, while also keeping the donor anonymous to third parties. Data-security measures are required, as well as safeguards against any unauthorised additions, deletions or modifications to donor test results. There must be no disclosure of infectious-disease test results.

Arrangements between the testing laboratory and the tissue establishment, or the clinical team responsible for use of the donated tissues or cells, should include agreed methods for the reporting of test results to ensure mix-ups are avoided and prevent misinformation. Laboratories and tissue establishments must have policies relating to the management of test results from a donor that may be pertinent to family members and other contacts of the donor or that have implications for public health.

Reporting procedures should ensure that accurate, rapid and verifiable results are provided. In addition, there must be a system in place to ensure prompt alerts using an immediate notification system when an indicative test result for an infectious disease occurs. Other precautionary measures in reporting may include [19] the following:

- where manual systems are still used (although they are not recommended), analysis reports should be cross-checked to ensure that the transcription of test results has been confirmed by two independent assessors (the 'four eyes principle');
- using computerised procedures for the transfer of test results from laboratory equipment to the laboratory data-processing management system (e.g. medical records) to eliminate the need for manual transcription of data or oral information;
- using clearly interpretable, computerised graphic symbols to highlight pathologic results;
- recording (semi)quantitative value (e.g. titre, IU/mL) of antibodies and/or the related positivity threshold next to the viral negative/positive result;
- recording the number of copies/mL (or, preferably, IU/mL) of nucleic acid measurement and the limit of detection, as well as (for qPCR systems) the linear range of the assay (if qualitative PCRs are used, then semiquantitative values should be recorded, e.g. Ct-Values, Cp-Values);
- using formal laboratory reporting structures and accreditation or certification pathways to improve quality standards;
- using widely recognised international units of measurement;

- mentioning systematically the name of the kit used (or making available on demand the data of the diagnostic assays, e.g. name, manufacturer).

5.7. Archived samples

If there are no national requirements a risk-analysis-based decision, based on the type of tissue/cell and the type of donor (autologous, allogeneic, living, deceased, multiorgan) must be performed to decide if, and for how long, archived samples must be retained. A storage time of at least 1 year after the distribution of the last tissue from the donor is recommended. In some countries, archiving donor samples may be required.

Archived samples may be used for several purposes: look-back testing involving a new infectious agent, development of more accurate or new tests, or if investigating a report of a serious adverse reaction in a recipient of tissues or cells. A documented risk assessment, approved by the tissue establishment's RP, should be carried out to determine the fate of all stored tissues and cells following the introduction of any new donor test that could reasonably be considered to affect safety or quality (see Chapter 2).

5.8. References

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Related material

- Appendix 18. Sample haemodilution algorithm
- Appendix 19. Example of validation of screening: infectious disease assays of blood from deceased donors
- Appendix 20. *Treponema pallidum* testing

Chapter 6: **Procurement**

6.1. Introduction

To ensure high standards of quality and safety during the procurement process for tissues and cells, it is recommended that a quality system be in place in the procurement organisation or the tissue establishment undertaking the process. This quality system must guarantee adequate training of all personnel involved, as well as written standard operating procedures (SOPs) that require documentation for all stages of the process. Procurement professionals should take measures to ensure appropriate safety and quality parameters are in place.

Procurement of human tissues or cells can take place only after donor consent/authorisation requirements have been satisfied, as described in Chapter 3. Tissues and cells must also be identified, packaged and labelled correctly (see Chapter 14) and then transported to the tissue establishment or clinical team for direct use, in accordance with established requirements.

Chapter 2 sets out the general quality-management expectations about the personnel, facilities, equipment, materials, procedures and documentation that should be applied when considering the quality and safety of tissues and cells for human application.

The definition of requirements related to procurement activities requires the acknowledgement that each of the various types of donation (e.g. deceased donation, either single tissue or multi-tissue, and living donation) represents a complex of processes and different risk factors that must be considered in order to assure the quality of the tissues and cells procured as well as the safety of (living) donors and recipients.

The criteria, including the location and standard of the premises, that apply in procuring tissues or cells from living donors are equivalent to those for the treatment of patients. However, in deceased donation there are additional considerations to take into account; as specified in Chapter 3, procurement from a deceased donor may take place not only in a hospital but in a mortuary or forensic department, and in those cases it is important to define conditions and requirements to guarantee the quality and safety of the procured tissues and cells [1].

Deceased donation of tissues or cells can also occur after organ donation, and in those cases sterility needs to be ensured throughout the whole procedure, including during organ procurement. If more than one tissue is to be procured from a single deceased donor, procurement may be performed by a multi-tissue team or by different tissue-specific teams.

This chapter provides guidance for tissue procurement in general, but with a specific focus on deceased donors and multi-tissue procurement.

6.2. Personnel

Procurement activities must be undertaken by personnel with appropriate qualifications, training, expertise and experience. This includes successful completion of a comprehensive technical and/or clinical training programme, including the broader ethical, legal and regulatory context of procurement.

The training programme must be tailored to the specific tissues or cell types to be procured and will also depend on whether the procurement is from living or deceased donors.

Persons undertaking procurement must be made aware of the risks and potential consequences if policies and procedures on procurement are not followed as directed in written SOPs and according to relevant legislation.

To promote compliance with donor-selection criteria and procurement procedures, the tissue establishment must have written agreements with each person, clinical team or third-party procurement organisation involved in carrying out procurement, as well as those collecting critical information used in donor selection. The written agreements must include detailed descriptions of expectations and responsibilities related to quality and safety measures, as well as any additional regulatory requirements. A written agreement is not necessary for individuals employed by the tissue establishment responsible for these steps but expectations and responsibilities pertaining to procurement must appear in their job description (see Chapter 2).

6.3. Facilities, equipment and materials

6.3.1. Facilities

Procurement activities must be authorised by the appropriate and competent Health Authority. Each procurement event must take place in an appropriate facility and follow the required clinical/technical procedures (see §6.4). The procedure must take into account the risk of microbial or other contamination of procured tissues and cells, and steps should be taken to minimise the risk. For reasons of privacy and control of contamination, access to the area where procurement takes place must be restricted during the actual procurement of tissues or cells. In addition, the donation of tissues or cells by living donors must take place in an environment that ensures their health, safety and privacy. A risk assess-

Table 6.1. **Factors and criteria to be considered in risk assessment of the procurement procedure**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	→			≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1-2 persons	→			≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the blood stream (infusion)

ment to determine the suitability of the procurement site, depending on the types of tissues or cells to be procured, must be carried out.

It is highly recommended that the facility where procurement takes place is:

a. of adequate size in the floor space, work-tops and benches that will be used;
b. appropriately located to ensure cleanliness and privacy;
c. furnished with sufficient and suitable lighting;
d. in a good state of repair;
e. free of pests; and
f. able to provide a sufficiently clean or cleanable environment that will not increase the risk of contamination of the cells or tissues during their procurement.

Before procurement, steps to minimise the potential for contamination must include cleaning of all work surfaces with an appropriate and effective disinfectant. The procurement area must also be cleaned appropriately after the procurement, including proper and safe disposal of single-use instruments, consumables and any other waste, including clinical waste that poses a biohazard. Any re-usable instruments will need to be cleaned and sterilised. If a tissue establishment (or third party carrying out the retrieval) uses the general services of the host facility to clean the procurement area and/or sterilise any re-usable instruments, the tissue establishment must have a written agreement with the host facility and the procedures used must be inspected and validated.

6.3.1.1. *Defining the requirements of a procurement area*

Procurement of tissues and cells may take place in various facilities, ranging from a hospital operating room, tissue establishment, hospital clinic, mortuary, funeral home or care home, to a donor's own home. These facilities can be broadly categorised as:

- operating theatre or equivalent;
- dedicated procurement area with routine air-quality monitoring and controlled cleaning (e.g. tissue establishment procurement room);
- dedicated clean area (controlled cleaning);

Table 6.2. **Example of musculoskeletal tissue recovery procedure with the specified characteristics**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤ 1h	1-2 h	2-3 h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 persons	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues or cells preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable clinical application in a well-vascularised site	large durable clinical application in a well-vascularised site	direct application into the bloodstream (infusion)

- non-dedicated area, with local cleaning of the procurement space.

A risk assessment based on the factors detailed in Table 6.1 will help define an appropriate procurement area, including air quality, depending on the level of risk and any subsequent steps taken during processing. Taking into account the criteria defined in Table 6.1, a risk assessment might reach similar conclusions to the cases exemplified in Tables 6.2 to 6.7. In each case, the shaded background shows the typical options in practice.

6.3.1.2. *Example of musculoskeletal tissue recovery procedure with the specified characteristics*

- Tissues are exposed to the environment for a long period of time (medium/high risk)
- The procedure is performed by two recovery members, plus one nurse responsible for tissue packaging and one circulating assistant (medium risk)
- During procurement, tissues are washed with sterile water to reduce surface microbiological contamination (medium/high risk)
- During processing an antibiotic decontamination will be applied to musculoskeletal tissues (medium risk)
- During procurement and processing the sampling method used is swabbing of each tissue so detection of contaminants might be missed (medium/high risk)
- Musculoskeletal tissues are usually used as durable grafts and implanted directly into a well-vascularised bed in the recipient (medium/high risk)

Probable risk assessment: It is considered a medium- to high-risk procedure and for this reason the conclusion of the risk assessment is likely to be that musculoskeletal tissues should be procured in an operating theatre environment or equivalent [4].

Table 6.3. **Example of sclerocorneal button recovery procedure with the specified characteristics**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤ 1h	1-2h	2-3 h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 person	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

6.3.1.3. *Example of sclerocorneal button recovery procedure with the specified characteristics*

- Tissues are exposed to the environment for a short period of time (low/medium risk)
- The procedure is performed by one recovery member (low risk)
- Before procurement, ocular surface is cleaned with a validated combination of iodine and antibiotic solution to reduce surface microbiological contamination (low/medium risk)
- No reduction of bioburden is applied during corneal processing/evaluation (high risk)
- After procurement, corneas are preserved into a culture medium allowing the evaluation of possible contamination (low risk)
- Cornea grafts are used as superficial coverage (low risk)

Probable risk assessment: It is considered a low- or medium-risk procedure and for this reason the conclusion of the risk assessment is likely to be that it is not considered necessary to procure eyes or corneas by *in situ* excision in a location with controlled, defined air quality. However, steps must be taken to reduce the bioburden on the ocular surface before procurement, especially for corneas procured by *in situ* excision, and a local sterile field must be created around the eye. If the whole eye is procured, further steps must be taken in the eye bank to reduce bioburden before excision of the corneoscleral disc. Furthermore, for corneas stored by organ culture, microbiological testing of the organ culture medium during corneal storage is essential to further mitigate the risk of microbiological contamination.

6.3.1.4. *Example of bone-marrow recovery procedure with the specified characteristics*

- Tissues are exposed to the environment for a short period of time during aspiration procedure (low/medium risk)
- The procedure is performed by a low number of recovery members, usually two (low/medium risk)
- No bioburden reduction is performed during recovery (5 points) or processing (high risk)

Table 6.4. **Example of bone-marrow recovery procedure with the specified characteristics**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤ 1h	1-2h	2-3h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 persons	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

- During procurement, a biopsy of bone-marrow is obtained for microbiological control (medium risk)
- Bone-marrow graft is used in immuno-suppressed patients and it is injected directly into the blood stream (high risk)

Probable risk assessment: It is considered a medium- to high-risk procedure and for this reason the conclusion of the risk assessment is that procurement needs to be performed in an operating theatre or similar.

6.3.1.5. *Example of peripheral blood stem-cell recovery procedure with the specified characteristics*

- Peripheral blood stem cells are collected and processed in closed systems without exposure to environment (low risk)
- The procedure is performed by one person (low risk)
- Bioburden reduction during recovery is not needed because of the use of a closed system (low risk)
- No reduction of bioburden is performed (high risk)
- At the end of collection/before processing, and at the end of processing, a blood sample is obtained for microbiological control (medium risk)
- Peripheral blood stem cells are injected directly into the blood stream (high risk)

Probable risk assessment: It is considered a low- or medium-risk procedure, mainly because of the use of closed systems during procurement and processing. Therefore, particular environmental requirements may not be necessary and the peripheral blood stem cells may be collected in a blood-donor clinic.

Table 6.5. **Example of peripheral blood stem-cell recovery procedure with the specified characteristics**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤ 1h	1-2h	2-3h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 persons	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

6.3.1.6. *Example of female gametes recovery through transvaginal procedure with the specified characteristics*

- Female gametes are collected in an open system during a procurement procedure that mostly takes on average 20-30 minutes (low risk)
- The collection procedure is usually performed by one person (a physician), and there is assistance from a second person (a nurse or midwife) (low risk)
- After collection, the oocytes are processed in an open system; they are collected from the follicular fluid and put in a small dish containing washing media supplemented with antibiotics; after this step, oocytes are individually selected and put in culture droplets containing media supplemented with antibiotics; these droplets are covered with oil (low risk)
- During processing, no sampling for microbiological control is usually done; according to Table 6.6, this would result in a high risk, but retrospective validation of this consolidated approach for the collection and subsequent processing of oocytes shows that there is a very low risk of microbial contamination
- Female gametes are subsequently used for fertilisation and the resulting embryos are considered a low-risk application because they are clinically applied in the intra-uterine cavity (low risk)

Probable risk assessment: It is considered a low-risk procedure, the risk being mainly reduced by the use of substances for bioburden reduction during procurement and processing, as well as by its clinical application. However, considerations of the donor's situation during the collection procedure (under the effect of sedatives) may recommend that procurement is performed in an operating theatre (where particular environmental requirements may be necessary). Additional specific situations in MAR are described in Chapter 27.

Table 6.6. **Example of female gametes recovery through transvaginal procedure with the specified characteristics**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤ 1h	1-2h	2-3h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 persons	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

6.3.1.7. *Example of skin recovery procedure with the specified characteristics (glycerol preservation)*

- Tissues are exposed to the environment for a short period of time: less than 1 hour (low/medium risk)
- The procedure is performed by two recovery members and one circulating assistant (low/medium risk)
- After procurement, tissues are placed into a container with glycerol that is intended to reduce the microbiological contamination (medium risk)
- During processing, a change of preservation media to higher glycerol concentrations is performed, obtaining a substantial microbial reduction (low/medium risk)
- During procurement, a culture of transport media is performed as sampling method to detect microbiological contamination (low/medium risk)
- Glycerolised skin grafts are used as superficial coverage into the recipient (low risk)

Probable risk assessment: It is considered a low- or medium-risk procedure and for this reason the conclusion of the risk assessment is likely to be that glycerolised skin grafts could be procured in a non-dedicated area with local cleaning, although procurement in more controlled areas will decrease the risk of contamination during procurement. Differences can apply if other preservation methods, like cryopreservation, are used and then a new risk assessment should be done.

6.3.2. Equipment and materials

Materials (i.e. consumables and reagents) and equipment (i.e. surgical instruments, packaging and containers) used during procurement must be managed in accordance with standards and specifications and with due regard for relevant national and international regulations, standards and guidelines for the intended use of the donated tissues and cells (see Chapter 2). Validated sterile instruments, CE (Conformité Européenne)-marked devices (where available) and sterile single-use materials (e.g. drapes,

Table 6.7. **Example of skin recovery procedure with the specified characteristics (glycerol preservation)**

Factor	Low		Risk		High
Duration of exposure of procured tissues/cells during procurement [2]	no exposure (closed system)	≤ 1h	1-2h	2-3h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment [3]	1 persons	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

gloves, fluids) must be used for tissue and cell procurement. Instruments or devices must be of good quality, validated or certified specifically (e.g. surgical grade) for procurement, and must be maintained in good working order. This must include visual inspection and scheduled calibration of devices, where appropriate, against relevant defined standards at specified intervals. Routine maintenance inspections (validation procedures), at least annually, of equipment used for procurement are encouraged and a re-validation assessment is required whenever repairs or modifications have occurred. Procurement personnel must receive appropriate training, supported by records, on the proper use of equipment.

Where possible, use of single-use instruments for procurement is recommended. When re-usable instruments are used, a validated cleaning, disinfection, packaging and sterilisation process for removal of infectious agents must be used and each event documented. A system must be in place that allows traceability and tracking of critical equipment and materials to each tissue- or cell-procurement event and to the donor.

In EU member states, critical reagents and materials must meet documented requirements and specifications, and when applicable, the requirements of Directive 93/42/EEC concerning medical devices and Directive 98/79/EC on *in vitro* diagnostic medical devices.

Personnel conducting procurement activities must be provided with protective clothing appropriate for the type of procurement. Usually, this will extend to being scrubbed as for surgery and involve wearing a sterile gown, sterile gloves, glasses and a face shield or protective mask. Approved materials necessary for reconstruction of a deceased donor's body must be provided to allow this step to be completed effectively.

6.3.2.1. *Identification, packaging, containers and labelling*

At the time of procurement, tissues and cells must be uniquely identified. They must be packaged so as to minimise the risk of environmental contamination. Labelling must be witnessed and the labels must be appropriate to ensure identification and traceability of tissues and cells. Labels must be resistant to storage conditions to avoid the loss of identification of tissues and cells.

Guidance on coding, packaging and labelling is provided in Chapter 14.

6.4. Procedures

Written SOPs for procurement must be in place, based on the requirements of the relevant Health Authority, the recommendations laid out in this Guide and the expectations of the tissue establishment or end-user needs. These SOPs must outline the correct steps to be taken for each stage of procurement. Procedures that ensure contamination control must be applied, including use of aseptic techniques, sterile materials and equipment and appropriate clothing for the personnel conducting the procurement (see §6.2 and §6.3.2). Review of procurement SOPs by an authorised person must be undertaken at least annually (or as required), and updates may be necessary owing to clinical, scientific or technical progress. Procedures must be authorised and appropriate for the type of donor and the type of tissue or cells procured, and must be standardised [5].

The SOPs must be readily accessible so that procurement personnel can follow the required steps, including:

a. verification of the donor's identity and what constitutes evidence of donor (or the donor family's) consent or authorisation (see Chapter 3);

b. assignment and appropriate use of a unique identifier/code (see Chapter 14);

c. knowledge of selection (risk) criteria required for donor assessment, including physical examination of the donor (see Chapter 4);

d. knowledge of the types of blood and other samples required for mandatory laboratory tests to ensure that they are of appropriate quality (see Chapter 5);

e. steps that minimise the risk of microbiological contamination during procurement (see this chapter, as well as Chapter 2, Chapter 10 and Chapter 17 and following);

f. procurement steps that protect the properties of the tissue and cells required for clinical use (see this chapter and Chapter 17 and following);

g. for deceased donation, how to reconstruct the donor's body so it is as similar as possible to its original anatomical appearance;

h. considerations for packaging, labelling and transportation of procured tissues or cells to the tissue establishment or, in the case of direct distribution, to the clinical team responsible for their human application or direct use (see this chapter, Chapter 12 and Chapter 14);

i. considerations for collecting, packaging, labelling and transporting samples of donor blood

or other samples to the laboratory for testing (see this chapter, Chapter 5 and Chapter 14);

j. procedures that protect the health and safety of the living donor (see Chapter 18 and Chapter 24 and following).

In addition, the tissue establishment is expected to have procedures in place to notify, without delay (see Chapter 16), other tissue establishments or the relevant Health Authority of all available information about:

a. knowledge of deviations from approved procedures that have occurred or that are suspected to have occurred; and/or

b. any serious adverse reaction in a living donor that may influence the quality and safety of the tissues or cells procured.

To minimise the risk of tissue or cell contamination by procurement personnel who may be infected with a transmissible disease, policies and procedures must be established and followed to address this risk.

Additional procedures and policies that minimise the risk of microbiological contamination during procurement must be considered (see also Chapters 17-32, including those listed here):

a. the maximum number of personnel permitted to be present during procurement must be defined and respected;

b. preparation of the donor's skin must follow the recommended standards of practice used for surgical patients and must occur at the beginning of procurement using an appropriate antimicrobial agent designed for this purpose;

c. the procedure for skin disinfection should account for the elimination of bacterial spores as well as vegetative micro-organisms and it should therefore include suitable disinfectants, their concentrations and durations of exposure;

d. before use, all materials and equipment must be visually inspected by procurement personnel to ensure that they meet specifications (e.g. sterile, seals not broken, equipment functioning as expected);

e. for deceased donation, it is advisable to procure tissue before the autopsy takes place but, if this is not possible, detailed procedures must be written to address the increased potential for contamination when procurement takes place after autopsy.

Procurement must include procedures that protect those properties of tissues and cells required for their ultimate clinical use. These are described more fully in Part B of this Guide (the tissue-specific chapters), but generally include:

a. *post mortem* procurement time limits – it is recommended that tissue should be procured within 24 h after death if the body has been cooled or refrigerated (with the aim of reducing microbiological growth) in the first 6 h after death, or within 12 h of death if the body has not been refrigerated; alternative time limits for procurement should be validated by quality assessments and tests for microbiological contamination; it may be possible to extend procurement times up to 48 h after death if processing has been validated to guarantee quality and microbiological safety, in which case the blood samples for serological testing should still be taken within 24 h after death to minimise the risk of haemolysis (see Chapter 5 for details on sample collection);

b. preservation of important anatomical structures and other tissue or cell characteristics;

c. temperature requirements during storage and transport to the next destination;

d. avoidance of delays in transport due to time limits in place for processing after procurement.

Instead of specialised procurement teams recovering different tissues from a deceased donor, a multi-tissue procurement team consists of a group of individuals who are trained to procure all tissues for which there is consent. The roles of the individual multi-tissue team members must be defined by SOPs.

The main advantages of a multi-tissue procurement model are:

a. better co-ordination, because all tissues are procured by the same team;

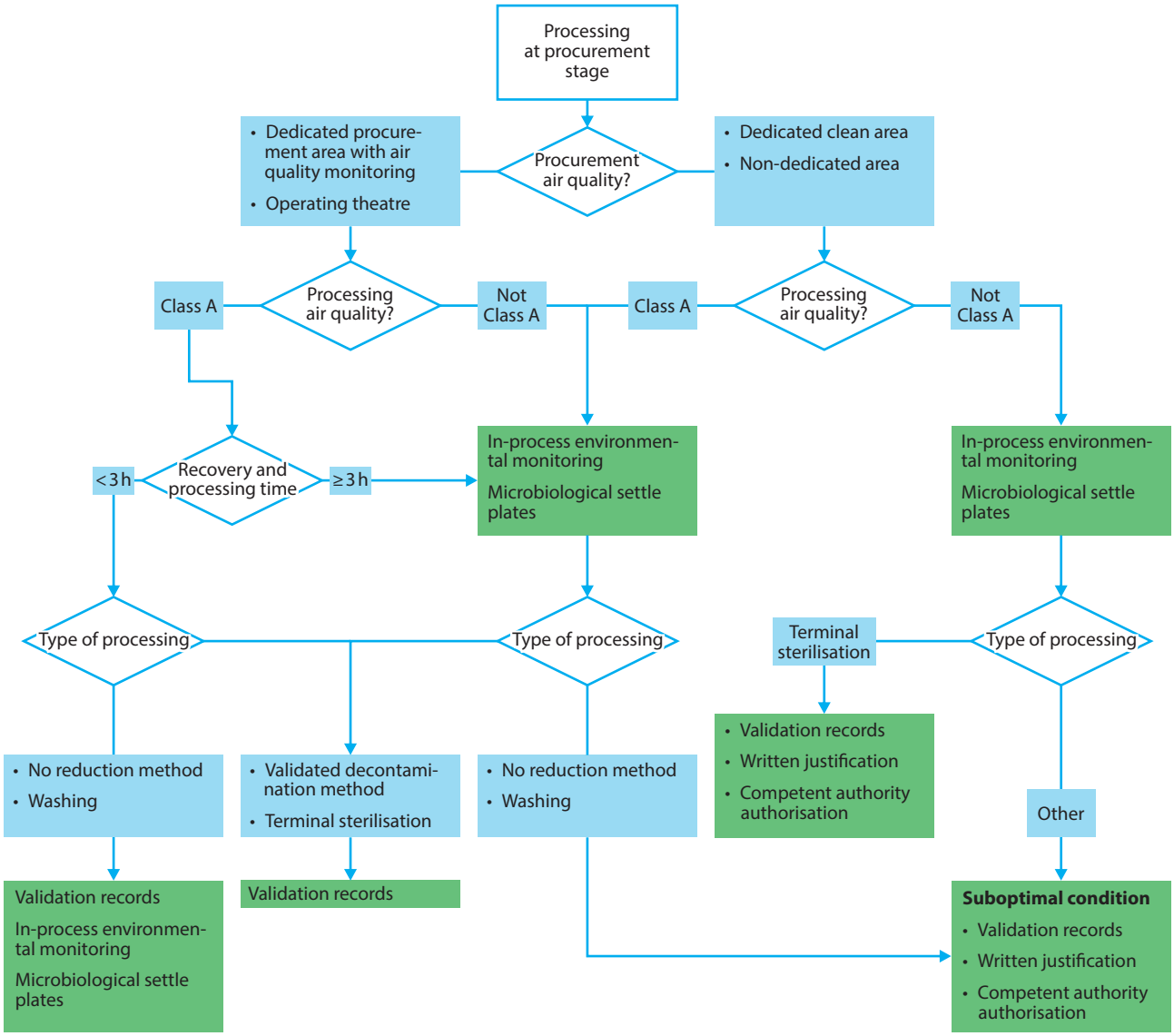
b. less time taken to procure all tissues, thereby decreasing the risk of microbial contamination because of long warm ischaemia times;

c. fewer equipment and consumables resources needed (e.g. same draping may be used to procure cardiovascular and musculoskeletal tissues).

In every deceased-donor procurement team, an appropriately trained senior person must take overall responsibility to ensure that SOPs are adhered to and that the following tasks are carried out to the required standards:

a. identification of the donor (Appendix 22);

Figure 6.1. **Environment quality: algorithm for processing at procurement stage**



b. review of donor documentation, including medical history, laboratory tests (if completed), lawful consent/authorisation;
c. physical examination of the donor (Appendix 15);
d. organisation and co-ordination of the procurement;
e. evaluation of abnormal procurement findings;
f. review of tissue packaging and labelling;
g. review of donor reconstruction;
h. completion of all required procurement documentation.

The sequence in which the various tissues are procured must be well defined to assure the quality of each type of tissue. The recommended procurement sequence, whether carried out by separate teams or by a multi-tissue team, is: skin, eyes/corneas, cardiovascular and musculoskeletal. Justification for this recommended procurement flow includes the following reasons:

- Skin is the first procured tissue because the donor is placed in a prone position to obtain skin from back and lower limbs, and the support provided by the presence of musculoskeletal tissues (in particular, bones) facilitates the procedure.
- Eyes are recommended to be procured after skin to avoid eye bleeding from the sockets if the donor has to be placed in a prone position following enucleation of the eyes.
- Cardiovascular and musculoskeletal tissues are recommended to be procured last because the same donor draping may be used. Some cardiovascular tissues (e.g. femoral arteries) may be procured simultaneously with the musculoskeletal tissues.

Where a tissue donor has already donated organs, all surgical approaches to obtain the organs must have been sutured to maintain as far as possible the sterility of thoracic and abdominal tissues before their procurement. If the procurement is performed simultaneously with organ procurement, the sequence varies: starting with the tissues from the cavities open for organ recovery, thorax and abdomen (arteries, heart for heart valves or vertebral bodies), then the recommended sequence of skin, eyes, cardiovascular and musculoskeletal should be followed. It is important that all the procurement teams involved know that tissues will be procured after organs, first to prepare the body before starting surgery, and second to guarantee sterile conditions during the whole procedure and to minimise the risk of cross-contamination.

Efforts should be made to ensure that procurement procedures do not unnecessarily interfere with funeral arrangements or other formalities such as religious or cultural rituals. If this is not possible, the donor's family must be informed at the time of consent. Timely and effective communication with all parties involved can help to meet expectations in regard to delays, as well as aesthetic considerations when tissues are procured from areas of the body that may be visible (e.g. if the body is to be viewed subsequently by the family and those attending the funeral).

6.4.1. Processing at the procurement stage

Microbiological safety during the procurement of tissues or cells must always be considered; but control of contamination and cross-contamination at the procurement site is typically less stringent than the controls applied in a tissue establishment (see Chapter 2, Chapter 10 and Chapters 17-32). Therefore, simultaneous undertaking of processing steps during the procurement phase, or in the procurement area, is not recommended and is avoidable. However, if processing, including shaping, cleaning, sizing and final packaging (for direct distribution) at the procurement site is unavoidable, its duration and extent should be limited to the minimum necessary, and a Grade A air-quality environment (surrounded by, at least, Grade D air quality) for the processing steps is desirable (e.g. a laminar flow cabinet located in the operating room). Records supporting the validation of the processing site must be available for inspection. If this level of control is not possible, an in-process (active) environmental-monitoring method must be used: preferably, active air monitoring using a viable particle counter and culturing method or, as a minimum control, using microbiological settle plates. Sample cultures of the tissues or cells procured should also be taken (see Chapter 10) and an appropriately validated culture method must be used (see Chapter 2). Ultimately, the procurement environment, if it is also used as a processing environment, must be specified and must achieve the quality and safety required for:

a. the types of tissues and cells procured;
b. the types of processing steps and tissues or cells that will be used (e.g. none; exposure only to antibiotics; a validated inactivation method; or, a validated sterilisation method);
c. the types of clinical application (as well as consideration of the immune status of the recipient, if applicable).

Selection of the use of suboptimal conditions must be supported by written justification and be authorised by the relevant Health Authority.

From the premise that processing at a procurement site is not considered Good Tissue Practice and all efforts should be directed towards avoiding this practice, Figure 6.1 shows recommendations for best practice when it is unavoidable. Four main factors are taken into account:

a. Procurement air quality: the environment where procurement is done. Two situations are possible:
  i. a dedicated area with air-quality monitoring, or an operating theatre,
  ii. a dedicated clean area, or a non-dedicated area.
b. Processing air quality: the environment where the processing step is done, following procurement. Two situations are considered:
  i. Class A (i.e. laminar flow cabinet placed in the procurement area),
  ii. non-Class A.
c. Recovering and processing time: duration of the whole process (including procurement and processing) where tissues are exposed to the procurement and processing environments. Times over 3 hours are considered as critical.
d. Type of processing: type of process to which tissues are subjected. Various procedures are considered:
  i. validated decontamination method,
  ii. terminal sterilisation,
  iii. other procedures, such as non-validated decontamination methods, washing …

Some considerations obtained from the algorithm (Figure 6.1) are:

a. When procurement is done in a dedicated clean area (without air-quality control) or a non-dedicated area, it is recommended to establish in-process environmental monitoring as well as using microbiological settle plates during the whole process.

b. When processing is not done in a Class A environment, or when the duration of the procurement and processing procedures is longer than 3 hours, it is recommended to establish in-process environmental monitoring as well as using microbiological settle plates during whole process.

c. When a validated decontamination method or terminal sterilisation is used, validation records are required, independently of the conditions of procurement and processing steps.

d. The following situations are considered to be suboptimal conditions, where validation records, written justification and authorisation from competent authority are required:

i. Procurement in a dedicated clean area (without air-quality control) or a non-dedicated area, with Processing not Class A;

ii. Procurement in a dedicated clean area (without air-quality control) or non-dedicated area, with Processing in Class A but non-validated decontamination method or no reduction used.

6.4.2. Temporary storage and transportation to the tissue establishment

Once the tissue is procured and until it arrives at the tissue establishment, critical variables related to maintaining the quality of the tissues or cells (e.g. temperature, sterile packaging) must be controlled (see Chapter 14). Records to demonstrate compliance with specified storage conditions must be completed and maintained.

6.5. Documentation

Procurement is a critical activity. Therefore tissue establishments must have procedures in place that address the retention of procurement records, which must include descriptive documentation of the steps taken, the materials and equipment used, and identification of the personnel involved. Such records must be clear and legible, protected from unauthorised amendments, retained and readily retrievable throughout a specified retention period, and must comply with data-protection legislation. Procurement records must be sufficiently detailed to facilitate robust and reliable traceability, to provide a complete history of the work undertaken and to be capable of linking the records to the particular donor of the procured tissues and cells (see Chapter 15). When tissues and cells are to be sent across national borders, potential language barriers should be addressed and a common language agreed for all documentation related to donors, tissues and cells.

A unique identifier (e.g. a donation number for a donation event and/or a donor identification number) must be allocated to the donor as well as the procured tissues and cells (see Chapter 14). This coding must be in place to ensure an effective and accurate system capable of tracking tissues throughout all stages, including an identifiable link to the procurement steps. For each donor, there must be a record containing the donor's identity (i.e. given name, family name, date of birth, sex). If a mother and child (both living) are involved in the donation, records must indicate not only the name and date of birth of the mother, but also the name (if determined) and date of birth of the child. These coded data should be entered in a registry maintained for this purpose.

In summary, before the procurement of tissues and cells may proceed, an authorised person (e.g. the team leader in a procurement team) must confirm and record the following as part of the procurement record:

a. donor identification;

b. that consent for the procurement has been obtained in accordance with local laws;

c. how and by whom the donor has been reliably identified.

To ensure that all steps are traceable and verifiable, the tissue establishment (or procurement organisation) must produce a report, recorded at the time of procurement, which must be forwarded without delay to the location where processing takes place. Care should be taken to maintain donor confidentiality if the procurement report is forwarded to the recipient's clinical team. This procurement report, depending on the type of donor, could contain the following:

a. donor identification data (given name, family name, date of birth and sex, as well as how and by whom the donor was identified, or donor identification in the case of an unrelated haematopoietic stem-cell (HPC) donor);

b. unique coding number, which will be either the donation identification sequence of the Single European Code for EU countries, or a code generated by a Health Authority or by use of an internationally recognised coding system such as ISBT 128 or Eurocode (Chapter 14);

c. the environmental conditions of the procurement facility, i.e. location or description of the physical area where procurement took place (see Appendix 21);
d. a list of observations made during the physical examination of the donor's body – but, for a living donor, only when such an examination is justified (see Appendix 15);
e. a description and identification of procured tissues and cells, including samples for testing of infectious diseases;
f. the identification of the person who has overall responsibility for the procurement session (including his/her signature);
g. date, time (where relevant, start and end times) and location of procurement;
h. the type, volume, manufacturer and lot/batch/serial number of reagents, additives and the tissue and cell transport solution(s) used;
i. name and address of the tissue establishment;
j. name and destination of the tissues and cells.

In addition, for procurement of tissues or cells from a deceased donor, this report must contain:
a. a sufficiently detailed summary of the events surrounding death;
b. the date and time of donor death and tissue procurement (and, where relevant, start and end times) to facilitate determination of the time interval from death to procurement;
c. the conditions under which the donor body was kept before procurement (whether or not the donor body was cooled or refrigerated and, where appropriate, the time when cooling or refrigeration began and ceased);
d. if possible, whether procurement took place before or after autopsy and whether or not an autopsy is planned;
e. when applicable, a description of other tissues and cells from the same donor sent to different tissue establishments, including their identification;
f. if applicable, information regarding reconstruction of the donor's body.

If procurement from a living donor involves a directed donation, the recipient's identification must be documented to avoid confusion.

6.6. References

1. Shiroff A, Gale S, Merlin M *et al.* Enhancing the tissue donor pool through donation after death in the field. *Prehospital and Disaster Medicine* 2013;28(2):187-90.
2. Bohatyrewicz A, Bohatyrewicz R, Klek A *et al.* Factors determining the contamination of bone tissue procured from cadaveric and multiorgan donors. *Transplant Proc* 2006;38:301-4.
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4. Mathur M, De A, Gore M. Microbiological assessment of cadaver skin grafts received in a skin bank. *Burns* 2008;35:104–6.
5. Fehily D, Brubaker SA, Kearney JN *et al. Tissue and cell processing: an essential guide.* Chichester: Wiley-Blackwell, 2012.

Related material
- Appendix 15. Physical assessment form (Dutch Transplant Foundation)
- Appendix 21. Sample form to assess working environment (NHS, UK)
- Appendix 22. Sample donor-identification form (NHS, UK)

Chapter 7: **Premises**

7.1. Introduction

In general, tissue establishments must have suitable facilities to carry out the activities for which accreditation/designation/authorisation or licensing is sought. This chapter provides generic guidance on the facilities used for processing, testing and storing of tissues and cells. Processing of tissue and cells, while exposed to the environment, must take place in an environment with specified air quality and cleanliness in order to minimise the risk of contamination, including cross-contamination between cells and tissues of different donors. This chapter gives guidance on creating, implementing and maintaining a validation master plan (classification and qualification) and monitoring plan in order to gain assurance that the cleanrooms are performing adequately and that the aseptic processing of tissue and cells is monitored (see also Chapter 2). Tissue- and cell-specific guidance on selecting the appropriate air quality for processing is given in Part B of this guide.

7.2. Requirements of storage facilities

Tissue establishments should have specific storage facilities/areas for the storage of tissues and cells. Such storage areas should be:

a. designated;

b. located in a secure area, and access must be limited to authorised personnel;

c. of sufficient capacity to allow orderly storage of the various categories of tissues/cells:

i. in quarantine;
ii. released for processing;
iii. rejected;
iv. returned;
v. recalled;
vi. for research use;

d. covered by an adequate management system, ensuring clear segregation of each category of tissues and cells. Physical and automated tissue-storage-management systems are both accepted as long as the risks of mix-up between categories and cross-contamination of tissues and cells of different donors are excluded. If any automated management system is used to manage the location of the tissues and cells, documented evidence should be provided to demonstrate the capability of the system to assure safe storage (see Chapter 13);

e. clean and dry, and maintained within an acceptable temperature range. Where special storage conditions are required (e.g. specific temperature and/or humidity) these should be specified, maintained and monitored. The necessary air-conditioning capacity for the storage area must be calculated, based on the actual heat load of the equipment and the environmental factors (see Chapter 9).

In addition, printed packaging and labelling materials may be considered critical and special attention should be paid to their safe and secure storage.

The design of a cryostorage room (e.g. storage rooms equipped with liquid nitrogen tanks or equip-

ment using liquid nitrogen) must comply with applicable regulations and safety requirements of the relevant country. Items related to safety should include at least:

a. good ventilation;
b. oxygen-level monitoring with a local audio and visual alarm;
c. visual surveillance from outside;
d. adequate space to contain the necessary freezers and tanks, including back-up systems;
e. easy access to all the storage devices, with a smooth pathway to and from the facility for liquid nitrogen supply and for prompt removal and transfer of tissues and cells in case of emergency;
f. personal protective equipment available for use, which may include items such as cryo-gloves, safety goggles, cryo-aprons and respirators;
g. a specific SOP already in place to support the safety issues.

A system to monitor all the alarms, including oxygen level in the room and the level of liquid nitrogen in the tanks, is essential. When applicable, monitoring of the temperature is recommended. Personnel must be trained to react to different alarms. Personnel need to be trained to use personal protective equipment.

7.3. Requirements of processing facilities

Processing facilities must be designed, classified, qualified and monitored to ensure that the air quality is appropriate for the processes carried out. International standards, such as the EU Guidelines to good manufacturing practices for medicinal products for human and veterinary use (known as GMP) [1] and ISO 14644 guidelines for Cleanrooms and associated controlled environments [2], provide information to help achieve the appropriate air quality.

Processing of tissues and cells should be carried out in cleanrooms. In these cleanrooms, the concentration of airborne particles (viable and non-viable) must be controlled to specified levels. Each processing operation requires an appropriate level of environmental cleanliness in the operational state to minimise the risks of particulate or microbial contamination. According to GMP, four grades can be distinguished.

- Grade A: The local zone for high-risk operations provided by localised airflow protection, such as laminar airflow workstations, isolators or restricted access barrier systems (RABS). Unidirectional airflow systems should provide a homogeneous air speed in the range 0.36-0.54 m/s (guidance value) across the whole of the Grade A area (GMP, Annex 1). Maintenance of the unidirectional airflow should be demonstrated and qualified.
- Grade B: For aseptic operations, this is the background environment for the Grade A zone. Lower grades can be considered as defined in the tissues- and cells-specific chapters of this guide (Part B). The risk-assessment tool for defining the air quality can be used to select the background environment for the Grade A zone (see §7.4).
- Grades C and D: Clean areas for carrying out less critical stages in the processing and storage of tissues and cells. These cleanliness grades can be considered where isolator technology is used.

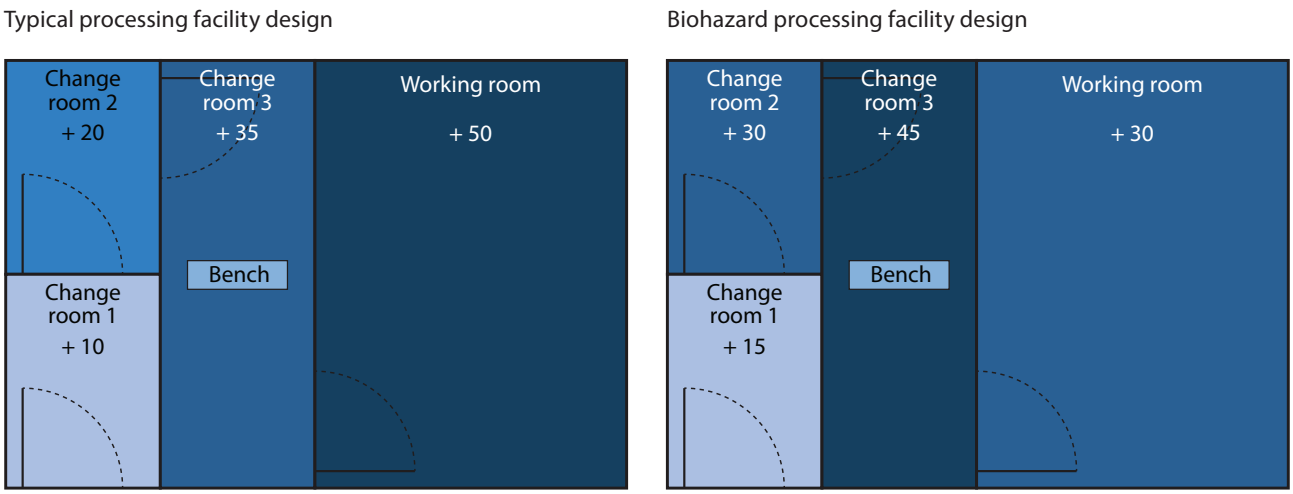
Different cleanroom standards are compared in Table 7.1 [1, 2]. Whichever classification is applied, facilities should have:

Table 7.1. **Air cleanliness classifications in Europe**

Classification		Maximal number of particles/m^3					
ISO 14644-1	EU GMP	ISO 14644-1		EU GMP			
				at rest		in operation	
		≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
ISO 5	A	3 520	29	3 520	20	3 520	20
	B			3 520	29	352 000	2 900
ISO 6		35 200	293				
ISO 7	C	352 000	2 930	352 000	2 900	3 520 000	29 000
ISO 8	D	3 520 000	29 300	3 520 000	29 000	not defined	not defined

Source: [1, 2].

Figure 7.1. **Schematic plans indicating air pressure differentials between adjacent cleanrooms**



a. floors, walls and ceilings of a non-porous material with smooth surfaces to minimise the shedding or accumulation of viable and non-viable particles and to permit the repeated application of cleaning agents and disinfectants;

b. temperature control and (based on risk assessment) humidity control;

c. a filtered air supply that maintains a pressure differential and airflow to adjacent cleanrooms of different cleanliness levels to prevent reversal of airflow direction between the segregated cleanrooms. A combination of negative and positive pressure can also be used to achieve specific biosafety requirements;

d. a documented system for manual or continuous monitoring of temperature, humidity, air-supply conditions, pressure differentials, and viable and non-viable particle numbers (for environmental monitoring, see below at §7.5.2);

e. a documented system for cleaning and disinfecting cleanrooms and equipment;

f. a documented system for gowning and laundry;

g. adequate space for personnel to carry out their operations;

h. adequate space for storage of sterile garments (if applicable);

i. access limited to authorised personnel.

Characteristics such as temperature and relative humidity are dependent on several factors (air changes in the room, number of personnel, heat load of the equipment, processing methods and external influences such as weather changes). Parameter settings should not interfere with the defined cleanliness levels. The environmental temperature and relative humidity should be set to guarantee the safety and quality of the tissue and cells, staff comfort, electrostatic charging and discharge. Energy consumption can also be taken into account. For relative humidity, the generally accepted guidance range is 30 % to 65 % (ISO 14644-4) [3].

To minimise the risk of contamination, a positive pressure should be created relative to adjacent cleanrooms of a lower grade. The pressure differential between adjacent cleanrooms of different grades should be 10-15 Pa (guidance values in GMP, Annex 1) with the maximal air pressure in the background environment for the Grade A zone (the working room in Figure 7.1) [1]. This forms a 'pressure cascade' to prevent reversal of airflow direction between the segregated cleanrooms and limits the entry of contamination into the cleanrooms of a higher cleanliness level.

Stringent biosafety requirements should be followed if processing tissues or cells from patients having known viral infections, e.g. hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV). The required precautionary activities or the need for a special contained laboratory (a processing room having reduced air pressure relative to the adjacent rooms) should be determined by documented risk analyses. Risk analyses should consider risks relating to cross-contamination of other tissues and cells processed at the tissue establishment. In addition, risk analyses should consider personnel safety according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work [4].

However, when working with viruses (see Chapter 30) the cleanroom installation must also

protect the environment, and therefore the processing room should have reduced air pressure relative to adjacent rooms (contained laboratories at biosafety level 2 and biosafety level 3). A possible solution is to increase the air pressure in the change rooms to result in the working room having reduced air pressure with respect to the last change room (see Figure 7.1). Another specific safety consideration when working with viruses for genetic cell modifications is the protection of the worker. For this reason the use of biosafety cabinets is mandatory. In many cases Class II biosafety cabinets are sufficient but, depending on the virus present, an isolator cabinet is recommended.

Residue manipulation is another issue when working with viruses. To protect the environment all material must be autoclaved before leaving P2/P3 (biosafety level 2/3) contained laboratories. The use of autoclaves inside these laboratories, or in between contained laboratories and the next room, is common. However, validating autoclaves in processing facilities is complicated by the use of water. One possible solution is to place all residues in hermetic containers and autoclave them in another room of the facility. If this is the case, these procedures should be validated.

Figure 7.1 shows schematic plans indicating the air-pressure differentials between adjacent cleanrooms of a processing facility for conventional or biohazard use. Conventional processing facilities are designed to protect the sample from any contamination and therefore there is an air-pressure increase in subsequent rooms of 10-15 Pa, with the maximum air pressure in the processing room. However, biohazard-processing facilities must be designed to protect both the tissue and cells and the environment. Therefore the installation must increase the air pressure in the change rooms, resulting in maximum air pressure in one of the change rooms, and the air pressure of the working room being less than in this change room.

7.4. Selecting the appropriate air quality for processing

According to GMP, aseptic processing must be done in a Grade A zone within a Grade B background environment. For tissue and cell establishments in the EU, there must be the equivalent of Grade A with a surrounding environment of at least Grade D (GMP classification). A less stringent processing environment may be acceptable if one of the following applies:

a. a validated microbial inactivation or validated terminal sterilisation process is applied; or,

b. if it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissues or cells concerned; or,

c. if it is demonstrated that the mode and route of application of the tissues or cells to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with transplantation of tissues and cells; or,

d. if it is not technically possible to carry out the required process in a Grade A environment – e.g. due to the requirements for specific equipment in the processing area that is not fully compatible with Grade A (Directive 2006/86/EC) [5].

Many national requirements are more stringent, requiring Grade A with a surrounding environment B or C for certain processes.

Table 7.2. **Risks of contamination that should be considered when determining air-quality specifications of processing facilities**

Risk	Explanation
Tissue or cell contamination during open *versus* closed processing.	Processes that are functionally 'closed' need a less stringent processing environment than processes where tissue and cells are exposed to the environment.
Effectiveness of the processing method to remove contaminants.	Some tissues, even though not terminally sterilised, can be treated with various antimicrobial agents; this reduces the risks of transferring any contaminants.
Suboptimal detection of contaminants due to the sampling method.	If the only option for final microbiological sampling is swabbing or testing of unrepresentative samples, the risk that contaminants will be undetected is higher than in processes where 5-10 % destructive testing of final tissue and cell grafts can be performed [6].
Transfer of contaminants at transplantation.	Tissues that are minimally processed, cellularised, or contain blood, blood vessels and lipids are more likely to support microbial contaminants than those that are blood- and cell-depleted. Method of application (i.e. permanent *versus* temporary) and site of transplantation both affect the risk of transfer of contaminants.

Source: Euro GTP Hot Topics guidance [16]

Table 7.3. **Qualification tests for cleanrooms, clean zones and laminar flow hoods**

Tests	Specification	Recommended time interval
Airborne particle count (classification test)	The total count of airborne particles (viable and non-viable) performed at rest and in operation, to determine cleanliness class	12 months
Airflow test	Average airflow velocity and air changes per hour	12 months
Air pressure difference	Differential pressure between different rooms	12 months
Installed filter system leakage test	Detection of leaks in the absolute filter and integrity testing of seals between filter and mounting arrangements	24 months or if the resistance across the filter changes abnormally
Temperature and relative humidity		12 months
Recovery test	The time required for a cleanroom to recover after a particle-generation event – normally tested for cleanrooms classified as Grade A or B. Maximum delay given by GMP Annex 1 is 15-20 min	24 months
Airflow direction test and visualisation	Airflow pattern type, i.e. unidirectional, non-unidirectional or mixed	24 months
Containment leak test	Detection of leaks on structure	24 months
Laminar airflow velocity (laminar flow hoods)	The average velocity must meet the specified acceptance criteria	12 months
Microbial contamination	The total count of viable particles performed in operation	12 months

As outlined in Table 7.2 the specification of the air quality of the processing environment should be decided on the basis of the particular types of tissue or cell and the processing method that is being applied. Based on a risk assessment, several factors (such as limitations of sampling methods, contamination during manipulation, use of antimicrobials, transfer of contaminants at transplantation) should be taken into consideration when determining the air-quality specifications, especially when less stringent conditions are applied. Where the risk of tissue or cell contamination during processing is high, and the chances of any contaminants being transferred to the recipient are high, a more stringent air-quality specification should be adopted. See also Chapter 8 and the tissue-specific recommendations provided in Part B of this guide.

The utilisation of isolator technology to minimise human interventions in processing areas may result in a significant decrease in the risk of microbiological contamination of aseptically manufactured tissue and cells. The air classification required for the background environment depends on the design of the isolator and its application. It should be controlled, and for aseptic processing it should be at least Grade D. Isolators should be used only after appropriate qualification. Qualification should take into account all critical factors of isolator technology, for example the quality of the air inside and outside (background) the isolator, sanitisation of the isolator, the transfer process and isolator integrity (checking for defective seals and pinhole leaks in the isolator gloves).

7.5. Qualification and monitoring

Processing environments and surrounding areas must be qualified and monitored in accordance with EN ISO 14644 [2, 3, 8, 9], EN ISO 14698 [10] and EU GMP Annex 1 [1]. The validation master plan should be built following a risk-assessment exercise aiming to define the extent and frequency of the qualification tests in a proportional manner to the risks identified. The validation plan should consider the initial and consequent qualification, as well as the at-rest and in-operation classification (see also Chapter 2). An example of a validation master plan is provided in Appendix 4 of this guide.

The qualification strategy should consider the monitoring strategy displayed over the process. Whenever a particular process is submitted to a continuous air-quality monitoring program, the frequency of qualification of cleanrooms, laminar airflow work stations, isolators and RABS might be adapted accordingly to a less stringent program.

7.5.1. Qualification

Qualification of cleanrooms and clean zones is required to support and verify the operating parameters and limits for the critical parameters. The specified acceptance criteria set should be verified, and therefore testing of certain parameters and specifica-

tions should be performed. The classification is part of the qualification of cleanrooms and clean zones and should be clearly differentiated from monitoring operational processes.

7.5.1.1. *Classification*

Classification is a method of assessing the level of air cleanliness against a specification for a cleanroom or clean-zone device by measuring the airborne particle concentration. For classification, the required tests and acceptance criteria should be defined in the approved classification protocol. Classification should be performed at rest and in the operational state.

For particle count, the minimum number of sampling locations related to the area of each cleanroom or clean zone to be classified is provided in EN ISO 14644-1. It divides the whole cleanroom or clean zone in sections of equal area and selects in each section a sampling location considered to be representative of the characteristics of the section. The position of the particle probe should be located at the same height and in the plane of the work activity.

The particle counter must have a valid calibration certificate. The frequency and method of calibration should comply with the requirement of ISO 21501-4 [11].

For classification, the airborne particles equal or greater than 0.5 µm should be measured. Classification in operation may be carried out during routine or simulated operations, with a specified number of personnel present.

The minimum air-sample volume per sampling location should be determined in accordance with EN ISO 14644-1 Annex A. Sequential sample techniques could be useful to classify a cleanroom or clean zone with a very low particle concentration at the class limit (EN ISO 14644-1 Annex D).

The cleanroom or clean zone has met the specified classification if the particle concentration measured at each of the sampling locations does not exceed the concentration limits as defined in the classification protocol.

Periodic classification testing should be performed annually in accordance with ISO 14644-1. This frequency can be extended, based on risk assessment, the extent of the monitoring system and data that are consistently in compliance with acceptance limits or levels defined in the monitoring plan.

7.5.1.2. *Other qualification tests*

Table 7.3 specifies optional test methods characterising the performance of cleanrooms and clean zones. The choice of tests should be based on factors such as the design of the installation, operational states and the required level of air cleanliness. The selected tests should be repeated as specified in Table 7.3 as a part of validation master plan. Deviations from the pre-set frequencies should be based on a formal risk assessment. All these tests should be undertaken by qualified professionals at least in an at-rest situation in accordance with EN ISO 14644-3 [9] which specifies ancillary tests related to other aspects of cleanroom performance such as pressure difference and airflow. The microbial load of the cleanroom should be determined in operational state as part of the cleanroom qualification. The recommended maximum limits for microbial contamination during qualification for each grade are given in Table 7.6.

Biohazard laminar-airflow hoods should also be certified to national or international performance standards at the time of installation and recertified annually.

7.5.2. Monitoring particle concentration

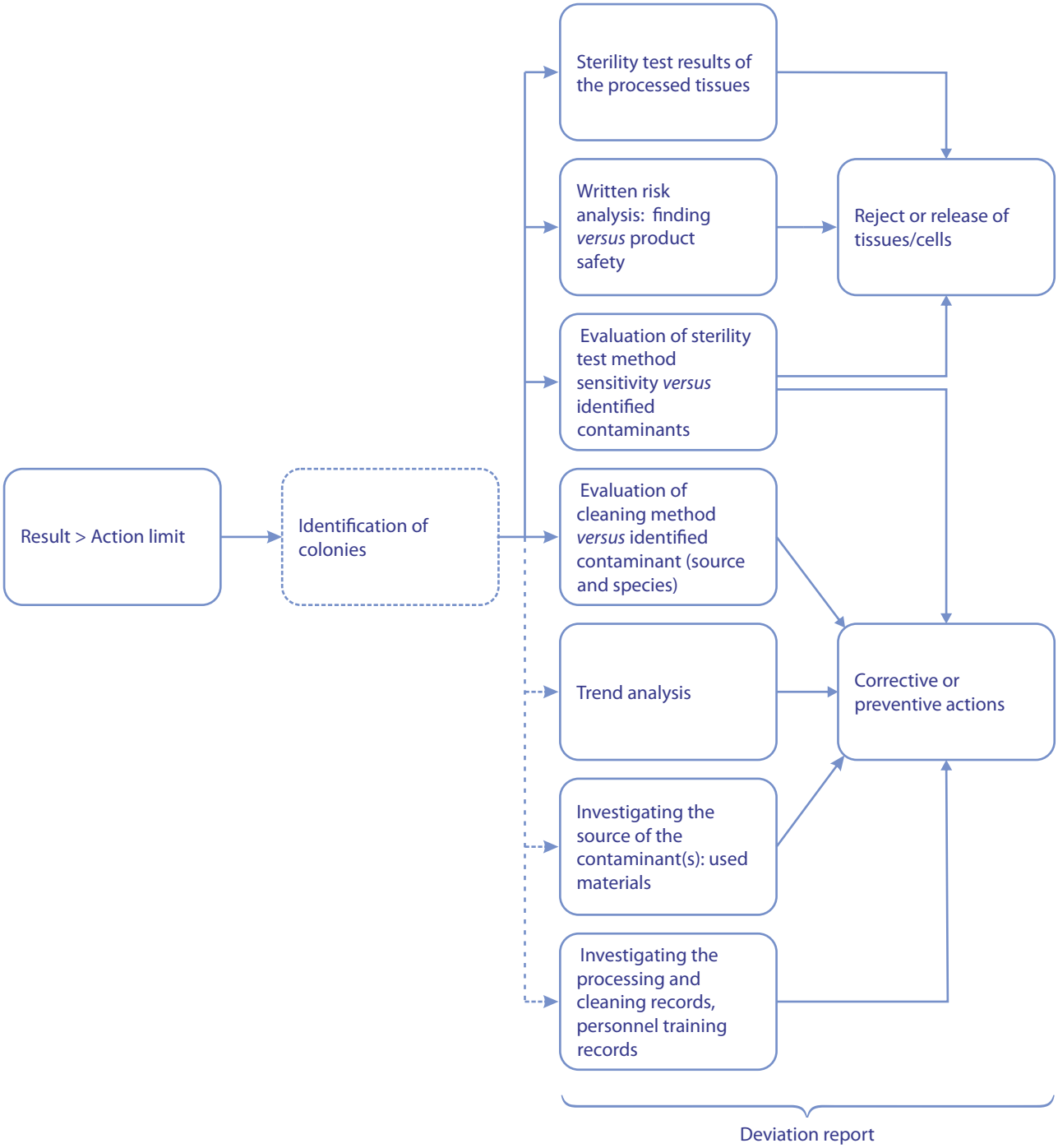
Monitoring particle concentration provides evidence of continuous compliance with the specified air-cleanliness class. ISO 14644-2 provides information for a monitoring plan for a cleanroom, related to air cleanliness by particle concentration.

Cleanrooms and clean zones should be monitored while in operation. Measuring locations should be determined on the basis of a formal risk analysis and the results obtained during qualification of cleanrooms. A monitoring plan, taking into consideration the level of air cleanliness specified, critical care points and performance attributes of the cleanroom, should be created and maintained.

Adequate alert and action limits should be set, based on the intent and purpose of monitoring, taking into account the nature of the process. For example, bone cutting may generate numerous particles, and corneal lamellar cutting may generate numerous aerosols. If the alert limits are exceeded, further investigation or increased observation are required. If the action limits are exceeded, appropriate corrective actions should be taken. Frequent and continuous high particle counts should raise concerns because they may indicate the possibility of pollution, problems with a heating, ventilating and air-conditioning (HVAC) system, or incorrect practices during routine operations. The performance of the monitoring systems and related trends should be periodically reviewed.

Monitoring may be continuous, sequential or periodic (indicating specified frequency).

Figure 7.2. **Decision tree: topics and actions to be considered if microbiological-monitoring results (number of colonies) exceed the action limit**



Note: In Grade A and B areas, detected colonies must be identified to the genus and species, and for other cleanliness according to the microbial-monitoring programme. Solid lines indicate minimal actions to be considered, and dashed lines indicate topics of broader investigations.

The system selected must be adequate for the monitoring operations required. If using sequential systems, particle losses because of the length of the tubes and kinks in the tubing should be considered. For airborne-particle counters, the frequency and method of calibration should be based upon current accepted practice as specified in ISO 21501-4 [11].

Selection of the particle monitoring system should also involve consideration of the risks generated by sampling during processing. The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of cleanrooms and clean-air devices.

The Grade A zone should be monitored with a frequency that allows detection of sporadic increases in particle counts which may exceed acceptable limits. It is recommended that a similar system be used for Grade B zones, though the sample frequency may

be decreased. The importance of the particle-monitoring system should be determined by the effectiveness of the segregation between adjacent Grade A and B zones.

The particle limits given in Table 7.4 for the at-rest state should be achieved after a short recovery time period of 15-20 min in an unmanned state after completion of operations.

Monitoring of Grade C and D areas should be performed in operation and in accordance with the principles of quality risk management.

Temperature, relative humidity and differential pressure of clean areas should be monitored every day.

7.5.3. Microbiological monitoring

Microbiological monitoring provides evidence of continuous compliance with the specified air-cleanliness class as well as evidence of contamination control of aseptic process operations and cleaning and sanitation methods. Microbiological monitoring is mandatory and should be done in accordance with:

a. EN ISO 14698 *Cleanrooms and associated controlled environments – Biocontamination control* [10]; or
b. EU GMP Annex 1 [1].

To define and control microbiological hazards it is necessary to identify the potential risks relating to each processing step and the potential risks of the tissues or cells themselves, as well as the probability of these risks and the mitigation actions intended to minimise the risks. Tissue establishments must have a monitoring plan that specifies:

a. acceptance limits of microbial contamination (action level, alert level);
b. sampling plan and frequency;
c. sampling methods and equipment (see Chapter 10);
d. sampling culture media and incubation of samples (see Chapter 10);
e. analyses and evaluation of results (including trend analyses);
f. handling of out-of-specification results.

Selection of the microbial monitoring method should involve consideration of the risks generated by sampling during processing. The sample sizes taken for monitoring purposes will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal qualification of cleanrooms and clean-air devices.

The frequency of sampling should take into account the processes and activities of the staff. Aseptic process operations performed in a Grade A or B environment should be monitored routinely. Background and surrounding areas could be monitored periodically.

Results of monitoring should be considered when making the decision whether tissues or cells can be released (Figure 7.2).

Recommended limits for microbiological monitoring of clean areas during operation are shown in Table 7.6. Alert and action levels for microbial contamination should be determined and the actions to be taken in the event that these levels are exceeded should be documented. The level should be specific to the area, determined on the basis of historical data and based on, for example, data from a single year. Levels should be reviewed periodically and, if necessary, updated if there are changes to processes.

The alert level emphasises an acceptable number for microbial contamination, but acts as a warning. Exceeding the alert level does not require corrective measures, but should trigger an investigation aimed at early detection of errors or deviations. The alert level is set at a lower level than the action limit. The action level emphasises a certain level of microbial contamination that necessitates immediate corrective action and corrective measures.

In Grade A and B areas, detected colonies must be identified by the genus and species, and for other cleanliness grades according to the microbial monitoring programme of the tissue establishment. Figure 7.2 describes topics and actions to be considered if microbiological-monitoring results exceed the action limit. Any presence of fungi or yeasts must be considered to denote deviation and should be identified.

After such results are obtained, tissue establishments should evaluate whether the finding will affect the risk that tissues or cells could have been contaminated during processing. Tissue establishments should also evaluate whether corrective or preventive actions should be initiated. All investigations that are carried out should be reported in a deviation report.

7.6. Avoiding contamination and cross-contamination

Entry of personnel and materials to the processing facilities, transit and exit of personnel and material through the processing area and the rules of use and clothing to be worn in them should be established to:

Table 7.4. **Recommended limits for airborne particle concentration during monitoring**

Monitoring		Recommended maximal number of particles/m^3					
ISO 14644-1	EU GMP	ISO 14644-1		EU GMP			
				at rest		in operation	
		≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	> 5.0 µm	≥ 0.5 µm	> 0.5 µm
ISO 5	A	3 520		3 520	20	3 520	20
	B			3 520	29	352 000	2 900
ISO 6		35 200	293				
ISO 7	C	352 000	2 930	352 000	2 900	3 520 000	29 000
ISO 8	D	3 520 000	29 300	3 520 000	29 000	Set a limit based on the risk assessment	Set a limit based on the risk assessment

a. minimise the risk of contamination of tissues and cells;
b. reduce the environmental bioburden;
c. protect staff from biohazards.

A written procedure designed to avoid potential contamination and/or cross-contamination from personnel and materials to tissues and cells should be in place.

Entry of personnel, tissues and cells and materials should be done through airlocks by following specified procedures to avoid the direct flow of non-treated air into cleanrooms. Both airlock doors should not be open simultaneously. An interlocking system or a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.

Only the minimum number of personnel required for efficient processing should enter the processing areas. The need for additional persons to be present in processing areas should be taken into account during risk assessment when the procedure is being designed.

High standards of personal hygiene and cleanliness are essential. Changing and washing must follow a written procedure designed to minimise contamination of clean area clothing or transfer of contaminants to the cleanrooms. Wrist-watches, make-up and jewellery must not be worn in clean areas. Outdoor clothing must not be brought into changing rooms that lead to Grade B and C rooms.

Table 7.5. **Minimum clothing requirements (adapted from EU GMP Annex 1)**

Classification	Clothing	Description
Grade D	Facemask	Depending on the process, at least beards and moustaches should be covered
	Cap	Hair should be covered
	Suit	A general protective suit
	Shoes	disinfected or sterilized shoes or overshoes
	Gloves	Dependent upon the process
Grade C*	Facemask	Depending on the process, at least beards and moustaches should be covered
	Cap	Hair should be covered
	Suit	A single or two-piece trouser suit gathered at the wrists and with high neck
	Shoes	disinfected or sterilized shoes or overshoes
	Gloves	Sterile, non-powdered rubber or plastic gloves
Grade A/B*	Facemask	Sterile, single-use. Headgear should totally enclose facial hair. Sterile eye protection/ coverage is dependent upon the process
	Cap	Sterile Headgear should totally cover hair, beards and moustaches; it should be tucked into the neck of the suit
	Suit	Sterile coverall
	Shoes	Sterilised footwear, boot-like structure to enable the trouser-legs to be tucked inside the footwear
	Gloves	Sterile, non-powdered rubber or plastic gloves

* In general, the protective clothing material should shed no fibres, and clothing should retain the particles shed by the body.

Required clothing should be chosen, based on the process and grade of the processing area. Minimum requirements modified from EU GMP Annex 1 are listed in Table 7.5. Clothing should be pocket-less, made of lint-free material, with tightly fitting fasteners at the neck, wrist and ankles. For each worker in a Grade A/B area, clean sterile (sterilised or sanitised adequately) protective garments should normally be provided at each work session (or slightly less often if monitoring results justify it) but in any case at least once a day. Masks and gloves should be changed at least after each working session. Gloves should be changed regularly during operations. Disinfection of gloves is acceptable in Grade C and D environments as long as direct contact with tissues and cells is excluded. When operators are trained in the use of good aseptic practices, documented with a successful process simulation, the operator glove sites can be considered as a non-critical surface [12]. The efficacy of disinfection depends on the disinfectant/type of gloves combination. Disinfection of some gloves has an influence on the tightness of the gloves [13]. A complete investigation should be performed and documented to evaluate the impact of glove disinfection.

Gowning procedures for personnel should be validated at least in Grade A/B areas to ensure that gowning materials and protocols are adequate. Samples should be taken from the surface in several fixed sites on clothing:

a. fingers of gloves;
b. facemask;
c. sleeve (forearm) of a suit;
d. front of the suit at chest height;
e. hood.

7.7. Cleaning

Appropriate sanitation of clean areas is of the utmost importance to satisfy environmental requirements. The cleaning process (both schedule and procedure) should be validated, and the validated cleaning process should be followed to achieve the required level of cleanliness. The cleaning validation should consider the influence of the time between processing and cleaning and the time between cleaning and processing to define how long the cleaning process can be delayed after processing and how long the cleaning process remains effective. All cleaning procedures should be documented. Cleaning should be done by personnel trained for the procedure, cleanroom environment, workflows and gowning. The rotation of disinfectants should be included in the disinfection programme to avoid any antibiotic resistance effect due to biocides [14] and to cover all the range of micro-organisms. Cleaning products are made up of broad-spectrum disinfectants containing quaternary ammonium compounds, stabilised chlorine dioxide, hydrogen peroxide and sodium hypochlorite.

Table 7.6. **Recommended limits for microbial monitoring (EU GMP Annex 1)**

	Recommended limits for microbial contamination			
Grade	*Air sample (CFU/m³)*	*Settle plates, diam. 90 mm (CFU/4 hours)**	*Contact plates, diam. 55 mm (CFU/ plate)*	*Glove print, 5 fingers (CFU/ glove)*
A†	1	1	1	1
B	10	5	5	5
C	100	50	25	not applicable
D	200	100	50	not applicable

Certain cleaning products might be detrimental for certain tissues and cells. Cleaning products should therefore not only disinfect the premises but also be safe for the human tissues and cells. Especially in ART centres, certain biocides might be detrimental for gametes and embryos, and therefore care should be taken in choosing the appropriate cleaning products and disinfectants [15]. Disinfectants and detergents used in Grade A and B areas should be sterile before use. Microbiological monitoring of the cleanroom should be undertaken regularly to detect development of resistant strains. Fumigation may be useful for reducing microbiological contamination on inaccessible surfaces.

Some tissue banks and cell banks accept material for autologous use from donors infected with HIV, HBV or HCV. In such cases, separate processing should be done and validated cleaning procedures applied. After processing, the surface should be decontaminated using disinfectant with specific label claims for blood-borne pathogens (e.g. HIV, HBV, HCV) or a freshly diluted bleach-based product in accordance with manufacturer's instructions, and the surface should be allowed to dry.

* Individual settle plates may be exposed for less than 4 h. Where settle plates are exposed for less than 4 h the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 h.

† It should be noted that for a grade A the expected result should be 0 cfu recovered; any recovery of 1 cfu or greater should result in an investigation.

Inactivation of prions should be considered if risk of prion contamination has occurred, e.g. if tissues or cells from a Creutzfeldt–Jakob disease-positive donor have been processed or stored. Prions are very resistant to inactivation. Published methods for prion inactivation include physical and chemical methods. Concentrated solutions of sodium hypochlorite achieve inactivation but other chlorine-releasing compounds are less effective. Sodium hydroxide (2 M) leads to substantial (but incomplete) inactivation. Other chemical procedures, such as use of proprietary phenolic disinfectants, are much less effective. Infectivity can survive autoclaving at 132-138 °C and, under certain conditions, the effectiveness of autoclaving declines as the temperature is increased. The small resistant subpopulations that survive autoclaving are not inactivated simply by re-autoclaving, and they acquire biological characteristics that differentiate them from the main population. Despite the limitations of autoclaving, combining autoclaving (even at 121 °C) with treatment using sodium hydroxide is extremely effective [16].

Storage facilities should be cleaned according to a schedule. Also, handling and disposal of wastes should include appropriate collection, storage and transportation procedures according to applicable European, national and local regulation.

7.8. References

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Chapter 8: **Processing**

8.1. Introduction

'Processing' means all operations involved in the preparation, manipulation, preservation, packaging and inactivation of micro-organisms in tissues or cells intended for human application. Storage is necessary at various stages from procurement to clinical use and must be controlled and documented to ensure that the required properties of the tissues or cells are maintained and that cross-contamination or loss of traceability is avoided (see Chapter 9: Storage and release). Packaging and labelling are described in more detail in Chapter 14.

The aims of processing tissues and cells include:

a. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of tissues or cells;

b. preservation of the required properties of the biological material, making extended storage for future use possible;

c. reducing the risk of disease transmission by the inactivation of micro-organisms or even sterilisation in circumstances where cell viability is not required;

d. improving the clinical performance of a graft by removing those elements that are not necessary for the success of the transplant.

Processing includes a range of activities such as (but not limited to) thawing, washing, cutting, grinding, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, separation, decellularisation, concentration or purification of cells, freeze drying, freezing and cryopreservation.

Although it can deliver great benefits, processing can also introduce risks. The potential risks include:

- microbial contamination from the environment or the operator, or cross-contamination from other tissues or cells,
- errors in identification or labelling,
- damage to the tissues or cells which reduces their clinical efficacy.

For these reasons, processing of tissues and cells must be carried out within a comprehensive quality-management system, must be documented using standard operating procedures (SOPs) and must be thoroughly validated, to demonstrate that the quality and efficacy of the final product have not been unacceptably compromised and that contamination or cross-contamination has not been introduced during processing.

Procurement is defined as the technique for obtaining different tissues directly from the donor, e.g. procurement of corneas by *in situ* excision or procurement of menisci by arthrotomy. However, if the eye was removed from the donor, and corneas and menisci were then excised at the procurement site, this activity would be classified as processing. Processing in the procurement facility, either during or after procurement, is not recommended because it is important to prevent microbial contamination, or cross-contamination of procured tissues. (See Chapter 6: Procurement.) This chapter provides generic guid-

ance on the processing of tissues and cells carried out by tissue establishments (TEs). Further, more specific, guidance is provided in Part B of this Guide. It is also important that TEs follow the Good practice guidelines for tissue establishments (GPG) to provide the key elements of their procedures, which should be defined and controlled within the TE's quality system. Where relevant, the GPG sections pertinent to the topic are referenced in each subsection of this chapter.

8.2. Receipt at the tissue establishment

Each TE must have a documented policy and specifications against which each consignment of tissues and cells (including blood samples from donors) is verified. These specifications must include the technical requirements and other criteria considered by the TE to be essential for the maintenance of acceptable quality. When the procured tissues or cells arrive at the TE, there must be documented verification of the consignment. Documents must be completed covering the transport – including the transport conditions, packaging, labelling and associated documentation – and samples (including blood) to ensure that they meet the requirements and specifications of the receiving establishment (and, in EU countries, the requirements of Annex IV of Commission Directive 2006/17/EC).

Upon receipt of the documentation, the procurement report and shipping record (if the donation was transported by a third party) should be cross-checked with the contents of the package. The packaging, the tissues and cells received, and any accompanying samples should all be examined to ensure that they have not been damaged or tampered with during transit.

The following should be checked and recorded:

a. (no) evidence of unauthorised opening or manipulation;
b. (no) signs of damage that might result in the deterioration of tissues and cells and (no) signs of incidents relating to storage;
c. transport conditions (unless a validated transport method has been used) and storage temperature and time in transit;
d. identification of the donor (donation number);
e. description of the tissues or cells (including number of units per device or ampoule);
f. procurement report including procurement date and time;
g. purpose of tissues and cells (i.e. for transplant/research);
h. status of the tissues or cells (e.g. quarantine);
i. associated samples (including blood).

The TE must ensure that the tissues and cells received are quarantined and stored in a defined, separated and adequate location under appropriate conditions until they, along with the associated documentation, have been inspected or otherwise verified as conforming to requirements. The acceptance or rejection of received tissues or cells must be documented.

The data that must be registered at the TE include:

a. consent/authorisation, including the purpose(s) for which the tissues and cells may be used and any specific instructions for disposal if the tissues or cells are not used for the purpose for which consent was obtained;
b. all required records relating to the procurement and donor medical/behavioural history (see §6.5);
c. for allogeneic donors, eligibility, i.e. a properly documented review of donor evaluation against the appropriate selection criteria by an authorised and trained person;
d. in the case of tissues and cells intended for autologous use, documentation of the possibility of medicinal allergies (such as to antibiotics) of the recipient.

Review of the relevant donor/procurement information, and thus acceptance of the donation, needs to be carried out by specified/authorised persons.

The TE must have documented procedures for the management and segregation of non-conforming tissues or cells, or those with incomplete test results for infectious diseases, to ensure that there is no risk of contamination of other tissues and cells being processed, preserved or stored.

If the material is not being transported by their own personnel, the TE should prepare an agreement to be signed by third parties that defines the responsibilities of each party in the transport of tissues and cells to the TE. Such transport should ensure the safety of the tissues and cells and maintenance of their temperature conditions, and prevent unauthorised access.

Quality-control checks of procurement and transportation methods should be reviewed regularly by TEs to ensure that the integrity of tissues or cells and the storage temperatures are maintained during procurement and transit.

8.3. Coding

TEs must ensure that human tissues and cells are correctly identified at all times. Upon receipt of the tissues and cells, the TE should assign a unique identification code to the material if this has not already been done at procurement. This code can then be extended to identify the different products and batches of tissues or cells obtained during processing.

Tissues and cells should be labelled at all stages of processing (see Chapter 14 for further guidance on labelling and Chapter 15 for further guidance on traceability). The label must include at least the following information:

a. unique identification;
b. identification of the TE;
c. type and characteristic of the product;
d. batch number (if applicable);
e. recipient name (if applicable).

The coded data must be entered in a register maintained for the purpose.

8.4. Processing methods

Tissues and cells should be appropriately processed, preserved and decontaminated for clinical use. TEs must address all processes that affect quality and safety through their quality system and associated standard operating protocols (SOPs).

TEs must ensure that the equipment being used, the working environment, process design, and validation and control conditions are in compliance with established quality and safety requirements (see Chapter 2). Each step of processing must be carried out under defined conditions to guarantee the quality and safety of tissues and cells, as well as the safety of TE personnel.

If a TE entrusts one of the stages of processing to a third party, a written agreement is needed between the TE and the third party. The TE must evaluate and select third parties on the basis of their ability to meet the established standards.

The recommended time limits between procurement, processing and storage are described in the tissue- and cell-specific sections of this Guide (see Part B). When appropriate, these maximum times from procurement (or circulatory arrest) until processing and storage must be defined. Procurement, processing and storage times must be documented in the records for tissues and cells.

8.4.1. Processing reagents

The reagents used in processing should be of an appropriate grade for their intended use, be sterile (if applicable) and comply with existing national regulations. Use of antibiotics during procurement, processing and preservation should be minimised, and, if used, information regarding the possibility of allergic reactions in the recipient must be included in the information provided to the end users. Whenever possible, reagents used for procurement, processing, decontamination and preservation should be approved for human use and should be CE (Conformité Européenne) marked. Reagents that are not of appropriate grade must undergo risk assessment and validation to confirm that they are suitable for their intended purpose. Reagents not approved for human use may be used if an equivalent reagent of appropriate grade is not available, if the use has been authorised by national authorities and if the use is supported by risk assessment. The origin, characteristic conditions for storage (physical, chemical, microbiological) and expiry dates of reagents should be monitored and recorded. Reagents should be used in a manner consistent with the instructions provided by the manufacturer. Critical reagents and consumables should have written specifications describing, if applicable:

a. materials, including:
  i. the designated name and the internal code reference;
  ii. the reference (if any) to a pharmacopoeia;
  iii. the approved suppliers and, if possible, the original manufacturer of the products;
  iv. a specimen of printed materials;
  v. certificate of compliance from the manufacturer.
b. directions for sampling and testing, or reference to procedures;
c. critical quality attributes, with acceptance limits;
d. storage conditions and precautions;
e. the maximum period of storage permitted before re-examination.

8.4.2. Processing techniques

8.4.2.1. *General principles*

Processing methods must not render the tissues or cells clinically ineffective or harmful to the recipient. They should be designed to ensure the safety and biological functionality of prepared tissues and cells. Processing methods should be validated to ensure

they achieve their objectives (see the general text on validation in Chapter 2 and §8.10 below).

Processing procedures must undergo regular re-validation to ensure that they continue to achieve the intended results.

Pooling of different tissues and cells from two or more donors during processing is not recommended. The only exception is where it is supported by a comprehensive risk–benefit assessment and it has been demonstrated to be the only way of providing sufficient clinically effective tissues or cells. If performed, traceability must be fully ensured.

8.4.2.2. *Procedures*

The main types of processing procedure that can be applied to tissues and cells include, but are not limited to:

- Cleansing of procured material by removal of extraneous tissues and bodily fluids is a common initial processing step. Commonly, scalpels, scissors and gauze wipes are used in this process.
- Separation is used to partition the specific type of tissue to be processed from another type, for example to divide dermis and epidermis, or amniotic membrane and chorion.
- Cutting and shaping allows initial preparation of procured tissues into the shapes and forms required for transplantation. Different types of cutting device can be used, depending on type of tissue. For cutting bone, different types of saw may be used, such as oscillating saws, bandsaws or rotary saws, whereas for soft tissues such as skin and tendon, scissors or scalpels may be used.
- Grinding, by different types of mill, is used to pulverise bone tissue into smaller pieces. Depending on the type of bone being ground, actively cooled grinding mills that dissipate heat may be used, to prevent the bone being damaged by excessive temperatures caused by grinding friction.
- Washing may be performed in one of three ways:
  - as an initial step in processing, e.g. to remove surface blood and lipids,
  - as an integral part of a process, e.g. to remove bone-marrow components from musculoskeletal allografts,
  - to remove traces of chemical compounds used during processing.

  Washing may also be used to decrease the bioburden of tissues. Several types of washing solution may be used, e.g. distilled water, 0.9 % NaCl, balanced salt solution, phosphate-buffered saline, or alcohols. The washing protocol utilised should be validated to demonstrate that it does not detrimentally affect the clinical efficacy or safety of the tissue [1, 2].
- Centrifugation may be used to concentrate and separate cells from a suspension or different fractions of suspensions, or to remove marrow, blood and lipid components from musculoskeletal allografts.
- Disinfection by soaking or rinsing in antibiotic or antimicrobial solutions is commonly used for decontamination of viable tissues that cannot be terminally sterilised, and as a stage in the processing of subsequently sterilised tissues to reduce the bioburden.
- Cell concentration and selection is used as an initial step for HPC processing or for *in vitro* cell cultures. This may also include the isolation of particular cell types, e.g. mononuclear cells from peripheral blood.
- Filtering procedure is used after bone-marrow collection.
- Decellularisation is a technique that aims to remove most of the cellular content of the tissue, leaving behind just the extracellular matrix (ECM). These extracellular matrices may be implanted directly or used as a scaffold for the manufacture of advanced therapy medicinal products (ATMPs). See Chapter 31 for a more detailed discussion of decellularisation.
- Demineralisation is a process of chemical removal of the bone mineral, resulting in exposure of biologically active bone morphogenetic proteins present in bone tissue. Demineralisation is usually performed using a dilute (0.5 M or 0.6 M) HCl solution.
- Freeze-thawing of tissues can be used as a processing step, for lysis of cells prior to washing procedures.

8.5. Preservation methods

Preservation of tissues and cells for long-term banking is central to the operation of a TE. It is essential that a preservation technique appropriate for the graft in question is selected. The selected technique must be capable of retaining the essential properties of the graft (e.g. viability, structural integrity) for the duration of the maximum possible storage period.

8.5.1. Types of preservation

Tissues and cells can be preserved by different techniques including, but not limited to, freezing, lyophilisation, cryopreservation, vitrification or glycerolisation.

Freezing is used for pre-processing storage of procured tissues, for in-processing storage between different processing steps of non-viable tissues and for storage of processed tissue awaiting release for transplantation. Freezing can disrupt tissues and cells [3]. Hence, the method of freezing used must take into account the eventual use of the tissues and cells.

Lyophilisation (or freeze-drying) involves dehydration of tissues by freezing and then reducing the surrounding pressure to allow the frozen water in tissue to sublimate directly from the solid phase to the gas phase. Lyophilisation prevents tissue autolysis and allows storage at room temperature.

Cryopreservation is a process where the biological and structural functions of tissues or cells are preserved by cooling to sub-zero temperatures in a cryoprotectant. This is used where cell viability must be maintained. The rate of cooling must also be controlled to prevent formation of ice crystals within cells, which can result in damage and loss of viability and integrity. Once cryoprotectants are added, tissues/cells may be placed in a freezing device (such as a controlled-rate freezer) that gradually reduces the temperature of the grafts.

Vitrification is ice-free cryopreservation. The crystallisation of ice is avoided by an extreme elevation of viscosity during cooling achieved by a combination of high cryoprotectant concentrations and rapid cooling.

Glycerolisation is a procedure for soaking tissues, mainly skin, in a concentrated glycerol solution. The glycerol binds water in the tissue and prevents degradative processes occurring.

8.6. Decontamination methods

Microbiological and viral safety are critical for cell and tissue transplants. Validated sterilisation and virus-inactivation processes are mandatory in many jurisdictions. In some countries a validated disinfection process may be accepted as an alternative to sterilisation.

It is important to clearly define terms when discussing the removal of micro-organisms and the inactivation of viruses. Typically, more than one process contributes to the overall effect. Initial steps generally serve to reduce and/or control the bioburden for the main process.

8.6.1. Types of decontamination

8.6.1.1. *Disinfection*

Disinfection is a term used for non-sterilising processes that kill bacteria, and/or fungi and/or spores, and/or inactivate viruses via a known, direct and quantifiable physical or chemical mode of action. In the preparation of tissue grafts, disinfection processes are either precursors to sterilisation processes or in some cases, where sterilisation is either not required or not possible, are themselves the primary mechanism for ensuring microbiological graft safety.

Generally, when used as a precursor to sterilisation, a disinfection process is intended to reduce the bioburden on the tissue prior to sterilisation such that it does not exceed a level that can be reliably completely eliminated by the sterilisation process.

When used as the main step for the removal of bacteria, spores and fungi and the inactivation of viruses, the process should be validated according to national requirements. In some instances, sterility tests on individual transplants may be required.

Some establishments rely upon a validated thermodisinfection process for the attainment of microbiological transplant safety [4]. Tissues that cannot be subjected to high temperatures or other disinfection procedures may be treated with antibiotic-based disinfection steps. The risk that antibiotic residues could remain in the tissue post-disinfection, and compromise post-disinfection sterility, must be considered [5]. Such processes should therefore be validated for both efficacy and residual antibiotic levels. It may be necessary to include information about such residues in the information provided to end users, as some patients may be allergic to certain antibiotics and other decontaminants, such as povidone iodine.

8.6.1.2. *Sterilisation*

Sterilisation is defined as a process that results in the complete absence of all cell-based micro-organisms capable of replication. In the preparation of tissue grafts a sterilisation process will usually also have to meet the requirements for virus inactivation.

Sterilisation processes can be based on moist or dry heat, chemical agents, irradiation or high pressure. Sterilisation processes used in tissue-graft preparation are held to exacting standards and must be validated according to national requirements.

Individual validation methods for processes used to sterilise tissue transplants are feasible, but the following established, standardised approaches are available:

a. Sterility Assurance Level (SAL). For sterilisation processes with a well-defined dose/kill relationship, a very high degree of sterility assurance can be achieved and quantified with an SAL. The SAL is expressed as an experimentally-derived number defining the likelihood of a contaminant surviving the process. The smaller the number, the higher the likelihood of sterility. For some classes of medical device and for some medicinal products, there is a European requirement that a sterilisation process must be validated to give a value of SAL $\leq 10^{-6}$ for the product to be labelled 'sterile'. This SAL means that the likelihood of non-sterility is 1 in 1 million. (This is often interpreted as meaning that in a theoretical batch with 1 million 'units', at least 999 999 units must be sterile. More relevant for tissue transplants is the interpretation that the process should result in all units being sterile in 999 999 from 1 million cycles.) At the time of writing there are no defined national requirements within Europe for sterilisation processes used to treat tissue transplants to be validated for a specific SAL. At the same time, when an SAL approach is used, a manufacturer may have difficulty convincing national authorities that a process with a 'lower' SAL should be approved. There is a very important limitation of the SAL approach for sterilisation processes used for the treatment of tissue grafts: the SAL method cannot be used to quantify the efficacy of virus inactivation. For this reason, in countries with the most stringent quality standards for tissue grafts, an alternative approach is considered appropriate.

b. Potency against a panel of bacteria, spores and viruses. Validation of potency can be achieved by application of the EN 1040 standard and/or Committee for Proprietary Medicinal Products (CPMP) guidelines [6, 7]. This type of validation requires that the maximum anticipated level of bacterial, fungal and viral contamination can be eliminated, by establishing the elimination capacity in terms of the number of log-scale reductions in the concentration of samples spiked with a panel of bacteria, fungi and viruses. The panel should cover Gram-positive and Gram-negative bacteria, spores and fungi, and should include known relevant 'resistant' species. In the case of viruses, relevant species of enveloped and non-enveloped viruses covering the range of virus particle sizes should be included [7].

8.6.1.3. *Virus-inactivation process*

The virus-inactivation process is based on the capability of the process to inactivate and/or remove virus. Validation of the virus-inactivation process will provide the evidence that the process will effectively inactivate or remove viruses that are known to contaminate the starting material or that could conceivably do so. The validation is based on a choice of virus similar to the virus that may contaminate a tissue. Such types include enveloped, non-enveloped, DNA and RNA viruses [7].

8.6.2. Sterilisation methodologies applicable to tissue grafts

8.6.2.1. *Irradiation*

Irradiation with gamma particles or accelerated electron beams can be used for the sterilisation and viral inactivation of tissue transplants. For the sterilisation component, such processes are well suited to validation according to SAL. Gamma radiation is effective in killing bacteria, fungi, spores and, to a more variable degree, viruses. However, depending on the dose and irradiation conditions, gamma radiation can have a negative effect on the mechanical properties of the grafts. Applying appropriate and validated irradiation conditions can substantially reduce these negative effects and protect the graft integrity. There are data that suggest treatment of allografts with less than 25 kGy does not affect the integrity of the allograft [8]. Depending on bioburden, a ≥ 25 kGy irradiation dose may be required for sterilisation, and depending on the nature and extent of viral contamination a dose of ≥ 34 kGy may be required for virus inactivation [9].

Such high doses and concomitant transient high temperatures (≥ 60 °C) are likely to have negative effects on transplant properties. It is, however, not possible to make generally applicable statements about the extent to which such effects will influence the clinical performance of transplants. The adverse effects of irradiation can be ameliorated by reducing the temperature and inclusion of radioprotectant chemicals [10, 11]. Irradiating musculoskeletal tissues in the frozen state retains the primary effects of gamma irradiation sterilisation (the breaking of covalent bonds by high-energy gamma rays) while minimising the secondary effects of the process (generation of free radicals). Overall, this can reduce the damage done to allografts by the irradiation process but may also provide some protection to micro-organisms.

8.6.2.2. *Peracetic acid-ethanol treatment*

This method is typical of the 'panel' approach in *b* above. In one example of a peracetic acid (1 %) ethanol sterilisation process, the method was tested in allogenic avital bone transplants with a thickness of 15 mm. The process led to a reduction of virus titres of more than 4 log10 TCID50/mL. For viable bacteria, fungi and spores, a titre reduction below the detection level (5 log10 cfu/mL) was achieved after an incubation time of 2 hours [12]. In the case of chemical treatment of tissues the question of potential residues could be a concern and the risk, if applicable, should be included in the 'instructions for use'.

8.7. Requirements for processing facilities

Facilities for aseptic and clean, non-sterile processing must be dedicated to this activity, and must be designed, qualified and monitored to ensure that the air quality is appropriate for the process being carried out (see Chapter 7).

8.7.1. Avoiding contamination and cross-contamination

In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during processing or storage, with tissues or cells from another donor, unless they are intentionally pooled. A separate set of clean, sterile instruments should be used for each donor. Where possible, these should be single-use and disposable. In some cases, e.g. for ocular tissue, single-use surgical instruments for procurement and processing are available and recommended. Each tissue or cell product should have a batch number that is also recorded in the processing records.

Pooled tissues or cells should be treated as a single batch, ensuring that full traceability to all the donations included in the pool is maintained. Due to the high probability of cross-contamination resulting from pooling, it must be supported by a thorough risk–benefit analysis.

8.8. Quality control

8.8.1. General considerations

Tests and procedures should be carried out to measure, assay or monitor processing, preservation and storage methods, equipment and reagents to ensure compliance with established tolerance limits. Written procedures must be in place that govern quality control at key stages during processing. The written procedures should include as a minimum the test method, the sample size and the acceptance criteria. The minimum requirements for evaluation of each type of tissue and cell are described in tissue- and cell-specific chapters (see Chapters 17-35). The results of all tests or procedures should become part of the permanent processing record.

If in-process controls are undertaken in the processing area, they should be carried out so that there is no risk to the processing steps being followed.

8.8.2. Microbiological testing

In many cases, it is not possible to exclude contaminated material during processing because the tissue originates from parts of the body which contain natural microbial flora, and pre-processing disinfection is not 100 % effective. The microbiological safety of tissues and cells is based on donor selection and minimisation of initial contamination, with protocols to control and monitor contamination being employed during the entire procurement process. Chapter 10 describes methods of microbiological control. Sampling and testing methods must be validated to demonstrate that the sampling method accurately represents the tissue, and that the testing methods are suitable and fit for purpose.

Various procedures exist for securing microbiological control, such as decontamination by antibiotics, or physicochemical methods. If physicochemical methods are to be applied, these procedures must be adapted to the type of tissue or cell and should be validated. The effectiveness of a decontamination or inactivation procedure should be shown for relevant micro-organisms in the tissue or cell preparation itself and not only in an aqueous solution. The risk that some micro-organisms may survive decontamination with antibiotics, but not be detected by post-decontamination microbiological testing, must be considered. This factor has been implicated in the death of, and serious injury to, patients [13, 14].

Non-conforming products must be identified and separated from conforming products. The fate of non-conforming products will be decided by the Responsible Person (RP) in charge of the TE.

8.9. Significant changes

A documented risk assessment approved by the RP must determine the fate of all stored tissues and cells following the introduction of any new donor

selection or testing criterion, or any significantly modified processing step, that enhances safety or quality. Guidance on risk assessment is provided in Chapter 2.

A documented change-control procedure must be followed before any significant change is implemented in processing. This must be supported by a comprehensive risk assessment. The purpose of this is to ensure that the change is justified, is documented and will not affect the quality of the product (see Chapter 2). All relevant persons should be involved in evaluation of the change.

As a minimum, the following aspects of any change in processing should be evaluated:

a. significance;
b. effect on quality;
c. need to update SOPs;
d. need to re-validate the process;
e. effects on quality-control (QC) analyses;
f. need to inform regulatory authorities;
g. need to train personnel;
h. effect on risk analyses.

8.10. Process validation

If processing is carried out according to GMP, the processing validation must also be done according to GMP guidelines. In the EU, Commission Directive 2006/86/EC allows for validation studies to be based on any of the following:

a. studies undertaken by the establishment itself;
b. data from published studies;
c. for well-established processing procedures, retrospective evaluation of the clinical results for tissues and cells supplied by the establishment.

Where validation is based on studies carried out by the establishment itself, reports should include at least the following elements:

a. a validation plan that specifies the critical parameters to be assessed and the acceptable result thresholds for these parameters;
b. a documented methodology;
c. all results obtained, described clearly and with relevant interpretation;
d. a declaration of validation acceptance or rejection signed by the quality manager (QM) or a person authorised by the RP.

Validation studies should be carried out by applying 'worst case' scenarios. The equipment used for validation studies should be fully qualified, and measuring devices should be calibrated to traceable standards. Validation experiments should be repeated at least in triplicate, though this will depend on the degree of variability in the data, to ensure reliably repeatable results. For an example of a validation study, see Appendix 4. Example of cleanroom qualification, Appendix 5. Example of incubator qualification, Appendix 6. Example of process validation – tissue transportation and Appendix 7. Example of method validation – oocyte vitrification.

Where validation is based on data from published studies, the relevant publications should be filed as part of the validation record. In this case, the TE should demonstrate that they can effectively reproduce the published process with the same results in their facility (operational validation). Copies of the relevant SOP and the results of the operational validation should be provided, to demonstrate that the process is equivalent to that applied in the scientific literature.

Where specific steps have been significantly modified or adapted, separate validation should confirm that these changes have not invalidated the method. There should be a signed declaration of validation acceptance or rejection by the QM or RP.

If validation is based on retrospective evaluation of the clinical results for tissues or cells supplied by the establishment (i.e. for well-established processing procedures), data should be collected and analysed that include the number of tissues or cells implanted following processing by the method under consideration, and the time period (start and end dates/times) during which these implantations occurred. It should be demonstrated that, where a vigilance system was already in place at the time, clinical users were informed of the procedure for reporting adverse reactions. There should be a signed declaration of validation acceptance or rejection by the QM or RP.

The procedures used to prevent or reduce contamination during processing may vary, depending on the type of tissue and how it is processed. However, they should all be fully validated. Decontamination methods, such as antibiotic soaking, should be validated to demonstrate effectiveness against a range of contaminants similar to those routinely found on the tissues or cells in question. Such studies should be designed to ensure that residual decontaminants (e.g. antibiotics) do not affect the validity of the microbial tests carried out on the product.

If the process includes a sterilisation or viral-inactivation step, process-specific validation studies

should be completed to demonstrate the log reduction achieved by the process.

Subsequent to process validation and during routine processing, TEs should monitor tissue and cell quality to ensure a state of quality control is maintained throughout the processing part of the product life-cycle. This will provide assurance of the continued capability of the process and its quality controls to produce finished tissues and cells that meet the desired quality and to identify changes that may improve product quality or performance. Relevant process trends (e.g. quality of incoming materials or components, in-process and finished product results, cases of non-conformance and defect reporting) should be collected and assessed to verify the validity of the original process validation or to identify required changes to the associated controls. Documentation and tracking of patient outcomes constitute a critical element of ongoing process verification. For new or significantly changed processes, a system to enable close clinical outcome monitoring should be agreed with clinical users.

8.11. References

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Related material

- Appendix 4. Example of cleanroom qualification
- Appendix 5. Example of incubator qualification
- Appendix 6. Example of process validation – tissue transportation
- Appendix 7. Example of method validation – oocyte vitrification

Chapter 9: **Storage and release**

9.1. Introduction

Storage is the maintenance of tissues and cells for clinical application under appropriate controlled conditions until distribution, and it occurs at various stages from procurement to clinical use. Storage must be controlled and documented to ensure that the required properties of the tissues or cells are also maintained during storage and that cross-contamination or loss of functionality, efficacy, clinical effectiveness and traceability is avoided.

The opportunity to store tissues and cells is also common during the process and brings great advantages:

a. preservation of the required properties of the biological material, making extended storage for future use possible;

b. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of tissues or cells;

c. reducing the risk of disease transmission by testing of infectious diseases and microbial contamination prior to release and clinical use, though this is only possible if the tissues and cells can be stored under defined conditions for an adequate time in order to await the test results;

d. performing and awaiting other quality-control results before release (e.g. cell counts, clonogenic assays for haematopoietic progenitor cells (HPC), residual moisture in lyophilised or dehydrated grafts).

Each tissue establishment (TE) must have a documented policy on tissue and cells release, and specifications against which tissues and cells are verified. These specifications must include the technical requirements and other criteria considered by the TE to be essential for the maintenance of acceptable quality. In general, release is the final evaluation and control that these specifications of tissues or cells are met. Only then, the tissues or cells can be distributed to the hospital and used for clinical application.

This chapter provides generic guidance on the storage and release of tissues and cells carried out by TEs. Further tissue and cells specific guidance is provided in Part B of this Guide.

9.2. Storage

9.2.1. General

Storage facilities for materials, tissues and cells are present in most TEs, procurement organisations, donation and transplant hospitals, organisations for human applications and pharmacies. These storage facilities must have policies and SOPs for all processes that affect quality and safety.

Storage facilities must ensure that the equipment being used, the working environment, the process design and the qualification, validation and control conditions are in compliance with established quality and safety requirements (see Chapter 2). Storage (during the process and after release) must be carried out as defined in the specifications for the specific tissues or cells to guarantee the quality and

safety of tissues and cells. Organisations must have a dedicated area or material for quarantine (temporary storage) and a plan for back-up storage. An inventory of tissues and cells stored must be performed regularly.

If an organisation entrusts one of the stages of storage to a third party, a written agreement is needed between the TE and the third party. The TE must evaluate and select third parties based on their ability to meet the established standards of quality.

The recommended time limits between procurement, processing and storage are described in the tissue- and cell-specific sections of this Guide (see Part B). Where appropriate, these maximum times from procurement (or cardiac arrest) until processing and storage must be defined to maintain quality, safety and clinical effectiveness of the tissues and cells. Procurement, processing and storage times must be documented in the records for tissues and cells.

Reference samples of tissues and cells for quality control should be stored under the same conditions as the tissues and cells themselves (e.g. HPC and cord blood reference samples).

9.2.2. Methods of storage

Following processing, tissues and cells should be stored according to currently accepted good practice, based on the best available scientific evidence and according to good manufacturing practice (GMP), as appropriate, for tissues and cells. All procedures associated with storage of tissues and cells must be documented in SOPs.

9.2.3. Storage temperature

Refrigeration devices/incubators containing tissues and cells should be suitable for the intended use, and the procedures for monitoring such devices should be appropriate so that tissues and cells are maintained at the required storage temperature. Regular monitoring and recording of temperature, together with suitable alarm systems, must be employed on all incubators, storage refrigerators, freezers and liquid nitrogen tanks (see Chapter 2). The functionality of the alarm systems must be checked regularly. Temperature ranges for storage of tissues and cells are shown in Table 9.1.

9.2.4. Requirements for storage facilities

Facilities for storage must be dedicated to this activity, and must be designed, qualified, validated and monitored to ensure appropriate conditions (see §7.2).

Although storage facilities do not need to strictly follow the same environmental criteria as the procurement and processing facilities, once the tissues and cells have been processed and stored/banked it is wise to invest in resources to maintain a secure, clean and stable environment for long-term storage.

The storage room must have a sufficient area in an appropriate place and must be designated for the specific purpose for which it is used; therefore, it must have enough space to allocate the number of tanks, refrigerators and/or freezers to store the expected number of samples. There must also be sufficient space for the movement of equipment, samples and personal. It is recommended that the room for manoeuvre is at least the size of the largest equipment or container. Furthermore, the access to the storage room must be through a door whose opening is larger than the diameter of the largest equipment in the room.

The room should be in a dry, cool, well-ventilated place, free from heat sources.

For security reasons access to the storage facilities should be restricted to authorised personnel. Appropriate alarms for temperature control and low levels of oxygen must be put in place, along with sensor systems in case of liquid nitrogen leaks. In case of loss of electrical power, as a part of the TE general emergency plan, the storage facility should have generators or uninterrupted power supply (UPS) systems.

Table 9.1. **Temperature range for storage of tissues and cells**

	Temperature range (°C)
Cryopreservation	< – 140
Deep frozen*	– 80 to – 60
Frozen†	< – 15
Refrigerated†	2 to 8
Cold or cooled†	8 to 15
Room temperature†	15 to 25
Organ culture	28 to 37

9.2.5. Selecting appropriate air quality for storage

In order to avoid heavy environmental microbial contamination, certain areas such as corridors or open access from outdoors should be avoided.

* Based on general practice.
† Based on the *European Pharmacopoeia* [1].

Storage rooms can be equipped with an HVAC (heating, ventilation and air conditioning) system for maintaining the temperature and humidity by adequate air exchange.

9.2.6. Environmental monitoring

Similarly, as with the processing rooms, if storage is carried out according to GMP and defined in the in-house environmental hygiene programme, monitoring systems for storage rooms may need to be put in place. The frequency of > 0.5 µm particle monitoring, as well as the implementation of alarm limits for particle concentration, will depend on the degree of stringency needed and the principles of quality risk management. Together with environmental airborne particle monitoring, microbiological contact-plate monitoring may be performed to demonstrate the degree of cleaning of the room (see §7.5.2 and §7.5.3).

9.2.7. Special safety measures for liquid nitrogen facilities

The organisation of the room must allow circulation and manipulation around the cryogenic tanks. The room must be clearly identified with pictograms indicating the dangers and the presence of personal protection equipment. The staff need to be specially trained and qualified for these working conditions.

Before entering the room, the staff must be able to ensure that ventilation is functioning correctly and be able to check the oxygen level. An adapted mechanical ventilation (extraction and fresh-air intake) system, working continuously, is mandatory, ensuring renewal of the air. Continuous control of the oxygen rate is ensured by several detectors, checked at least once a year. They are put in the lower areas of the room where the ventilation is at its weakest. The oxygen rate or alarm status must also be visible outside the room.

Following nitrogen evaporation, leading to an oxygen level equal to or lower than 19 %, an alarm (visual and audible) is activated and staff must then leave the room and await normalisation of the oxygen level. The alarm is connected to a continuous monitoring station allowing alerts for the staff working in the room and the rescue or assistance staff, if necessary.

Tank-filling systems should be designed to minimise evaporation of nitrogen. They should be equipped with safety valves and rupture discs, and external degassing valves for long lines.

The room should be equipped to allow visual surveillance from outside. It is important to prevent condensation on the tanks and the formation of ice inside them. The floor should be covered with a material resistant to low temperatures and high loads, allowing easy movement of the tanks without shock. The tanks are to be maintained and checked annually.

The entrance door of the room is equipped with an oculus allowing surveillance from outside. The opening of the door is outwards. Gloves with long sleeves able to protect against cold, with non-combustible capacities, and safety glasses (EN166) or visors protecting the face are to be made available to the staff. (For further details, see §7.2).

9.2.8. Avoiding contamination and cross-contamination

Storage conditions must prevent mix-ups, contamination and cross-contamination of tissues, cells, supplies and reagents (see also §7.6) [2, 3, 4]. Areas designated for storage of cells and tissues in process, under quarantine, and released for distribution must be established and controlled. In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during storage, with tissues or cells from another donor, unless they are pooled intentionally. Every effort should be made to avoid cross-contamination of material. Tissues and cells immersed in liquid nitrogen should be double-wrapped during storage or stored in a validated high-security primary container especially designed for liquid nitrogen (depending on the storage system, type of sample and after risk assessment). This may not be mandatory for reproductive cells; see §27.7.8, Processing of samples from seropositive donors in partner donations.

This is highly important for storage with liquid nitrogen owing to the accumulation of microbial contaminants in liquid nitrogen storage vessels, as well as to avoid cross-contamination among samples. The seals and the material employed must be validated for their use at the designated storage temperature and for the conditions of use, to demonstrate that the packaging and labelling can retain their integrity under such conditions.

In principle, to avoid contamination of tissues and cells, also the entry of personnel to the storage facilities should be reduced to a minimum.

Additionally for periodical cleaning, a cleaning plan for the sanitation of the storage equipment should be implemented, depending on the type of equipment. During the cleaning process, a back-up

unit must be used to provide the same safe conditions as the storage equipment. The emptying for cleaning and maintenance should be scheduled in advance and needs to be performed following an SOP.

9.2.9. Types of storage

9.2.9.1. *Quarantine storage*

All human tissues and cells that are stored before having determined their suitability must be kept under quarantine. Quarantined tissues and cells should be physically separated and visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from released tissues. An SOP must describe how to categorise quarantined and released tissues and cells.

Specific areas must be defined for tissues and cells in quarantine and for tissues and cells that have been released; a separate area must be dedicated to the storage of medium and other materials used during the process of preparation. Access to storage facilities must be restricted to authorised persons. If the storage devices are located in a shared facility with other users, they must be securely locked.

9.2.9.2. *Short-term storage*

Because of processing methods and the life span of cells, some viable tissues and cells can only be stored for a short period of time (e.g. cornea for about 4-5 weeks).

9.2.9.3. *Long-term storage*

If cell viability should be maintained for a longer period, other strategies such as cryopreservation or lyophilisation need to be considered.

Obtaining and analysing the critical clinical outcome data can provide evidence to be used to verify the safety and efficacy of the storage procedures.

9.2.9.4. *Storage at an organisation responsible for human application*

Organisations responsible for human application (ORHAs) have short- and long-term storage for tissues awaiting inspection, surplus or unused tissues and tissues recalled by TEs. Therefore, a system for identification of tissues and cells throughout any phase of the clinical application at the ORHA must clearly distinguish tissues and cells released from quarantine and/or discarded ones (see Chapter 12).

9.2.10. Expiry date

To ensure the maximum safety and quality of tissues and cells, it is mandatory to specify a maximum storage time with an expiry date for each type of storage condition. The chosen maximum storage period should be validated, based on data from published studies, stability testing by the establishment or evidence-based facts (e.g. retrospective evaluation of the clinical results for tissues and cells supplied by the establishment). When determining the maximum storage period, several factors should be considered. These include (but are not limited to):

a. possible deterioration of the required properties of tissues and cells;
b. risks related to microbiology;
c. package integrity over time;
d. expiry of storage solutions;
e. stability at the storage temperature;
f. overall risk assessment of quality assurance: donor evaluation, donor testing (kits), quality criteria (viability, functionality after thawing), regulations.

When relevant for the type of tissue or cell, the time of procurement should also be indicated. In certain specific cases, it may be possible to prolong the expiry date, especially in the case of cryopreserved HPC (i.e. cord blood), reproductive tissues and cells for partner donation or in cases of fertility preservation (see Chapter 27 and Chapter 28), e.g. MAR/ART do not have expiry dates, and HPC are retested before release, even after decades of storage.

9.2.11. Storage validation

If storage is carried out according to GMP, the storage validation must be done according to GMP guidelines. Further details can be found in Chapter 2: Quality management, risk management and validation.

Regarding storage validation, homogeneity and reproducibility are fundamental requirements of tissues and cells storage/banking. Stored material should be checked to ensure that both maintenance and documentation are updated and that any changes have been accordingly recorded.

Storage design should prevent accidental release and distribution of tissues or cells.

In case of dissolution of the establishment, facilities must have third-party agreements for transferring stored usable tissues and cells to another storage facility or for disposing of the unusable tissues and cells.

9.3. Release

9.3.1. Release procedure

Release is the act of certifying compliance of a specific tissue or cells or batch of tissues or cells with the requirements and specifications. Before any tissues and cells are released, all relevant records (including donor records, processing and storage records, and post-processing quality-control test results) must have been reviewed, approved and documented as acceptable by an authorised and trained person according to the relevant local SOP and national regulations. There must be an SOP that details the specifications, circumstances, responsibilities and procedures for the release of tissues and cells.

At the time of release, donor records and tissue- or cell-processing records should be reviewed to ensure that the material is suitable for clinical use and implantation. The review should include:

a. approval of donor eligibility by the RP or designated person;
b. review and approval of the processing and storage record (including environmental monitoring records);
c. final evaluation of the label and container to ensure traceability, accuracy and integrity (these tasks can be delegated);
d. results of screening tests on incoming material and in-process controls;
e. specifications for final release of tissues or cells based on testing results used to determine final release (e.g. quality controls such as viability or cells count, microbiology test results; if necessary and justified, the final release can be undertaken on a 'negative-to-date' basis);
f. absence of any serious adverse reactions and events (from previous or same donation) over the time period; in cases of MAR, the final clinical users must report the clinical outcome even in the absence of any SAE or SAR;
g. in cases of MAR/ART (gametes and embryo donation), check that the total of permitted live births does not exceed the national regulations;
h. if the tissue or cells are used autologously, the same review should be performed; if there are positive test results, it is up to the decision of the RP and the treating physician whether the tissue or cells can be used, but in any case the final decision has to be justified and recorded.

The items indicated in the release record should contain at least:

a. the procurement file and/or release statement of the person responsible for procurement;
b. type(s) of tissues and cells processed and/or stored (number of units per device or ampoule);
c. quantitative and qualitative description of the tissues and cells processed, preserved and/or stored;
d. date and time of each stage of processing and storage, identification of persons responsible for each step and the identifying reagents and materials used (batch number and expiry date);
e. status of tissues and cells at all stages of processing and storage (i.e. quarantine, release for therapeutic use, release for manufacture of medicinal products, *in vitro* research, etc.);
f. use of antibiotics, antibiotic composition and incubation period (if applicable);
g. type and amount of reagents used;
h. procedures and records concerning the processing of tissues and cells (if applicable);
i. processing data (preparation, culture technique, incubation, treatment chemicals, confirmation of clean room status);
j. data on techniques of decontamination, sterilisation or viral inactivation;
k. results of specific quality testing, depending on tissue and cell type (e.g. human leukocyte antigen (HLA), histology, radiology results, tissue or cell viability, number of CD34 cells);
l. procedures and records concerning the preservation of tissues and cells (e.g. cryopreservation method, trace of the cooling curve, glycerolisation, lyophilisation), if applicable;
m. date and time of storage;
n. method of storage;
o. storage temperature;
p. expiry date, if applicable;
q. identification of tissues and cells (i.e. donor identification code and product code). Further information on coding, labelling and packaging can be found in Chapter 14.

Access to registers and recorded release data must be restricted to authorised persons. These records must be kept for a minimum of 30 years after clinical use or discard of tissues and cells.

The person responsible for the release of tissues or cells (RP) should sign a statement that specifies fulfilment of all legal requirements, tissue and cells specifications and quality-release criteria as defined by the TE, thereby releasing the tissues and cells for storage in an inventory of tissues and cells that are available for human application. If release can not be approved, the tissues or cells need to be discarded or

can be made available for research/educational use, if a specific consent for research/educational use was given.

Released tissues should be physically separated and made visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from quarantined tissues. The TE must provide clinical users with instructions for using the tissue or cells. Clinical users must be reminded that they must report any adverse events or reactions to the TE and are responsible for traceability to the recipient (see Chapter 15).

If the tissues or cells do not comply with all specifications, there can be an exceptional release under some conditions (see §9.3.2). If autologous tissue is released with positive test results (e.g. infectious disease marker, microbiological controls), the clinical user must be informed in written form before the tissue is distributed to the hospital.

9.3.2. Exceptional release

In exceptional circumstances, a TE may agree with the organisation responsible for human application of tissues and cells (ORHA) and the treating physician that tissues or cells that do not meet the normal criteria for release can be released and used in a specific patient after giving consent, based on a risk–benefit analysis taking into consideration the alternative options for the patient and the consequences of not providing the tissues or cells. For more information, see Chapter 12.

9.3.3. Risk assessment

A documented risk assessment approved by the RP must determine the fate of all stored tissues and cells following the introduction of any new donor-selection or testing criterion or any significantly modified processing step that enhances safety or quality. Guidance on risk assessment is provided in Chapter 2.

9.3.4. Disposal of human tissues and cells

There must be a documented policy for disposal of tissues and cells that are unsuitable for clinical use. Records should include details of date, involved personnel, method of and reasons for disposal. The material should be handled appropriately and disposed of in a manner compliant with local control of infection guidelines. Human tissues, cells and other hazardous waste items should be disposed of in such a manner as to minimise the hazards to the TE's personnel or the environment, and should be in conformity with applicable European, national and local regulations.

Disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and the human body. For HPC and autologous tissues and cells it is necessary to document that the conditions for disposal defined in the consent prior to collection have been met, including (where applicable) the option to transfer the tissues and cells to another facility if the designated recipient is still alive after the agreed storage period.

In case of MAR/ART, the donors and patients must declare in writing the destiny of their reproductive material when the maximum storage period has ended (see Chapter 27). A specific signed consent is needed for the acceptance of the final disposal of a given sample.

Disposal of cellular therapy products must include a pre-collection written agreement between the storage facility and the designated recipient or the donor, defining the length of storage and the circumstances for disposal of cellular therapy products [5].

9.4. References

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Chapter 10: **Principles of microbiological testing**

10.1. Introduction

This chapter addresses the control and examination of microbiological, endotoxin and mycoplasma contamination of human tissues and cells, a process which is critical in ensuring the quality and safety of human tissue and cell grafts used for human application. This chapter also defines the approaches to, and requirements for, effective and meaningful microbiological testing of preparations of tissues and cells and the environments in which they are processed. It describes general principles that should be adopted in developing a comprehensive strategy for microbiological testing, which are based on the use of the *European Pharmacopoeia (Ph. Eur.)* microbiological test methods. However, specific characteristics of human tissues and cells must be considered and methods may be adapted accordingly. Guidance on microbiological testing for particular tissue and cell processes is provided in tissue- and cell-specific chapters.

All facilities that procure, process or store tissues and cells should have access to the services of a microbiology laboratory with a fully implemented quality-management system and access to the advice of a suitably qualified expert microbiologist.

10.2. Microbiological examination of donors

Although they do not forecast what will be the microbiological status of the tissues, microbiological blood cultures can be a useful tool for the diagnosis of bacteraemia and other infections in deceased donors [1] and living donors (e.g. stem-cell donors) of tissues and cells, in addition to the required serological examinations. The blood samples must be of sufficient quantity, collected properly within a suitable timeframe, prepared/stored appropriately so as to ensure proper testing and accompanied by relevant clinical information. Compliance with aseptic techniques has to be assured in order to evaluate the origin of a detected contamination properly.

For blood cultures, a clear protocol should be followed that addresses skin disinfection, the amount of blood obtained and the number of blood cultures. Skin disinfection is an important prerequisite for reliable results to avoid secondary contamination of the sample. At least a two-step alcoholic disinfection (preferably accompanied by sporicidal disinfection) should be done. Larger quantities of blood as well as several independent blood cultures improve the probability of detecting a bacteraemia in the donor. It is recommended to take at least 2-4 blood cultures (each aerobic and anaerobic), ideally at different time points and from different vessels. The blood cultures have to be incubated for ≥ 5 days.

- Blood samples for culture can be obtained before or after circulatory arrest. The results of blood cultures using samples collected before circulatory arrest provide useful information about the clinical status of the donor (e.g. infections with objectionable organisms) and can be a useful supplementary tool for evaluation of donor suitability. Blood cultures are valuable in evaluating the quality and safety of specific

tissues and cells especially if the tissues and cells are not terminally sterilised. The evaluation depends on numerous factors and should be based on knowledge of the particular settings, e.g. time points of blood withdrawal [2-8].

- In the situation where blood samples to perform blood-culture tests are obtained sometime after cardio-circulatory arrest (in which organs, tissues and cells may be at a higher risk of endogenous microbiological contamination) the information provided by blood cultures may be questionable because the course of *post mortem* distribution of micro-organisms is (to a large extent) unknown, and the results can be influenced by agonal spread and *post mortem* bacterial translocation. The conditions under which the deceased donors are stored may be highly variable. On the other hand, information about *post mortem* bacteraemia and fungaemia at the time of procurement may also be useful to assess the quality and suitability of tissues and cells.

The main objectives of *post mortem* cultures in the context of tissue and cell donation are three:

i. to record infections that could be either clinically unsuspected or clinically suspected but not proven *ante mortem*,
ii. to evaluate the efficacy of antimicrobial treatment in the case of procurement of tissues, and
iii. to check contamination from the procurement team and environment as well as cross-contamination between tissues or cells preparations.

The theories supportive of the ambiguity of *post mortem* culture results are agonal spread and *post mortem* bacterial translocation. Although agonal spread is less common than assumed and prevention is difficult, it appears that *post mortem* bacterial translocation is completely overcome if the body is refrigerated soon after death [2, 3]. Manipulation of the deceased donor can lead to dissemination of micro-organisms from the lung and visceral organs to the heart. However, published data suggest that neither agonal nor *post mortem* spread can be expected to produce false-positive cultures if the body is appropriately cooled, if the tissue procurement is performed within 24 hours after death and if the samples for microbiological culture are collected early during tissue procurement, with minimum possible manipulation of the deceased donor before manipulation of the gastro-intestinal tract [2, 3]. Nevertheless, a positive culture, in the particular context of organ, tissue or cell procurement, may signify contamination or cross-contamination during procedures, indicating problems with *post mortem* microbial spread, recovery techniques and/or environment. These possibilities should be evaluated because they may affect the suitability of particular tissue and cell preparations and of other tissues or cells that eventually could have been affected by cross-contamination, or may imply that particular attention should be applied to decontamination and/or sterilisation methods.

10.3. General considerations for microbiological testing

The approaches outlined in this section cover the microbiological testing of procurement, processing, storage and release of tissues and cells. Microbiological testing by sampling and culturing of the tissues or cells is the most direct measure of microbiological contamination.

10.3.1. Sampling

In principle, microbiological testing must be carried out on representative pre-processing samples of the procured tissues or cells and on post-processing samples of every final tissue and cell graft, since a number of microbial contaminants can firmly adhere to or persist inside tissues and cells [9]. Sampling must be completed immediately before packaging or as late as possible during the procurement or manufacturing process. In cases where the nature of the procured tissues and cells or final tissue and cell grafts does not allow sampling, an alternative sampling approach may be undertaken. For example, transport, storage, rinsing, washing or culture solutions can be used instead of the primary samples. If an appropriately validated terminal sterilisation process is applied, parametric release allows tissue establishments to replace microbiological testing of samples drawn from the finished product as a release criterion with acceptance criteria for the control of identified process parameters.

In addition, where applicable, in-processing samples should be tested on a regular basis according to a risk assessment, considering the nature of the tissues and cells and critical processing and storage steps. In-process testing should be performed at relevant steps of the procurement or production process, such as:

- bioburden testing before a terminal sterilisation procedure or decontamination procedure (e.g. antibiotic soaking);

- microbiological testing after a stage of decontamination or inactivation, before final storage, though ideally a disinfectant- or antibiotic-free period should precede sampling if a validated terminal sterilisation method is not applied;
- microbiological testing after washing steps or changing of the storage medium, particularly when decontamination processes cannot be applied.

10.3.2. Testing for bacteria and fungi

Aerobic, anaerobic and fungal testing of tissue or cells samples under appropriate incubation conditions is the most direct qualitative measure of microbiological contamination and must be conducted for pre-processing samples of the procured tissues and cells and on post-processing samples of every final tissue and cell graft. Considering the nature of the procured tissues and cells and any subsequent processing steps, the microbiological testing approach should follow the procedures outlined in the *Ph. Eur.*, in particular chapters 2.6.1, 2.6.12, 2.6.13 and 2.6.27. Deviations from such standards should be justified, and alternative test methods must be validated in accordance with chapter 5.1.6 of *Ph. Eur.* Factors such as samples containing antibiotics or very small sample amounts may affect the sensitivity of tests leading, in the worst-case scenario, to false-negative results. Testing should be extended to specific micro-organisms known to represent potential contaminants on the respective tissues or cells where transmission may become relevant for infection [10]. These micro-organisms may not be detectable with common culturing media; therefore, additional tests for specific infectious agents should be undertaken. If applicable, an exclusion list for non-acceptable micro-organisms should be compiled.

If release of the tissues or cells is necessary before the end of the officially verified/required testing period, negative-to-date reading of the results may be carried out. In this case, intermediate results of the final testing in combination with intermediate or final results of in-process testing are used for tissue or cell release. The clinician using the relevant graft must be notified so they can decide if its application is clinically justified. Final testing is still ongoing after the tissue or cell graft is released and will be completed in line with the above-mentioned requirements. If micro-organisms are detected after tissue or cell release, predefined measures such as identification and antibiotic sensitivity of the species must be carried out and information must be provided immediately to clinicians caring for the patient. Alternative, rapid microbiological methods should be considered, especially for preparations of tissues and cells with a short shelf-life. Independent of the applied method, the fitness for purpose must be shown according to *Ph. Eur.* 2.6.27 or 5.1.6. Deviations from these standards should be justified.

10.3.3. Testing for mycoplasma

Depending on the type of preparation and manufacturing process, it can be necessary to complement the microbial test concept by additional tests for specific infectious agents such as mycoplasma (*Ph. Eur.* 2.6.7). To identify and assess the contamination risk of specific tissues or cells, a sufficient number of samples from different tissue or cell batches should be examined. Possible sources for mycoplasma contamination include the cellular starting material itself or its procurement, animal- or human-derived raw materials such as untreated sera, personnel in the clinic or manufacturing site, and the entire manufacturing process.

Mycoplasma can penetrate sterilising-grade filter membranes with a nominal pore size of ≤ 0.2 µm. They also lack a rigid cell wall, which makes them unsusceptible to antimicrobial agents that target the cell wall. Furthermore, many broad-spectrum antibiotics inhibit the proliferation of mycoplasma but do not kill them. Hence, elimination with antibiotics is difficult.

Contamination with mycoplasma represents a potential risk for the patient because of transfer of infectious microbial agents into a potentially immunocompromised patient, but the effect of mycoplasma contamination on the tissue or cell preparation might also be critical. Mycoplasma are known to alter cell function, leading to an alteration of gene expression, cellular signalling and metabolic activity.

Testing should be conducted at manufacturing steps at which mycoplasma contaminations would most likely be detected, such as after pooling or collection but before washing steps. Mycoplasma are cell-associated micro-organisms that may even locate within the cell, so testing should always include the tissues or cells, if possible.

10.3.4. Testing for bacterial endotoxins

The need for routine testing for endotoxins is dependent upon the intended application of the tissues and cells, and the estimated impact of endotoxins on the recipient. For example, endotoxins in

pancreatic islets will negatively affect insulin production and the outcome of transplantation. If deemed necessary, endotoxin testing should be carried out according to *Ph. Eur.* 2.6.14 and 5.1.10. In any procedure in which animal products are used (e.g. collagenase, trypsine), endotoxin testing should be done.

The presence of endotoxins in tissues or cells can result in responses ranging from fever to irreversible and fatal septic shock. Endotoxins are the lipid portions of the lipopolysaccharide (LPS) macromolecule structures of Gram-negative bacteria, and of some cyanobacteria, which form an integral part of the cell wall. During bacterial growth, membrane complexes containing endotoxins may exist, bound to the cell surface or shed in small amounts into the environment, but they are released in greater concentrations on the death and lysis of the cell. These complexes accumulate both *in vitro* and *in vivo*, and release is enhanced by cell lytic compounds and antimicrobial agents.

Endotoxins have important roles in cell cultures because they can alter the evolution of cell cultures and thereby impair the safety and efficacy of the cell graft. The potential sources of endotoxins in cell cultures are:

a. glassware and plastic ware used in the laboratory;
b. washing solutions or water used to prepare media and solutions;
c. media and sera used during cell culture;
d. any components and additives.

Hence, it is recommended to use raw materials certified to be free of endotoxins by their manufacturers and to utilise pre-sterile, single-use items whenever practicable.

Each laboratory that works with cell cultures should have a specific risk assessment and risk analysis that should include when and how to carry out an endotoxin test, together with all microbiological controls that are considered necessary. It is recommended to carry out an endotoxin test on the final cell graft before release to the patient.

Several methods have been applied for the determination of endotoxin levels in a sample. Today the most widely used assay method for endotoxin is the Limulus Amoebocyte Lysate (LAL) assay based on the reaction of LPS with a clotting protein secreted by the horseshoe crab *Limulus polyphemus*. There are three basic LAL methods: the gel clot, turbidity measurement and chromogenic assay. The latter two may be used as kinetic methods and are claimed to be more sensitive, being able to detect 0.001 international (endotoxin) units/mL [11]. Several factors – such as pH, protein concentration, metal ions and some chemicals – can influence the sensitivity of LAL tests. Different endotoxins may differ markedly in their activity by weight in both pyrogen testing and LPS-specific methods; the reference standard may also vary between laboratories, making comparisons difficult. Consequently, endotoxin activity is expressed universally in international units (IU) against an LPS standard preparation of a reference strain of *E. coli*. As a guide, 1 IU is considered to represent approximately 0.1 ng (10^5 fg) of endotoxin and is equivalent to a weight between 2 and 50 fg/cell or between 2 000 and 50 000 bacterial cells, depending on the bacterial species.

In terms of a risk threshold, the maximum permissible level of endotoxin in injectable cell grafts is 5.0 IU per kilogram of body weight, which defines whether a cell graft is pyrogenic or non-pyrogenic [12]. For injectable cell grafts the total amount of endotoxin that can be administered to an adult human of 70 kg should not exceed 350 IU (70 kg × 5 IU). However, the critical amount of endotoxin required to initiate the sepsis cascade is unclear and depends greatly on the virulence of the infecting organism. Endotoxin concentrations in clinically septic episodes reportedly range from non-detectable to 12 IU/mL in plasma, with an approximate mean of 2.5 IU/mL [13].

Due to the variability in the responses of cells to endotoxins, it is not possible to state the critical level at which endotoxins begin to interfere with the function and growth of cells. The endotoxin limit that can be accepted in cell grafts is based on the route of administration (intravenous or intrathecal), the threshold pyrogenic dose and the volume of the injected cells. For certain cellular grafts, ones that must be administered immediately and that cannot be cryopreserved without damaging the viability and quality of cells, the availability of a rapid testing method for endotoxin testing is fundamental. The bacterial endotoxin test (BET) quantifies only the amount of endotoxin. The monocyte activation test (MAT) detects or quantifies substances that activate human monocytes or monocytic cells to release endogenous mediators such as pro-inflammatory cytokines. Consequently, the MAT will detect the presence of other pyrogens in the test sample [14-16]. The method is highly sensitive (≈ 10 pg/mL of endotoxin), but physicians should take into consideration that this method is also able to detect non-endotoxin pyrogens. More recently, several electrochemical and optical biosensing techniques using various detection platforms have been developed, some offering high sensitivity (0.01-1 ng/mL) for LPS [17].

10.3.5. Microbiological testing required for specific processing methods

10.3.5.1. *Processing using closed systems*

For cells in which a closed system is used for processing and for cell therapies where no further cell-cultivation steps are conducted, repeated testing steps are not suitable and do not yield more information on the microbial status of the cell graft. In such cases, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point may be applicable. For microbial testing of haematopoietic progenitor cell preparations, methods need to be validated before use, e.g. matrix validation [18].

10.3.5.2. *Processing with terminal sterilisation*

For preparations that undergo a validated sterilisation process, the requirements of *Ph. Eur.* 5.1.1 should be considered where methods of sterilisation are described. In particular, it must be shown that adequate precautions have been implemented to minimise the microbial contamination before sterilisation and that tissues and cells with an acceptably low degree of microbial contamination have been used as determined by bioburden testing (see §10.3.6).

Procedures and precautions employed for sterilisation are to be such as to give a sterility assurance level (SAL) of $\leq 10^{-6}$ [19].

If the release of tissues and cells sterilised in their final container (terminal sterilisation) is intended to rely on process data only, and not on final tissue or cell testing for sterility ('parametric release'), then validated procedures for all critical production steps and a fully validated sterilisation method must be applied. This approach includes validation of procurement of tissues or cells, transportation, washing, antibiotic treatment and other processing steps, packaging and storage. In some countries authorisation by the competent authority is needed for such an approach.

10.3.5.3. *Processing that includes decontamination of tissues and/or cells*

Procedures applied for decontamination of tissues and cells are usually limited to approaches in which the vitality and functionality of the preparation can be maintained. For instance, treatment with antibiotics and anti-fungal agents is employed widely to achieve reduction of the microbial load in tissues and cells. Because of virulence mechanisms such as facultative intracellular parasitism, biofilm formation and persistence in a resting metabolic state without growth (which is reversible upon withdrawal of antimicrobial agents or stress conditions), micro-organisms can escape these treatments.

Further processing after the decontamination step should be conducted without antimicrobial agents. Methods for testing of final tissue and cell grafts must be evaluated carefully with respect to possible inhibition of microbial growth due to decontaminating agents or their residues.

10.3.5.4. *Open processing without terminal sterilisation*

Most tissues and cells, including preparations which have been decontaminated, are exposed to the environment at certain processing stages between procurement and packaging. Without terminal sterilisation, the environmental contamination risk during open processing must be avoided to the greatest possible extent. The requirements for microbiological sampling and testing are expected to be most stringent in these situations, and aseptic conditions must be maintained during procurement, transportation and the whole manufacturing process.

If open processing takes place without terminal sterilisation, the sampling and microbiological assessment should include the starting material, the transport solution and any solutions used to wash tissues and cells. *Ph. Eur.* 2.6.1 provides a means of verifying that the tissues and cells are sterile. Alternatively, depending on the nature of the tissue or cell-based preparation, the approaches in *Ph. Eur.* 2.6.27 or *Ph. Eur.* 5.1.6 may be applied. If a preparation is not required to be sterile or cannot be rendered sterile, *Ph. Eur.* 2.6.12 and 2.6.13 can be employed (as described in §10.4.3) allowing quantitative enumeration of micro-organisms or qualitative methods to determine the culture's negative or positive status. Such testing may require use of validated methods employing special media and/or conditions to enable growth of such micro-organisms and their detection. In addition, the final tissue or cell graft should be tested to ensure quality and safety for clinical use.

10.3.6. Bioburden testing

Knowledge is necessary about the acceptable quantitative microbial load of the starting material before decontamination procedures and/or terminal sterilisation [19]. Bioburden is usually expressed as a measure of the numbers and variety of micro-organisms on a surface or volume.

For *post mortem* procured human tissues, the quantification of microbial load of the starting material prior to processing and preservation is an essential prerequisite to inform the potential infection

risk of tissue grafts to an intended recipient. Although bacterial and fungal infection post-transplantation is rarely reported, some virulent microbes when present in high numbers on a tissue surface may form cytotoxic exoproducts such as proteases and toxins, which can have a deleterious effect on the cellular integrity or structural properties of the graft [20, 21]. In practice, estimation of bioburden involves:

a. an approximate determination of the numbers of microbes in a tissue or cell sample,
b. identification of the species present, and
c. establishment of acceptance/rejection criteria based on the species identified.

For the assessment of the microbial load of tissues and cells, *Ph. Eur.* test methods 2.6.12 and 2.6.13 can be employed. Where appropriate, tissues or cells can be sampled by a representative tissue or cell sample suspended in a broth medium and, less commonly, by a culture swab or suspension of an initial wash of the specimen. Although swab-based methods have a low efficiency of recovery, generally less than 20 % [22, 23], they allow a crude estimation of viable microbial contaminants as heavy, moderate or light and the identification of individual species helps to inform decisions as to the potential hazard of the contaminant. Rigorous sonication/mechanical shaking methods, exposing representative tissue samples to an extraction fluid with a surfactant, can increase the recovery efficiency [22].

Table 10.1. **Pathogenic, highly virulent micro-organisms that could result in tissue discard unless treated with a disinfection or sterilisation process validated to eliminate the infectivity of such organisms [26-29]**

Staphylococcus aureus
Pyogenic *Streptococci*, *Enterococcus* spp.
Non-fermenting micro-organisms: *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomas maltophilia*, *Sphingomonas paucimobilis*, *Burkholderia cepacia*
Sporulating micro-organisms: *Bacillus* spp. (*B. anthracis*, *B. cereus*), *Clostridium* spp.
Enterobacteriaceae (*Escherichia coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp.)
Anaerobic Gram-negative micro-organisms (o.a. *Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp.)
Yeast and filamentous fungi (moulds)

Note: This suggested list is not exhaustive. Some chapters in Part B of this guide have a more detailed list of pathogenic, highly virulent micro-organisms for the specific tissues or cells.

Coagulase-negative *Staphylococci* and non-pyogenic *Streptococci* are generally the most frequently isolated organisms [24] and are often susceptible to antibiotic decontamination procedures [25]. Efficacy studies focusing on the usually expected initial bioburden of tissues or cells, as well as the type and concentration of effective antimicrobial agents, should be carried out. Based on those studies, temporary treatment schemes and an exclusion list of specific contaminants for the incoming material should be determined, based upon not only the category type of tissue but also upon the method by which the tissue was processed. Table 10.1 provides a list of pathogens that could result in the discard of the tissues unless they are treated (e.g. cryopreserved musculoskeletal tissues that cannot be 'sterilised' and can only be 'disinfected').

10.4. Conditions and methods of microbiological testing

For each procedure, aerobic and anaerobic testing must be conducted under incubation conditions that are appropriate for the detection of tissue- or cell-specific bacteria and fungi (yeasts and moulds) as well as bacteria and fungi of environmental or clinical origin. If indicated, control tests must be carried out for specific micro-organisms that may not be detectable with the culturing media recommended in the relevant chapters of *Ph. Eur.* (e.g. *Mycobacterium* spp., fastidious micro-organisms).

10.4.1. Sterility testing (*Ph. Eur.* 2.6.1)

Conditions for sterility testing are detailed in Table 10.2. Precautions should be taken against microbial contamination during a test (*Ph. Eur.* 2.6.1 and 5.1.9). At the least, sub-cultivation should be carried out in a Grade A laminar airflow cabinet, properly disinfected before the test, and no other activity should be conducted at the same time. The preferred microbiological control procedure is dependent on the sample material.

10.4.1.1. *Membrane-filtration method*

This method uses membrane filters having a nominal pore size ≤ 0.45 µm whose effectiveness to retain micro-organisms has been established.

10.4.1.2. *Direct inoculation method*

The direct inoculation method is suitable for solutions and tissue samples (i.e. solid substances). Sample quantities are listed in *Ph. Eur.* 2.6.1.

For both methods, microbial growth media are assessed macroscopically for evidence of microbial growth. During incubation, at least one intermediate reading and one final reading should be made. If the test carried out on the sample material results in tur-

bidity of the culture medium, sub-cultivation must be carried out (see *Ph. Eur.* 2.6.1).

Ph. Eur. does not include specific microbiological growth media for the detection of fungi because fungi are detected along with aerobic bacteria in soya-bean casein digest medium. However, other media and /or incubation temperatures may be used, provided that they pass the growth-promotion and validation tests (according to *Ph. Eur.* General Notices on alternative methods). Examples of alternative incubation temperatures that might be used, if equivalence with *Ph. Eur.* has been demonstrated, are shown in Table 10.3.

10.4.2. Microbiological testing using automated culture systems (*Ph. Eur.* 2.6.27)

10.4.2.1. *Incubation conditions*

Use of a validated automated culture system may be advantageous, especially for liquid samples containing cellular material which already results in turbidity of the culture media immediately after inoculation.

Incubation in automated culture systems should be carried out over at least 7 days. The testing time can be adapted to specific requirements arising from the characteristics of the preparation. For example, if risk assessment identifies potentially slow-growing micro-organisms such as *Cutibacterium acnes (Propionibacterium acnes)* the testing time could be extended up to 14-21 days.

The time and temperature of incubation may be too limited to sufficiently account for a broad range of contaminating micro-organisms found in the environment or in tissues and cells. Hence, the incubation conditions detailed in Table 10.3 are recommended as alternatives on the basis of risk assessment, taking into account the expected microbial flora and environmental conditions. Testing times should be validated.

Preparations of tissues and cells with a short shelf-life may be released based on an intermediate readout of the test before the test period is completed (negative-to-date result). In the case of a positive readout during the test period after release of the tissue or cell graft, identification of the microbial species and a resistogram must be carried out and the information immediately forwarded to the caring physician.

10.4.2.2. *Sample volume*

For automated culture systems, sample volumes recommended up to 10 mL can be inoculated per culture bottle. Very small sample amounts of < 1 mL may bear the risk of an increased sampling error, leading to false-negative results, if only a low microbial count is present in the tissues or cells. Certain conditions, such as the usual small initial microbial count and early sampling during the production process or delayed microbial growth in the tissues or cells due to inhibiting substances or unfavourable temperature, are reasons for this. Therefore, a large amount of sample should be envisaged for inoculation (if applicable and appropriately validated).

10.4.2.3. *Samples without antimicrobial additives*

Microbial growth media without any neutralising additives of microbial growth inhibitor should be used. If sensitive organisms are not identified as a possible contaminant in the risk assessment, it is not necessary to add a fastidious organism supplement.

10.4.2.4. *Samples with antimicrobial additives*

Microbial growth bottles with resin or activated charcoal neutralising additives of microbial growth inhibitor should be used (if membrane filtration cannot be carried out due to the nature of the sample).

The type, amount and mixture of antimicrobial agents used in manufacturing processes (and therefore present in samples for microbiological testing) is highly variable. Culture bottles containing adsorbing substances are established for the testing of patients' blood with therapeutic doses of a limited number of antibiotics or antimycotics. Therefore, such samples must be validated very thoroughly for residual antimicrobial activity to prove the suitability of the chosen method.

Table 10.2. **Incubation conditions for sterility testing**

	Culture medium	Incubation temperature °C	Testing period
Aerobic	Soya-bean casein digest medium (TSB)	20-25	14 days
Anaerobic*	Fluid thioglycolate medium	30-35	14 days
Fungi	Soya-bean casein digest medium (TSB)	20-25	14 days

* Fluid thioglycolate medium will also detect aerobic bacteria.

Table 10.3. **Alternative incubation conditions for microbiology testing***

	Aerobic incubation	Anaerobic incubation
Option 1	20-25 °C normally (automated system); 30-35 °C if necessary (automated system)	30-35 °C (automated system)
Option 2	35-37 °C (automated system); and, where relevant, additional incubation at a lower temperature (manual method)†	35-37 °C (automated system)
Option 3	30-32 °C (automated system)	30-32 °C (automated system)
Option 4	30-32 °C (automated system)	35 °C (automated system)

* Testing period is ≥ 7 days with an automated growth-based method and may be extended up to 14 days. Testing period is 14 days with a manual method.

† Where relevant, incubate in addition at 20-30 °C. Incubation can be done using commercially available microbiological media, either aerobic bottles intended for automated systems or tryptic soy broth (TSB).

10.4.2.5. *Period between inoculation of culture bottles and incubation in an automatic culturing system*

Inoculated culture media bottles should be placed into the automated culture system as soon as possible; if a delay occurs, they should be maintained at 15 °C-20 °C [30]. If a delay period of 12 hours is exceeded, the results of the automated culture system must be verified by subculture unless otherwise validated. For some automated systems and delayed kinetic culture bottles, manufacturer's specifications mention as acceptable a delay up to 48 hours at room temperature. However, given the variability in terms of delay time, pre-incubation temperature and type of micro-organism, a delay exceeding 12 hours should be validated.

10.4.3. Bioburden testing (*Ph. Eur.* 2.6.12 and 2.6.13)

The tissue-associated bioburden of aerobic mesophilic bacteria and fungi can be quantitatively enumerated either by membrane filtration or plate-count methods according to *Ph. Eur.* 2.6.12. The preferred microbiological recovery procedure is dependent on the type of sample. Different mechanical techniques can be used, alone or associated, to extract micro-organisms of the product before filtration or plate-count method (e.g. sonication, crushing, blending, shaking). The addition of a surfactant, such as polysorbate 80, can improve the extraction. The extraction method should be validated. A correction factor can be calculated from the validation data. The tissue-containing jar can further be sonicated for 5 minutes (47 kHz) followed by mechanical shaking for 30 minutes (200 strokes/minute) on a linear reciprocal shaker [22].

10.4.3.1. *Membrane-filtration method*

An appropriate volume of the extraction fluid is filtered through at least two appropriate filters (e.g. 0.45 µM pore size nitrocellulose filter). One filter should be transferred to the surface of a casein soya-bean digest agar plate for determination of the total aerobic microbial count (TAMC) and one filter to the surface of a Sabouraud-dextrose agar plate for determination of the total combined yeasts/moulds count (TYMC). The casein soya-bean digest agar should be incubated at 30-35 °C for 3-5 days and the plate of Sabouraud-dextrose agar at 20-25 °C for 5-7 days. In addition, anaerobic bacteria can be enumerated by transfer of a filter to an appropriate medium plate (e.g. thioglycolate agar) and anaerobic incubation at 30-35 °C for 3-5 days. The microbial count can be calculated as colony-forming units (CFU) per unit weight or volume (gram or millilitre) or surface area of tissues and cells.

10.4.3.2. *Plate-count methods*

At least two Petri dishes for each level of sample dilution for each medium are prepared, either by the pour-plate method or by the surface-spread method. Plates of casein soya-bean digest agar, Sabouraud-dextrose agar and anaerobic medium agar are incubated as stated above. Plates should be counted which show the highest number of colonies less than 250 for TAMC and 50 for TYMC corresponding to a given dilution. The arithmetic mean per culture medium of the counts is used to calculate the number of CFU per unit weight or volume (gram or millilitre) or surface area of tissues and cells.

For the determination of the absence or limited occurrence of specified micro-organisms, test conditions are described in *Ph. Eur.* 2.6.13, in which the growth-promoting, inhibitory and indicative properties of respective media are prescribed.

10.4.4. Alternative methods for control of microbiological quality (*Ph. Eur.* 5.1.6)

Alternative rapid microbiological methods (RMM) based on novel technologies that provide automated, faster and more sensitive microbiological results as compared with classical or conventional methods may be used to test in-process samples, to demonstrate sterility of cell grafts that have a shelf-life much shorter than the required testing time for the current compendia sterility test (e.g., 2-5 days *versus* a 14-day testing period) or in other special circumstances; see Table 10.4.

Such alternative methods must be validated. The validation process includes validation for the intended use, and equivalence or non-inferiority to the compendia methods.

Table 10.4. **Alternative methods for control of microbiological quality**

Growth based methods	Electrochemical
	Gas consumption/production
	ATP bioluminiscence
	Turbidimetry
Direct methods	Cytometry • solid phase • flow cytometry
Cell components methods	Phenotypic
	Genotypic • RT-PCR • Genetic fingerprinting

10.5. Validation of microbiological testing methods

10.5.1. Growth-promotion testing

Each batch of the microbiological culture medium used for microbiological testing must be tested for its growth-promoting capacities as well as being used to test for the microbial strains listed in the relevant *Ph. Eur.* chapters. In general, it is recommended to include in the assays any possibly relevant microbial contaminants from the respective tissue preparation or cell preparation or the environment – for instance, *Cutibacterium acnes (Propionibacterium acnes)* and *Micrococcus* spp. – because of their specific growth properties.

Growth-promotion testing can also be done for the plates and media used in environmental monitoring. Growth-promotion testing must show that the media are suitable to consistently recover environmental contaminants (if they are present). The standardised list should comprise organisms based on the literature and/or environmental isolates, and should include a reasonable range of 'representative' micro-organisms that could be encountered in manufacturing environments (e.g. Gram-positive rods, Gram-positive cocci, filamentous moulds or yeasts, Gram-negative rods). The list should contain a minimum of five unique microbial strains [19].

10.5.2. Method suitability

The method must be verified in the presence of the intended sample material (e.g. transport medium, final tissue or cell graft). The basis for method verification is the 'method-suitability test' laid down in the relevant chapters of *Ph. Eur.*

The same conditions must be chosen as for routine testing (e.g. culture conditions, sample type, sample amount). The method-suitability test must be undertaken using the bacterial and fungal species indicated in the relevant chapters of *Ph. Eur.* For microbiological methods not described in *Ph. Eur.*, validation is required, as described in Chapter 5.1.6 of *Ph. Eur.*

It is recommended to complement the microbial spectrum by tissue-specific and/or contaminating micro-organisms, if found to be applicable to the process, such as *Cutibacterium acnes (Propionibacterium acnes)* and *Micrococcus* spp., which are typical skin contaminants.

For instance, *Cutibacterium acnes (Propionibacterium acnes)* is not readily accessible to skin disinfection due to its prevalence in the sebaceous glands, and detection of this species in tissue preparations is not unusual. *Cutibacterium acnes (Propionibacterium acnes)* grows under anaerobic or microaerophilic conditions as a 'slow-grower' and is associated with particularly long detection time, so it may be included in method validation. If any other micro-organisms are considered to be relevant during processing and if present in the environment, they should also be included in validation studies.

The sensitivity of the chosen method should be shown, as described in the relevant chapters of *Ph. Eur.*, by experimental studies. Applicability of this method must be assessed in connection with its impact to ensure microbial safety of the tissues and cells.

Each micro-organism species should be tested to evaluate the capacity of the method to allow a good detection of the micro-organisms. The efficacy of the method to extract the micro-organism should be repeated with different samples (minimum 3) with a

minimum of one strain. A strain of a bacterial aerobic germ is commonly chosen for this step, showing thus the robustness of the method.

For comparison, a positive control (without tissues or cells) must be included in the test for each test strain.

Inoculated media must be incubated under the conditions applied in routine testing (temperature, duration) and checked for growth at regular intervals.

Test assays and controls must be evaluated in predetermined intervals during and at the end of the testing period. Samples for subculture must be taken from positive detected tests as quickly as possible. In the case of microbial growth, the micro-organisms must be identified.

If inhibition of microbial growth by the sample material is identified at validation, the method must be adapted in an appropriate way, for instance, using a higher volume of the culturing media or addition of binding or enzymatic substances that inhibit antimicrobials.

10.5.3. Documentation and interpretation of results

All materials used and working steps undertaken must be documented. Interpretation of results should include at least the following factors:

a. assessment of the growth of micro-organisms in the presence of the tissues or cells to be tested and in controls;

b. specification of the microbial count for evaluation of the method;

c. period of time until a positive result has been detected for test assays and positive controls;

d. proof of identity of inoculated micro-organisms.

For negative controls or test assays without detection of micro-organisms, the total testing period and results of the subculture (including the methods used) must be specified.

10.6. Interpretation of results and actions to be taken

In general, source material that demonstrates contamination must be rejected unless the preparation undergoes decontamination and/or terminal sterilisation, and the detected quantity and quality of micro-organisms can be reliably inactivated or removed by the intended procedure, or if it is justified by exceptional clinical circumstances (refer to Part B for specific examples).

Contaminated source material should be rejected if processing includes decontamination (but not terminal sterilisation) and if risk assessment considering the intended route of administration cannot exclude risk to the recipient even if adequate antimicrobial treatment is initiated. Such source materials should be evaluated on the basis of qualitative (exclusion list for objectionable micro-organisms) and quantitative (microbial count, bioburden) microbiological control tests, and specifications should be given. The decontamination procedure should be shown to be suitable to remove or destroy the type and number of contaminants allowed in the source material. In particular, multi-drug resistant micro-organisms – e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE) and extended-spectrum beta-lactamases (ESBL) – and possible toxin-producing micro-organisms, such as *Pseudomonas aeruginosa*, *Streptococcus* group A, *Staphylococcus aureus*, *Clostridium* and *Bacillus,* as well as yeasts and filamentous fungi, need to be evaluated carefully, and if appropriate, the tissues and cells should be rejected.

In the case of locally acquired contamination or a local infection, the microbiological result applies only to the tissue where the contamination was detected and to tissues that could have been cross-contaminated. If bacteraemia, septicaemia (anamnestic or blood culture) or any other distribution of the objectionable micro-organisms (at procurement, storage, transport, manufacturing) cannot be excluded, other tissues should be rejected.

For contaminated autologous preparations, or preparations received from a specific allogeneic donor, whereby a repeated procurement cannot be conducted or involves a high degree of risk, risk assessment based on the urgency of the application, judgment of infection risk and treatment options must be conducted. In any application of such preparations, measures must include full identification of the contaminating micro-organism and their resistograms, as well as adequate prophylaxis of the donor/recipient if the tissues or cells must be used.

10.7. General considerations for environmental microbiological monitoring

Guidance for planning and carrying out environmental monitoring of cleanrooms, clean zones, processing methods and other equipment is described in Chapter 7.

Table 10.5. **Environmental microbiological monitoring methods**

Method	Air or surface qualitative or quantitative	Notes
Volumetric sampling	Air quantitative	Can be used to quantify bacteria and fungi suspended in the air surrounding the open product. Active sampling can be used to detect homogeneous suspensions of micro-organisms in the air, but it is not a reliable measure of the sporadic contamination that occurs during operations. The chosen device should be shown to correspond to current standards of sensitivity and detection. In general, the manufacturer of the sampling instrument will recommend sample sizes, and this recommendation must be taken into account in the design of sampling strategies. Sample sizes ≥ 1 m^3 should be taken for each measurement. If this sample size results in an unreadable number of colonies, reduced volumes may be employed to monitor Class C and D areas if justified. The effect of capture-plate drying during sampling and transport to the microbiological laboratory should be determined by a validation study. Time limits should be set to ensure that micro-organisms remain viable up to the point of transfer to an environment for detection of growth promotion.
Settle plates	Air qualitative	This is the only method that can provide continuous monitoring of micro-organisms. Can be used to detect bacteria and fungi that descend in a column of air over the plate. Need to determine how long the plates can be open (usually 2-4 h). Exposed plates may be replaced by unused ones so that total time of exposure is reached.
Contact plates	Surface qualitative	Plates or strips can be used. Contact plates can be used to detect contamination by micro-organisms in the immediate vicinity of the work area. The pressure and duration of contact have a significant influence on microbial yield (recommendation: uniform pressure for 10 s). Tested surfaces must be cleaned after sampling.
Swabs	Surface qualitative	Used for wiping of surfaces that cannot be sampled with plates or strips. A swab dampened with sterile physiological (0.9 %) NaCl solution is used to test dry surfaces. A dry swab is used to test damp surfaces. The sample material collected is then wiped onto an agar plate. The sample area should be ≈ 25 cm^2, if possible. Tested surfaces must be cleaned after sampling.
Glove prints	Glove or fingertips qualitative	Fingertips are the most likely area to come into contact with microbial contamination on work surfaces, on materials or arising from the operator and then transferred onto products. Glove prints (all five fingers) should be taken to assess this possibility. Usually placed on contact plates after processing or before changing gloves. Gloves must not be disinfected immediately before samples are taken. A firm and even pressure should be applied for ≈ 5-10 s taking care not to damage the agar surface.

Table 10.6. **Incubation conditions for environmental microbiological monitoring**

	Culture medium	Incubation temperature	Incubation period
Aerobic Fungi	Trypticase soy agar irradiated	20-25 °C + 30-35 °C	3-5 days + 2-3 days
Alternative incubation conditions			
Aerobic*	Trypticase soy agar irradiated	30-35 °C	2-3 days
Fungi	Sabouraud agar	20-25 °C	5-7 days

* When applicable, consider also anaerobic testing in the same culture conditions as aerobic testing but in anaerobic atmosphere.

Microbial samples can be taken using four sampling methods: volumetric air sampling, settle plates, contact plates and glove prints – or fingerprints. A non-selective culture medium, permitting growth of the expected micro-organisms, and containing additives to overcome the residual effect of biocides and cleaning agents, should be selected. Additives inhibiting residual biocides and cleaning agents are an essential component of the culture medium.

At present no commercial neutraliser is able to inactivate all biocides. The choice for a sanitation and disinfection programme with a specific cleaning agent and biocide must be well considered. The concentration of residue left on the surfaces after cleaning depends on the type of biocide and the sanitation programme. The culture medium used for environmental monitoring has to be appropriately validated for the growth of diverse bacteria and fungi, and it must be possible to demonstrate that the resi-

dues generated by the sanitation programme do not interfere with micro-organism recovery. Table 10.5 summarises the characteristics of these sampling methods.

10.7.1. Incubation of samples

Environmental monitoring samples should be incubated at a minimum of two different temperatures to detect bacteria and fungi. Incubation conditions for environmental microbiological testing are detailed in Table 10.6. Incubation for 3-5 days at 20-25 °C followed by incubation at 30-35 °C for an additional 2-3 days has been shown to be sufficient to detect most bacteria and fungi. The method chosen should be validated and standardised very carefully. Alternative methods are acceptable if high recoveries (> 90 %) of micro-organisms of interest can be demonstrated consistently [31].

If micro-organisms are expected in the environment, and cannot be detected using standard media for environmental monitoring with the temperatures recommended above, the procedure must be adapted accordingly.

10.7.2. Data analyses

Reading of plates should be done according to a defined, standardised procedure. Identification of CFU should be undertaken according to the environmental monitoring programme of the tissue establishment. According to EU GMP, detected CFU in Grade A areas must be identified to the genus or species; in Grade B areas, detected CFU should be identified.

10.8. References

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Chapter 11: **Distribution and import/export**

11.1. Introduction

This chapter describes the requirements for distribution of tissues and cells including unfinished products and defines recommended controls for their import and export. The term 'distribution' should be understood to mean transport and delivery of tissues or cells intended for human application. The term 'unfinished' should be understood to describe tissues and cells just after procurement or during processing. 'Transport' is meant as the act of transferring a tissue or cellular product between facilities under the control of suitably trained, designated and authorised staff at the distributing and receiving facilities. 'Shipment' is a type of transport where the transfer of the tissues or cells from the distributing to the receiving facility is carried out by means of a contract with a third party, usually a specialised logistics company. The entire distribution chain must be validated appropriately, including the equipment used, to ensure the maintenance of critical transport or shipment conditions.

The terms 'import' and 'export' should be understood to include all processes and procedures that facilitate the entry or exit of tissues and cells, whatever their step of processing, to/from a single country. Import/export controls must ensure that the quality and safety of the tissues or cells are in compliance with this Guide.

Tissues and cells can be transferred by a tissue establishment (TE):

- to a clinical facility within the same country, where they will be applied (i.e. distribution);
- to another TE within the same country for local distribution.

Cross-border movement of tissues and cells includes transfers:

- to a TE outside the country (i.e. export);
- from another country to a clinical facility or TE in the country (i.e. import).

For transfers of tissues or cells between countries that are within the European Union (EU), usually referred as 'distribution', the legislation does not require import/export controls to be in place provided that these tissue and cell preparations come from a duly authorised TE and their processes have also been authorised by the national competent authority. However, several EU member states opt to apply more stringent requirements than those in the directives and consider this movement in the same way as import/export involving countries outside the EU (referred to as 'third countries'). Written agreements might be needed between countries in some member states (see §11.5.5).

11.2. Release

Prior to distribution, a comprehensive record review should ensure that all elements of collection, processing and storage have met the established quality criteria, including identity of the product (see Chapter 9). In a case of incomplete eligibility of the donor, the product must be released only for documented urgent medical need (see §12.5 on release). An

alternative plan of transport or shipping should be available in case of emergency situations, to prevent possible clinical complications to the recipient. The courier should be able to contact the receiving facility on a 24-hour basis in case of delay during transit.

11.3. Transport

The choice of mode of transport should take into account any general regulations governing transportation of biological substances and any specific handling, storage or transportation recommendations provided by the TE.

Critical transport conditions, such as temperature and time limit, must be defined to ensure maintenance of the required properties of tissues or cells [1]. When transport is carried out under storage conditions, the impact on transport time is minimal. Unfrozen products are usually transported refrigerated (2 to 8 °C), or cooled (8 to 15 °C) or at room temperature (15 to 25 °C); frozen products are transported deep-frozen (– 80 to – 60 °C in dry ice), frozen (< – 15 °C with ice packs) or cryopreserved (< – 140 °C in liquid nitrogen vapour phase). When the transport device does not allow the temperature conditions to be maintained over time, a time limit must be validated to guarantee that the storage conditions during transport do not affect the quality of the product.

For unfrozen products, such as bone marrow, there are conflicting recommendations for storage and transportation – e.g. 4 °C *versus* room temperature [2, 3, 4] – so the transplant centre is normally requested to define the transport conditions they wish to be applied. 'Room temperature' should be defined as a controlled temperature range with defined values for the upper and lower limits. Special attention should be paid to shipment at room temperature when the planned journey length is over 6 hours. Special attention should also be paid to refrigerated conditions: when the pharmacopoeia sets the temperature range between a lower limit at + 2 °C and an upper limit at + 8 °C, the risks of damage should be considered for cells or cornea exposed at + 2 °C temperature [5, 6, 7]. For cells and tissues potentially contaminated during the procurement, refrigerated transportation is generally recommended in order to prevent the risk of bacterial proliferation.

If the tissues or cells require specific environmental conditions, the capacity of the transport container to maintain the required environmental conditions, and the length of time that these conditions can be maintained by the transport container, should be determined by validation and documented. For instance, if liquid nitrogen is used to maintain very low temperatures, the dry-shipper must contain sufficient absorbed liquid nitrogen to maintain the storage chamber temperature < – 140 °C for a defined period of time, at least 48 h beyond the expected time of arrival at the receiving facility. Where temperature control is critical, data loggers should be used to monitor temperature during transport or shipment, with data downloaded from the device providing a graph to show that temperature was within the acceptable range at all times. Temperature indicators can be also used to indicate exposure to extremes of temperature.

Containers/packages should be secured and labelled appropriately (see Chapter 14).

Written agreements should be in place for the shipment of tissues and cells. In EU member states a written agreement must be signed between the shipping company and the TE to ensure that the required conditions will be maintained. This document must describe what should happen if the tissues or cells are damaged or lost during shipment (see also Chapter 2) and must cover a requirement that any related serious adverse events should be identified and reported to the Health Authorities (see Chapter 16).

11.4. Allocation

The allocation of tissues and cells should be guided by clinical criteria and ethical norms. The allocation rules should be equitable, externally validated and transparent.

The procedures for distribution of tissues and cells by authorised TEs must comply with the criteria laid out in the sections below.

It is mandatory for EU member states to have procedures in place for the management of requests for tissues and cells. The rules for allocation of tissues and cells to certain patients or healthcare institutions must be documented, and made available in appropriate circumstances, in the interests of transparency.

11.4.1. Visual examination

Packaged tissues or cells should be examined visually for appropriate labels, expiry date, container integrity and security, and any evidence of contamination prior to being dispatched (see Chapter 14).

11.4.2. Medical competence

Distribution for clinical application should be restricted to hospitals, physicians, dentists or other qualified medical professionals and must comply with all applicable national regulations.

11.4.3. Documentation

The place, date and time of pick-up and delivery (including time zone where relevant) and identity of the person receiving the tissues and cells should all be recorded, and this record should be maintained in the TE from which the tissues or cells are distributed.

Any transportation must be accompanied by specific documentation attached to the package (see Chapter 6, Chapter 12 and Chapter 14).

11.4.4. Recall and return procedures

An effective recall procedure must be in place in every TE, including a description of the responsibilities and actions to be taken in the case of a recall. This must include procedures for the notification of the relevant Health Authority/ies and all the facilities/institutions potentially affected by the recall.

A documented system must be in place for the handling of returned products, including criteria for their acceptance into the inventory, if applicable.

For further information see Chapter 15.

11.5. Import and export

11.5.1. Underlying principles

Import and export between countries should be done only through legally authorised tissue establishments that can guarantee that they have sufficient competence to evaluate safety and quality and also can guarantee that they have adequate systems to meet traceability requirements. They should be specifically authorised for one or more of the following:

a. import and/or export of human tissues and/or cells intended for human application;

b. import and/or export of tissues or cells intended for the manufacture of medicinal products derived from human tissues and/or cells (with the exception of tissues/cells that have been substantially manipulated, such as cell-lines or cell banks);

c. import of procured human material intended for processing, storage or banking in a TE or cell establishment in their country.

As a general rule, if organisations responsible for human application, manufacturers of advanced therapy medicinal products, clinical practitioners or individuals identify a need to import tissues or cells, they should organise this through a written agreement with a licensed TE in their own country. Third-party agreements must specify the terms of the relationship and the relevant responsibilities, as well as the protocols to be followed, to meet the required performance specifications.

11.5.2. Import

TEs that wish to import tissues or cells should be able to demonstrate that the need cannot be adequately met by comparable material available from sources within their country or that there is another justifiable reason for the import. They should also be able to justify the import in terms of accessibility, quality, speed of supply, risk of infection, quality of service, cost-effectiveness or scientific or research needs. They should ensure that any material intended for import is consistently sourced under the legal and ethical requirements of their country and the exporting country. If the importing TE cannot satisfy itself that ethical standards are in place in the country of origin, the tissues or cells should not be imported.

The safety and quality characteristics of the tissues or cells to be imported should be equivalent to those in place within the importing country. Imports should be accepted only from countries that have established procedures to authenticate the legitimacy of exporters and the provenance of the donated material they supply. Exporters should be asked to provide evidence of compliance with the regulations that they are required to observe before any orders are placed with them.

Companies that act as distributors, often also carrying out import and export activities, have responsibilities equivalent to those of TEs for ensuring the equivalent safety and quality requirements, for maintaining traceability and for having adequate vigilance systems in place. Fulfilment of these requirements implies having suitably trained, designated and authorised staff (including those with medical expertise) to evaluate donor-selection criteria and reports of adverse incidents and reactions.

See also §11.5.5 below for EU requirements for import.

11.5.2.1. *Routine importation*

The importing TE should assess whether the supplying TE complies with the quality and safety recommendations in this Guide and document that assessment, which includes respect for the fundamental ethical principles of consent, non-remunerated donation, anonymity and respect for public health. The evaluation should include at least the following:

a. the general quality and safety systems at the exporting establishment, including organisational chart, staff training, facilities, processing methods, validation studies, traceability and biovigilance systems, licences and accreditation (including lab certification/authorisation) and donor blood testing;

b. a review of the safety and quality of individual dispatches of tissues or cells (i.e. confirmation of donor consent, verification of donor sample testing and the results, donor eligibility records, description of the tissue or cells, transportation arrangements, etc.).

Potential language barriers should be considered and a common language agreed upon for all donor and tissue- and cell-related documentation.

A service-level agreement or contract between the exporting and importing TEs that clearly defines roles and responsibilities is a basic requirement. Agreed procedures for the transport of the tissues and cells from the country of origin to the TE in the importing country should form part of the contract and should specify the methods to be followed to ensure maintenance of the required environmental conditions, of the package integrity and of compliance with agreed timeframes. Such transportation should be direct, without intermediate stops when possible, using an approved courier. The courier or transportation service must provide records of pick-up and delivery to the TE so that complete traceability is ensured.

The agreement should specify how tissues and cells will be identified. Unique identifying codes should allow traceability and a formal and unambiguous identification of all tissues and cells (see Chapter 14).

Agreements between importing TEs and suppliers in other countries should include provisions for the performance of audits at the exporting facility and should require that any changes to authorisation status be immediately communicated to the importing TE.

11.5.2.2. *'One-off' importation*

There may be cases where exceptional or one-off importation is necessary for a single patient. In these cases, the importing TE should ensure that there exists a documented evaluation of the safety and quality of the tissues or cells being imported. The importing TE should keep the documentation obtained from the supplying TE for the time period specified in national regulations (e.g. 30 years in EU member states).

In limited cases (e.g. in emergency situations or for immediate transplantation) the import of certain tissues and cells may be directly authorised by a Health Authority, which should take all the necessary measures to ensure that imported tissues and cells respect the national quality and safety standards.

11.5.3. Customs and security clearance

For clearance of Customs, all tissues and cells supplied from abroad require a clear description of the content of the consignment and its destination and must be labelled as described in Chapter 14. It is important that frozen tissues or cells, which are usually packed in dry ice or stored in a dry-shipper, as well as fresh cells and tissues for urgent medical need, must not be delayed at border crossings. Viable tissues and cells for clinical use must not be exposed to irradiation devices; instead they should be subject to a visual inspection.

However, it should be noted that a study published in 2002 concluded that even 10 passages through the hand-luggage control system resulted in no harm to haematopoietic progenitor cells (HPC) and lymphocytes in terms of viability and potency. Interestingly, the radiation dosage of passage through the hand-luggage control system is of 1.5 + 0.6 μSv compared to a radiation dose of 60 μSv received by the HPC during a 10 h flight [8]. The lack of data on long-term effects suggests that, in line with the precautionary principle, the non-irradiation rule should be followed for the time being. Therefore, it may be expedient for the importer to inform Customs of a prospective consignment and any enquiries by Customs should be answered promptly. The agreement with the exporter should define responsibilities for meeting the cost of transport, refrigeration and/or storage at a Customs facility for any items that may be detained pending Customs enquiries.

11.5.4. Acceptance at the tissue establishment

Each importing establishment should have a documented procedure and specifications against which each consignment of tissues and cells, together with its associated documentation, is verified for compliance with the written agreement in place with the exporter. Any non-compliance should be reported to the exporter. Consignments should be examined for any evidence of tampering or damage during transport.

Tissues and cells should be stored in quarantine in an appropriate secure location under defined conditions until they, along with their associated

documentation, have been verified as conforming to requirements. The acceptance or rejection of received tissues and cells should be undertaken and documented in accordance with the guidance shown in Chapter 13.

11.5.5. EU requirements for importing tissues and cells

In April 2015, a new implementing directive on procedures for verifying equivalent standards of quality and safety of imported tissues and cells was adopted by the EU. Commission Directive 2015/566/EU stipulates that tissues and cells must be imported into the EU by an importing TE authorised for such imports by competent authorities. An importing TE is defined in the directive as:

> a tissue bank or a unit of a hospital or another body established within the Union which is a party to a contractual agreement with a third country supplier for the import into the Union of tissues and cells coming from a third country intended for human application.

Directive 2015/566/EU also lays down the obligations of the importing TEs and the competent authorities of EU member states who need to verify that imported tissues and cells meet quality and safety standards equivalent to those in place in the EU legislation for tissues and cells. These new requirements aim to facilitate the exchange of tissues and cells with non-EU countries while ensuring high standards of quality and safety are applied whatever the origin of the imports.

The procedures laid down in the new directive mirror closely the verification systems already in place within the EU. That is, procedures on the authorisation and inspection of importing TEs are laid down, specifying the information and documentation that needs to be provided or made available to Health Authorities in EU member states when TEs apply for import authorisations. Such information and documentation relates to the importing TE itself and the non-EU country suppliers it plans to use as a source of tissues and cells.

Another key element of the 2015 directive concerns the need for written agreements between importing TEs and their non-EU-country suppliers. Several minimum requirements for such agreements are listed in the text with a view to ensuring that the roles and responsibilities of each party are clear and fully undertaken to ensure equivalent quality and safety standards are met. Annexes to the directive describe the minimum requirements in the information and documentation to be provided by importing TE applicants when applying to be accredited, designated, authorised or licensed for the purpose of import activities, the content of the authorisation certificate for importing TE and the information to be provided regarding the third-country supplier.

The directive allows a limited number of exceptions to certain procedures for situations where certain tissues and cells are imported on a one-off basis. A 'one-off import' is defined in the directive as

> the import of any specific type of tissue or cell which is for the personal use of an intended recipient or recipients known to the importing tissue establishment and third country supplier before the importation occurs. Such an import of any specific type of tissue or cell shall normally not occur more than once for any given recipient. Imports from the same third country supplier taking place on a regular or repeated basis shall not be considered to be 'one-off imports'.

Those tissues and cells imported under direct authorisation of the competent authority of an EU member state (i.e. in emergency situations or for immediate transplantation) are not affected by the new procedures. An 'emergency' is defined in the directive as

> any unforeseen situation in which there is no practical alternative other than to urgently import tissues or cells from a third country into the Union for immediate application to a known recipient or known recipients whose health would be seriously endangered without such an import.

In the EU, distribution and shipment of all cells classified as advanced therapy medicinal products (ATMPs) are within the responsibility of a marketing authorisation holder and supervised by national/EU authorities for medicinal products.

Where an EU country imports from a non-EU country and the ultimate destination is a different EU member state, then the tissues or cells should fulfil the quality and safety requirements of both EU countries (i.e. with one EU country acting as the point of entry into the EU and the other as the final receiver of the tissues or cells).

11.5.6. Export

Tissues or cells should not be exported if there is an unmet clinical need for the material in the country of origin. Exported material should be procured, used, handled, stored, transported and disposed of in accordance with the consent that has been given by the donor. Tissues and cells should be exported only to countries that have proper controls on the use of donated material. They should be

exported only for the purposes for which they can lawfully be used in the country of destination, and exporters should satisfy themselves beforehand that the human tissues and/or cells will be used for a *bona fide* clinical application or research.

TEs should ensure that the quality and characteristics of the tissues and cells to be exported are equivalent to those of the tissues and cells implanted in their own country and are required in the country of destination.

11.6. International co-operation

For some transplant patients, including sensitised patients, it may be difficult to find a match within their own country. In these cases, co-operation between countries is necessary and in some cases it may be necessary to search worldwide to identify suitable donors. International co-operation and exchange of tissues and cells is necessary to increase the chances of providing tissues and cells for patients in life-threatening situations. For these reasons, it is important to ensure that there is good co-operation between organisations that allocate internationally. Registries should be in place for all imported and exported tissues and cells to ensure transparency in the process.

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Chapter 12: Organisations responsible for human application

12.1. Introduction

An organisation responsible for human application (ORHA) is a healthcare establishment or a unit of a hospital or another body that carries out human application of tissues or cells. Once tissues and cells, which must be ordered by a clinician or other authorised person, arrive at an ORHA, the responsibility for maintaining the quality-assurance chain is transferred to that organisation. The ORHA must store and handle tissues and cells correctly according to the instructions of the supplying tissue establishment (TE). The ORHA must also maintain traceability and biovigilance, which includes responsibility for immediately reporting serious adverse reactions and events to the TE and to the Health Authority, participating in the investigation and, where required, implementing corrective and preventive actions. This chapter is based on the guidance Vigilance and Surveillance of Substances of Human Origin (SoHO V&S) published by the EU and on the basis of good practice that has been described in the American handbook for practitioners [1, 2, 3].

12.2. Decisions on using and ordering tissues and cells

A serious adverse reaction (SAR) is defined in EU Directive 2004/23/EC as an unintended response that is fatal, life-threatening, disabling or incapacitating, or which results in, or prolongs, hospitalisation or morbidity. The risk of an SAR in a recipient of tissues and cells is considered to be very low, especially if the tissues and cells have been highly processed or terminally sterilised. However, the human application of tissues or cells is not free of risk and examples of SARs are documented in the World Health Organization's Notify Library [4], hosted by the Italian National Transplant Centre. Clinicians must, therefore, give careful consideration to the risks and benefits of the human application of tissues and cells, and the feasibility and availability of alternative options.

Somatic tissues and cells for allogenic application are donated altruistically for the benefit of patients in need and are often in short supply; therefore, only the required amount should be ordered, to minimise the likelihood of wastage. Healthcare professionals responsible for the storage and preparation of human tissues and cells for clinical application should receive appropriate training to ensure their compliance with all applicable technical and legal requirements that assure the quality and safety of the supplied tissues and cells.

12.3. Choosing a supplier of tissues or cells

In most cases, procured tissues and cells require processing and storage at the TE before their distribution to ORHAs for human application. In cases of direct distribution, procurement organisations send procured tissues and cells directly to the ORHA for immediate transplantation without any intermediate steps such as processing or storage.

Before requesting tissues or cells, the ORHA should confirm that the supplying TE, or the pro-

curement organisation in the case of direct distribution, is compliant with all relevant legal and technical standards and requirements for the lawful provision of tissues and cells that are safe and of appropriate quality. In the case of procurement of haematopoietic progenitor cells (HPC) or lymphocytes for unrelated allogeneic use, there is no direct interaction between the procurement organisation and the ORHA before cell procurement. This means that the ORHA does not directly select the procurement organisation that will procure the HPC or lymphocytes. Suitable donors are identified through donor registries. The registry is responsible for ascertaining whether the procurement organisation complies with appropriate quality and safety standards, including traceability of the procured cells.

TEs must be authorised, accredited, designated or licensed (collectively referred to in this chapter as 'authorised') by an appropriate Health Authority. This authorisation must specify the types of tissues or cells that can be accepted and the permitted activities, including procurement, donor testing, processing, storage and distribution, undertaken by the TE. The TE must be inspected regularly by the Health Authority to confirm compliance with legal requirements and quality and safety standards. Health Authorities must also authorise, where appropriate, the direct distribution of tissues and cells to ORHAs for immediate clinical application from abroad.

Using only appropriately authorised TEs ensures that the donors of tissues or cells have been selected and tested correctly, and that all quality system requirements are in place for the procurement, processing, storage and distribution of tissues or cells. ORHA may consider it appropriate to conduct a quality audit of a supplying TE.

To ensure that the quality and safety standards and the respective responsibilities of TEs and ORHAs are clearly set out and fully understood by both parties, there should be a formal service-level agreement (SLA) or contract in place between the supplying TE and the ORHA. These written agreements should be signed, dated and reviewed regularly (as defined by the parties concerned), but sooner if changes are required. They must comply with relevant laws and regulations. Where an ORHA and the supplying TE are within the same healthcare institution, responsibilities should be specified in the overall quality-system documentation.

Service-level agreements should include:

a. contact details for relevant persons in both parties, including the TE's Responsible Person (RP);

b. procedures for ordering and the delivery of tissues or cells, including liability for transport;

c. a statement that storage and preparation of tissues and cells for human application at the ORHA must comply with all relevant and specific instructions provided by the TE, including adherence to expiry dates;

d. procedures at the ORHA for the lawful disposal of unused tissues or cells or remnants of tissues and cells after human application;

e. procedures, if permitted, for the return of tissues or cells to the TE;

f. responsibility for maintaining traceability and biovigilance, including procedures for the timely reporting and investigation of adverse reactions and adverse events, including 'near misses', and procedures for the management of tissue and cell recalls and look-backs;

g. procedures, where permitted, for reporting of relevant clinical outcome data relating to the quality, safety and efficacy of the applied tissues or cells by the ORHA to the TE (see §12.14).

Where novel tissues and cells are to be supplied by a TE, or where novel clinical applications of processed tissues and cells are intended, authorisation by the Health Authority is needed. The extent of clinical follow-up needed to evaluate the efficacy of the applied tissues and cells should also be agreed between the Health Authority, TE and ORHA (see Chapter 29).

It is strongly recommended that ORHAs should obtain tissues or cells directly through a TE. If a broker (meaning here: an organisation that mediates for a payment between an ORHA and a TE in tissue and cell distribution) is used, the ORHA must verify that the distributing TE is authorised appropriately, that the broker has an agreement signed with the TE, and that the tissues and cells are supplied on a non-profit basis from voluntary unpaid donations.

Tissue establishments should distribute gametes, embryos and germinal tissue only to other authorised TEs or ORHAs for human application under the supervision of a clinician or other appropriate healthcare personnel.

12.4. Importing tissues or cells from other countries

If an ORHA needs to import tissues or cells from another country, it is good practice to make arrangements through a local TE to locate and communicate with the exporting TE. In the EU, tissues and cells from a third country outside the EU must be

imported through a TE authorised for importation by an EU Health Authority. Commission Directive 566/2015/EU sets out the procedures for verifying the equivalent standards of quality and safety of tissues and cells imported from third countries. The only exceptions to this rule are tissues and cells imported for direct distribution or for cases where there is an urgent clinical need. In the former, imported tissues and cells may be distributed directly for immediate clinical application provided the supplier is authorised for this activity. Urgent clinical cases include any unforeseen situation where there is no alternative other than to urgently import tissues and cells from a third country for immediate application to a known recipient or known recipients whose health would be seriously endangered without such an import. In both of these cases, the Health Authority must authorise the import directly.

The importing and exporting TEs must liaise with each other to ensure that the equivalent standards of quality and safety required by the importing TE are applied. In the EU, any TE that is authorised by a Health Authority in its own member state may provide tissues or cells directly to ORHAs in other member states. However, some member states have implemented more stringent regulations that require formal import procedures to be followed, even if the tissues and cells come from another EU member state. It is important to be aware of the national legislation in place for the importation of tissues or cells from another country.

12.5. Exceptional release

In exceptional circumstances, an ORHA may agree with a TE that tissues or cells that do not meet the normal release criteria can be applied in a specific individual on the basis of a risk–benefit analysis, taking into consideration the alternative options for the individual and the consequences of not providing the tissues or cells. The risk assessment should be documented before acceptance of the exceptionally released tissues or cells. The recipient's clinician should liaise with the TE's RP in conducting the risk assessment and risk–benefit analysis for the intended recipient. These discussions and conclusions should be documented. The treating clinician should sign an agreement accepting clinical responsibility for the exceptional release. The recipient, where possible, must be informed as part of the normal consenting procedure of the intention to use tissues or cells under exceptional release (see §12.6).

12.6. Recipient consent

Although donors are carefully selected and tested, there remains an albeit small risk of an adverse reaction in a recipient of tissues or cells. Recipients must therefore be made aware both of the risks and benefits of the intended treatment to be able to give informed consent. The Notify Library is an invaluable source of information for clinicians when evaluating the risks associated with the human application of tissues and cells [3]. Where the collection of clinical follow-up data is proposed, recipients may need to provide consent for the sharing and secondary use of their data in accordance with national legislation and guidance. In the EU, the General Data Protection Regulations (GDPR, Regulation EU 2016/679) provide certain exemptions from the need for consent for the collection and use of such data under Articles 6.1 (e) and 9.2 (h). However, recipients must be informed about the collection and use of their clinical data and, although they do not need to give consent, they do have the right to refuse to allow their data to be collected and used. Moreover, they can request to have their data withdrawn at any time even though they may not initially have refused their permission.

The information given to a prospective recipient should include at least the following:

a. a description of any adverse outcomes that have been reported for the given type of tissue or cell application;
b. an estimate of the frequency of the adverse outcomes described;
c. whether the treatment is consolidated or if it involves novel methods of processing/clinical application;
d. information on alternative treatments, if available.

Once the appropriate information has been given, the recipient, if willing to proceed, should then consent to the treatment, according to national requirements. The recipient should confirm:

a. that the risks associated with the human application of the tissues or cells have been explained and the information has been understood;
b. that they accept the risks in light of the potential benefits.

It is strongly recommended that a specific consent form should be signed by the recipient in the case of any novelty, both at the TE, such as introduction of new processing methodology, as well as at the ORHA, such as introduction of new clinical applica-

tion procedures for the tissues or cells (see Chapter 29).

12.7. Centralised *versus* devolved management of tissues and cells

Tissues and cells are either delivered directly to the relevant department or operating theatre in an ORHA (i.e. devolved management of tissues and cells) or they are delivered to a single, dedicated location under the direct supervision of an authorised healthcare professional (i.e. centralised management of tissues and cells). The advantage of devolved management is that the relevant department or unit in the ORHA with the appropriate specialist knowledge takes responsibility for the tissues and cells, whereas under centralised management there may be a more uniform approach to biovigilance and traceability and to ensuring compliance with quality and safety requirements for storage, handling and inventory control of the tissues and cells.

Regardless of the model applied for the management of human tissues and cells, all activities associated with receipt, storage, handling and follow-up should be incorporated into the existing quality-management system of the ORHA. The roles and tasks of officially designated persons should be clearly specified in standard operating procedures (SOPs).

12.8. Incoming inspection at the ORHA

When tissues and cells are received by an ORHA, appropriate personnel should verify and record that:

a. the tissues or cells received correspond to what was ordered and to the information in the accompanying documentation, which must be complete and legible;

b. the shipping containers and primary containers are labelled with the required information – including, where appropriate, the Single European Code (SEC) – and that labels are affixed and legible (see Chapter 14). Separate accompanying documents should provide information that is not included in the primary container label;

c. the shipping container and primary container are intact;

d. the specified expiry dates of tissues or cells have not been exceeded;

e. the transport temperature range was monitored or maintained adequately and is acceptable. For tissues or cells that are transported at low temperatures, maintenance of the required transport temperature can be confirmed by data readout from a temperature logger placed in the shipping container or by a residual coolant in the container (e.g. water ice for refrigerated tissues or cells and dry ice for frozen tissues or cells). The supplying TE should be able to provide, on request, a validation report to show that the required temperature can be maintained in the shipping container for a period of time that exceeds the maximum duration of transport.

Where the delivered tissues or cells do not comply with the above requirements, the ORHA must liaise with the TE to decide the correct course of action, which could include disposal of the tissues and cells or their return to the TE.

Tissue establishments should provide the ORHA and the end-user clinician with documentation of the donor consent, risk assessment and testing, tissue-related information and tissue-processing details, while ensuring that such information does not compromise the confidentiality of the donor. Alternatively, the TE could provide a statement to the effect that the donor and the tissues comply with all the TE's quality and safety standards and legal requirements for donor consent and testing, along with specific information about the characteristics of the tissues and cells required by the end-user clinician.

12.9. Package insert/instructions and temporary storage before use

Once tissues or cells have been distributed by a TE for clinical use, appropriate storage and handling become the responsibility of the ORHA. Instructions should be available in the package insert that accompanies the tissues or cells that describe the appropriate storage conditions and the proper handling procedures to be followed before clinical application. These instructions must be followed precisely by the ORHA.

Tissues and cells are stored under various temperature conditions, depending on their type, method of preservation and packaging. Where a specific storage temperature is necessary from receipt to clinical application, the storage device (refrigerator, freezer, liquid nitrogen storage tank, incubator, etc.)

should be regularly maintained and calibrated and should be secure, i.e. with restricted access. It should be dedicated to the storage of healthcare products and cleaned according to a defined protocol and frequency. It should have functional alarms, and emergency back-up storage capacity should be present. Storage procedures should address the steps to be taken if the temperature is outside defined limits or in the event of equipment/power failure. Failure to monitor and maintain controlled temperatures can result in waste of a precious resource and, if tissues or cells are used, serious adverse outcomes due to deterioration in their quality. All records pertaining to storage temperatures should be retained for at least 10 years.

During the storage of tissues and cells at the ORHA before clinical application, they must be kept together with their associated documentation or else the documentation must be reliably linked to the tissues or cells and easily accessible. The accompanying documentation should specify the presence of particular additives or reagents that may adversely affect the recipient (e.g. antibiotics, allergens). If there is no package insert accompanying the tissues or cells, they should not be used.

Some EU member states regard short-term storage of tissues or cells at an ORHA as a licensable activity that requires specific authorisation from a Health Authority. Therefore, it is important to be aware of the national legislation in place for the storage of tissues or cells at an ORHA.

12.10. Inspection of the container, documentation and tissues or cells

Before application, the container must be inspected and the accompanying documentation must be reviewed and confirmed to be complete and legible. The label should be checked and compared with the description on the package insert to confirm that the material is indeed what was ordered for the patient and is what is shown on the label. The packaging and the contents should be inspected for any signs of damage during transport. Where temperature during transport and storage at the ORHA is critical, there should be confirmation that the required temperature has been maintained.

In the case of tissues, the graft should be examined once the container has been opened to confirm that the anatomical characteristics are as shown on the label (e.g. left *versus* right femur, aortic *versus* pulmonary heart valve).

Tissues to be used in surgery should be specified and their use documented in the surgical checklist.

12.11. Preparation of tissues or cells before use

Instructions for opening the container or package, and any required manipulation/reconstitution (e.g. thawing, washing, rehydration), as well as information on expiry dates after opening/manipulation and the presence of any potentially harmful residues or reagents that may adversely affect the recipient (e.g. antibiotics, ethylene oxide), must be provided on the label or in the documentation accompanying the tissues or cells.

The handling instructions provided by the TE for the preparation of tissues and cells for human application should be followed precisely. Any departure from the instructions provided by the TE is at the discretion of the clinical user, who must take full responsibility for any adverse outcome resulting from not adhering to the instructions provided by the TE.

12.12. Surplus or unused tissues or cells

Tissues or cells remaining from a clinical procedure must not be used in another patient; any residue should be discarded as clinical or anatomical waste, in accordance with national rules, or returned to the supplying TE for appropriate and lawful disposal. Similarly, a single unit of tissues or cells (e.g. two halves of femoral head delivered in one container) must not be used in more than one separate patient. Activities that are routinely performed to finally prepare tissues and cells just before their clinical application, e.g. shaping of tendons or bone grinding for impaction grafting, are not considered as processing and do not require notification to the RP at the supplying TE.

Tissues or cells provided to one ORHA should not, in general, be sent to another ORHA for clinical application. Within the EU, this would be defined as distribution and it would require specific authorisation. However, such transfer of tissues and cells may be acceptable where the TE manages the process and the quality and safety requirements of the tissues and cells are not in any way compromised.

Tissues or cells that are received and not subsequently used in one department of an ORHA may be sent to a different department or operating theatre in the same ORHA. However, the details of such activity must be specified either in the overall quality-system documentation or in an SLA between the different

departments. There may be nationally established rules for such circumstances.

The documentation that accompanies the tissues and cells should specify whether they can be returned to the TE if not opened or used, e.g. if the patient is not well enough for surgery or if surgery is cancelled for another reason. Tissue establishments that do accept the return of unused and unopened tissues or cells must be able to confirm that the required storage conditions have been maintained, that the packaging has not been tampered with, and that the quality and safety of the tissues and cells has not been compromised.

12.13. Traceability

Coding and traceability are addressed fully in Chapter 14 and Chapter 15. In the EU, ORHAs are required to maintain traceability records from the point of receipt of the tissue until 30 years after clinical use or another final disposal. These records (mandatory in the EU) must include:

a. identification of the supplying TE or procurement centre;
b. identification of the clinician/end user/facility;
c. type of tissues or cells;
d. unique product identification;
e. identification of the recipient;
f. date of application.

Details of the tissues or cells applied should be in the recipient's record and in the logbook of the treatment room or operating theatre where they have been applied. However, these records alone are not adequate to permit rapid tracing of patients who might be at risk from a particular donation or processing batch. The ORHA should also have an electronic or paper log where all received, transplanted and discarded tissues or cells are recorded. This should provide a robust two-way audit trail to facilitate rapid identification of tissues and cells in the case of a recall by the TE or the Health Authority, or identification of recipients where the TE has been notified of a serious adverse reaction or serious adverse event that may have implications for one or more recipients treated at the ORHA. Careful consideration should be given to where and how this log will be archived for the required period, and the person(s) responsible for its maintenance and safe storage should be clearly identified and documented.

Some TEs require the ORHA to return a traceability form or card providing details of the recipient for each tissue and cell supplied. A copy of the card should be retained in the recipient's medical record. The details should be sufficient to unambiguously identify the recipient and the applied the tissues and cells: i.e. at least three points of identification for the recipient and a unique identifier (e.g. SEC in the EU) for the tissues and cells. Returning the card does not release the ORHA from its responsibility to maintain the above-mentioned traceability records for 30 years after clinical use or another final disposal. Where cards or forms are returned to the TE, the manner of documentation should adhere to national data-protection regulations and should ensure that confidential information is stored in secure systems and that the recipient's privacy is not compromised in any way.

It is highly recommended that when individuals who have been treated with human tissues or cells are discharged from an ORHA, their discharge documentation should specifically mention this fact. Hence, general practitioners looking after the patient in the longer term will be able to associate unexpected symptoms with possible transmission or other reactions from the tissues or cells applied. Moreover, general practitioners should be advised to report any suspicious or unusual findings to the ORHA.

12.14. Recipient follow-up and clinical outcome registries

Depending on the healthcare system, a routine clinical follow-up of the tissue or cell recipient is performed either by ORHA or by another healthcare organisation. The extent and duration of this routine clinical follow-up should, where possible, be standardised for each tissue and cell product and application.

Clinical follow-up data are to be kept at the ORHA in the recipient's records and may be submitted to national/international clinical outcome registries. If clinical follow-up data are collected, see section 12.6 for discussion of the implications for recipient consent. In some countries there may be legal obligations to collect clinical follow-up data. In addition to registries for which clinical outcome data entry is mandatory, there are registries based on voluntary reporting of clinical data. Some of them are national, others international; some are maintained by scientific or professional associations whereas others are held by Health Authorities.

Clinical outcome registries provide 'real-world' data that may give a more realistic overview of outcomes compared with single-centre studies. While randomised clinical trials are considered to provide the highest level of evidence, it is not always possible to apply the results more generally outside the strict

inclusion/exclusion criteria of such studies. Registries fulfil an important role in allowing studies on large data sets that can be used for determining recipient outcomes for a wide range of conditions, evaluating factors that influence clinical outcome or that may increase the risk of adverse events, and for validation of TE protocols and practices [5]. Registries have been established for several tissue and cell products including HPC, medically assisted reproduction and corneal transplantation.

Currently, there are two ongoing EU projects concerning the importance of recipient follow-up as a source of information for both TEs and Health Authorities; namely, EURO-GTP II (Good Practices for demonstrating safety and quality through recipient follow-up) and VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation).

The outputs of these projects will provide tools and assessment criteria for evaluating and verifying through clinical outcome studies the quality and safety of novel tissues and cells processing methodologies, or novel clinical indications/applications of consolidated tissue/cell products. Another project, which is part-funded by the EU, aims to establish a European Cornea and Cell Transplant Registry (ECCTR), which builds on the experience and expertise of three major corneal transplant registries in, respectively, the Netherlands, Sweden and the United Kingdom.

12.15. Adverse events and adverse reactions

Vigilance and surveillance (V&S) is addressed in Chapter 16. Effective V&S relies heavily on all healthcare professionals involved, from procurement through to application.

SARs, as defined in EU Commission Directive 2004/23/EC and in section 12.2 above, may be detected during or after procurement in living donors or after application in recipients of procured tissues or cells. As SARs in recipients might result from many diverse factors associated with the clinical procedure or with the recipient's underlying condition, clinicians might not consider the applied tissues or cells to be the cause of or a contributory factor to the SAR. However, there is an obligation, legal in the EU, for ORHAs to report known or suspected SARs to the supplying TE, which then must report to the Health Authority.

Serious adverse events (SAEs) are defined in EU Commission Directive 2004/23/EC as any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to transmission of a communicable disease, to death or to life-threatening, disabling or incapacitating conditions for patients, or which might result in, or prolong, hospitalisation or morbidity. If detected by the ORHA, they must also be reported to the TE and the Health Authority.

Tissue establishments that supply tissues and cells should provide ORHAs with clear instructions on how to report SARs and SAEs, preferably using standardised documentation. In general, any suspected adverse reaction or event should be reported immediately by the ORHA to the TE that supplied the tissues or cells, before it is confirmed or investigated, to enable the TE to take appropriate precautionary actions to prevent harm to other recipients and to involve the TE in the investigation process. The ORHA has a key role in supporting and contributing to the TE's investigation of suspected adverse reactions and events.

12.16. Management of recalls and reviews

There are various reasons why a TE may recall tissues or cells that were distributed to an ORHA. A recall may be related to the receipt of new information on the donor's medical or behavioural history that implies a risk of disease transmission, or it may be related to the discovery of an error in processing or a fault or contaminant in a reagent or solution used in processing. It may be instigated by the TE or required by the Health Authority.

When a TE issues a recall, it will be necessary to trace very quickly all the recipients of the particular batch (or donation) of tissues or cells implicated. The existence of a centralised logbook or electronic database that maintains a two-way audit trail of tissues and cells received, with dates of use or disposal and identification of recipients, will greatly facilitate conducting a recall. In many of the most significant cases of disease transmission arising from tissue and cell transplantation, it has not been possible to trace the fate of some of the tissues supplied for clinical use. This could leave some patients at risk and without appropriate follow-up and treatment. In these situations, centralised management of tissues and cells in the ORHA should facilitate effective action.

A review may also be required as part of an investigation of the safety of particular tissues or cells that have been applied to patients in the past. It may require recalling patients for additional testing or other investigations. Again, maintenance of a

two-way audit trail is essential for effective identification of potentially affected patients.

12.17. References

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Chapter 13: **Computerised systems**

13.1. Introduction

Computerised systems are playing an ever-increasing part in the management of business operations, including those related to healthcare. Tissue establishments (TEs) and donor registries may use a wide range of computerised systems. These can range from simple stand-alone computer systems that use a software package to track and trend data to fully integrated systems that control a range of processing steps and present data that will allow release of tissues and cells for clinical applications. In some cases these systems are relied upon to record consent and donor identity. Computerised systems may also have a role in controlling the facility (premises) or ensuring that required environmental conditions, such as air-pressure differentials or particle counts, are maintained (e.g. a building-management system).

Errors and malfunctions of computer systems can go unnoticed and might have serious consequences. Changes in software must be managed carefully to ensure that data have not been corrupted or reorganised in a manner that changes their meaning or impact. A review conducted for the UK Secretary of State for Health, published in 2010, reported the discovery of a systematic error in the documentation of wishes of organ donation that had probably occurred in 1999 and which potentially affected the records of > 900 000 individuals [1].

Computerised systems help to bring efficiency to processes. However, if they record critical information with an impact on donation, processing and release of tissues and cells, they must be selected and validated just like any other piece of critical equipment [2].

13.2. Planning the implementation of a computerised system

Figure 13.1 describes the different steps in implementing a computer system. It illustrates the design flow and documents related to specific phases (life-cycle documentation), together with the division of responsibilities between supplier/vendor and user with regard to testing, user instructions, maintenance, system improvements and access to source codes. Diagram A reports the models for system software categories 4-5 and diagram B reports the simplified model for system software category 3 (as in Table 13.1). Before implementation of a computerised system at a TE, it is advisable that the user has close contact with their information technology (IT) department, or an IT consultant independent of any supplier of computerised systems.

The TE needs to:

a. define the system by generating a written description of the functions that it is designed to carry out, and all human interactions, i.e. functional and non-functional requirements: user requirements specifications (URS). The URS will be the basis for subsequent testing and verification of the developed/supplied system. A list of minimal requirements for the computerised system includes (but is not limited to):

i. the need to manage calculations and printouts (e.g. reports and labels),

ii. the need for data protection (personal access to the system or parts of the system),
iii. the duration of and options for record storage (in general, 10 years is required for quality-system-related data and 30 years for traceability-related data in the EU),
iv. backup conditions ensuring restoration of the effective data,
v. the need to connect with other computerised systems/registries (social security registries, administrative systems, financial systems),
vi. the need for encryption in case information is transferred over an open network,
vii. the need for CE labelling (in EU) if patient data, or data relevant for diagnosis or treatment of patients, are to be included in the system,
viii. the need for audit trails (registration of GMP-relevant changes or data deletion);
ix. read-only access of QA to the computerised system and its audit trail(s);
x. handling of data originating/accumulating from measurements;
xi. e-archives for long-term storage of data,

b. evaluate the different systems available and choose one that meets the established requirements (though the degree of user-friendliness and maintenance should also be taken into consideration);

c. audit the developer/manufacturer to ensure that they can provide a product that meets regulatory requirements.

These steps should ensure that the user has all the necessary information about the system to be purchased and that the IT department or IT consultant has received the relevant technical information. It is recommended that the developer/supplier of the computerised system receives proper information about the surrounding/other systems that have to be linked to the system to be purchased. This course of action also minimises the need for 'work around' by the user (which can be a source of error).

The computer system that manages the activities of a TE usually includes hardware, software, peripheral devices and documentation such as manuals and standard operating procedures (SOPs). For further information, refer to the International Organization for Standardization ISO/IEEE 12207:2017, ISO/IEC/IEEE 29148:2018, ISO/IEC 27001:2013 and ISO/IEC 27007:2011 [3, 4, 5, 6].

13.3. Verification and testing

The guidance in Chapter 2 on the verification of new equipment should be taken into account. The verification of computerised systems in a TE should be incorporated in the general validation plan of the centre, which should include:

a. the identity of the computerised systems and interfaces that are subject to verification;

b. a brief description of the verification strategies for different categories of computerised systems, as well as other validation activities;

c. an outline of the protocols and related test procedures for all verification activities of the computer system (the reporting requirements for documenting the verification exercises and related results should also be defined);

d. the identity of key personnel and their responsibilities as part of the verification programme.

The level of verification required for computerised systems is dependent on the criticality of the systems to the quality and safety of the tissues and cells. Therefore, a criticality rating based on a risk assessment should be applied to all computerised systems in place. The method of verification of these critical systems depends on the type/category of software used. Table 13.1 gives some examples with suggested approaches to verification.

Verification should be commensurate with level of risk, intended use and potential implications of malfunction to quality and safety.

Before verification of a newly installed computerised system can be carried out, a full set of documentation that is as detailed as necessary to ensure appropriate operation of the system must be in place. The documentation should include:

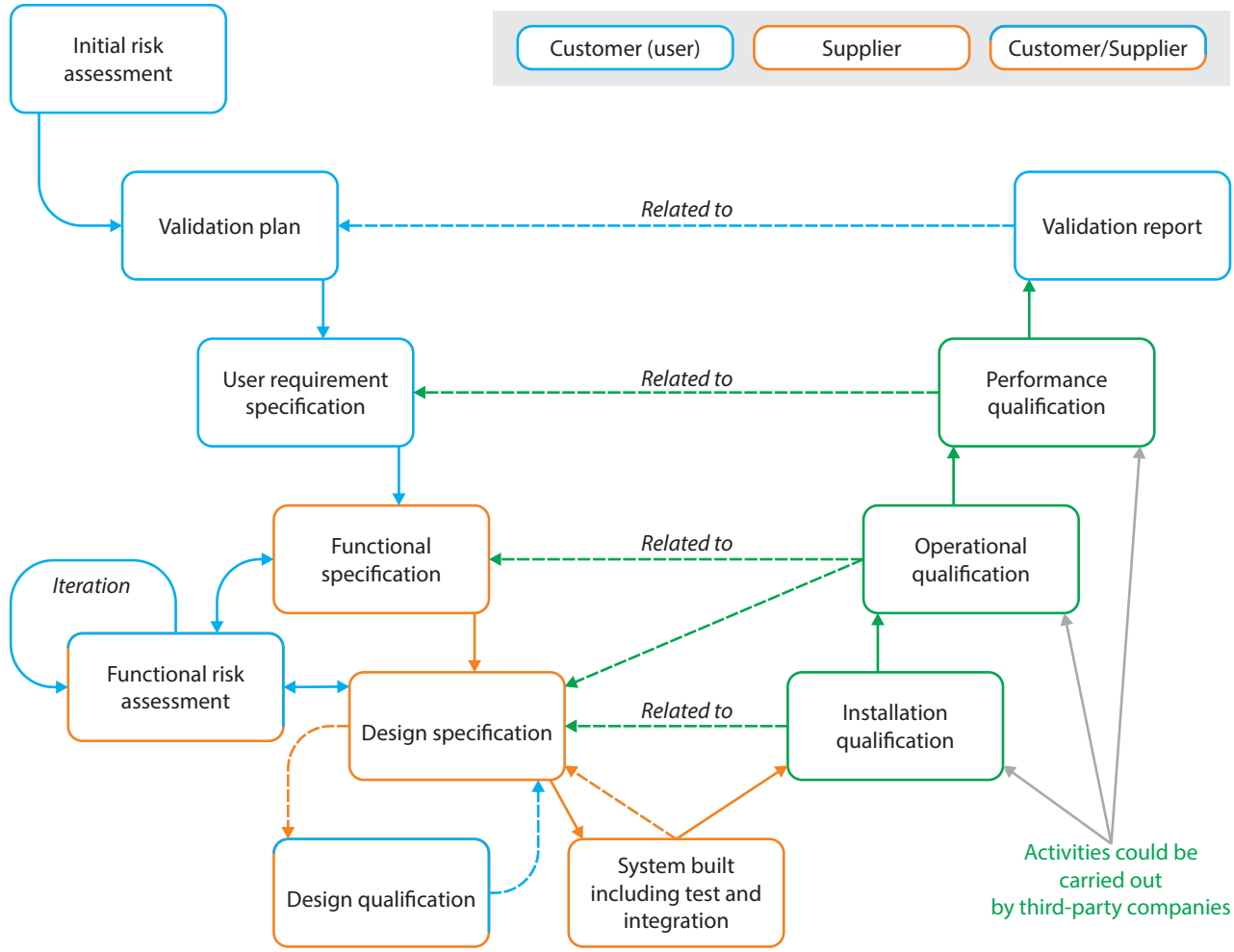
a. a detailed specification (inventory) of the hardware, software and peripheral devices, including their environmental requirements and limitations;

b. diagrams or flowcharts of the system's operations that describe all component interfaces, a network diagram and all database structures (e.g. file sizes, input and output formats) – if applicable: i.e. for system software categories 4 and 5;

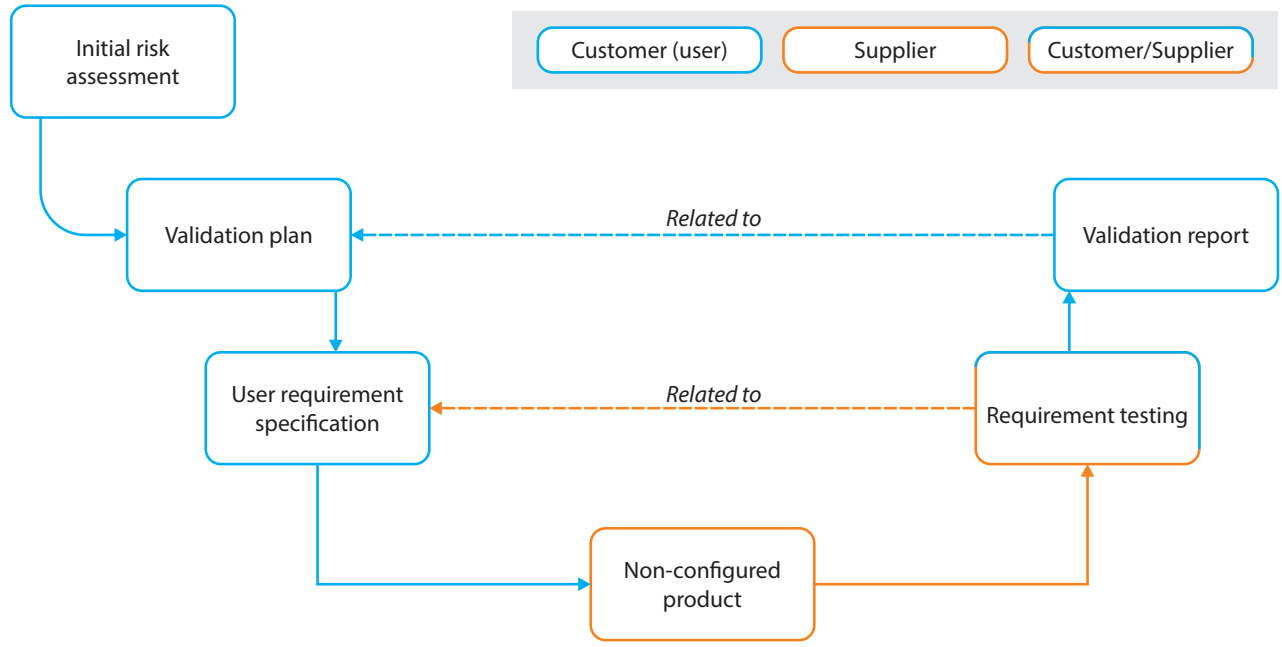
c. SOPs that describe how the system is used. The user should develop the SOPs, based on the instructions for use provided by the software developer and the internal procedures of the establishment. In particular, SOPs should address all manual and automated interactions with the system, including:

Figure 13.1. **'V' model diagrams for computer system implementation**

A. Models for system software categories 4-5



B. Simplified model for system software category 3



Dashed lines indicate relationships between testing and specification documents. Section A (top) describes system software categories 4-5; section B describes category 3 (categories as in Table 13.1).

i. routine backup, maintenance and diagnostic procedures, including assignment of responsibilities;
ii. safety leading indicators [5, 6, 7];
iii. 'work-arounds' for system limitations;
iv. procedures for handling errors, including assignment of responsibilities;
v. procedures for handling disasters and contingency planning, including assignment of responsibilities;
vi. procedures for supervised changes to incorrect data;
vii. procedures for verification of a change;
viii. a training system that includes manuals, documentation and procedures for training.

Verification documents and the results of tests undertaken and approved by the supplier/vendor or developer of the system must be part of the documentation supplied to the user. The user can then carry out tests according to a predefined and documented test plan [9]. Types of risk to consider include inadequate design of a system, errors that may occur in use (errors of use or system defects) and loss or distortion of data [10]. Testing should involve the entire system, and in the manner in which it is expected to perform routinely in the establishment. Testing may be done by a third party but, in that case, must also include personnel from the TE. The organisation for ownership, system management, maintenance and support, and the plan for regular internal revisions, should be included in the quality-management system (see Chapter 2)

The following types of basic testing are examples of what should be conducted initially and when new versions of the software are installed:

a. Functional testing of components
Initial qualifications are usually carried out by supplier/vendor, who will provide the user with documentation related to the tests performed. At minimum, documents should include
i. details of the methods employed to conduct verifications and testing of requisites stated in the URS document,
ii. qualification documents with results of tests (test scripts) for each functionality, including test procedure, expected result, test result, acceptance criteria, and
iii. conformity statement with relevant signatures.
For traceability and to facilitate quality assurance review and follow-up, it is recommended that any supporting documentation (e.g. print screens) be included to verify the specific test case.

b. Data migration
The process for data migration should be defined, documented and tested appropriately. This should ensure full maintenance of traceability, including archiving of data (if necessary).

c. Environmental testing (installation and verification instructions, IVI)
In the actual operating environment, functional tests are carried out to demonstrate that
i. the software systems work appropriately with the hardware;
ii. all applications of the software perform appropriately with the software operating system;
iii. appropriate information passes correctly through system interfaces, including appropriate data transfer to or from other laboratory and automated (e.g. serology testing, cell counting) systems (if applicable);
iv. accessories such as barcode scanners perform as expected with the barcodes in use (if applicable);
v. printed reports are formatted appropriately and correctly;
vi. personnel are trained and use the system correctly;
vii. the system performs appropriately at peak production times and with the maximum number of concurrent users;
viii. backups restore data in a correct way.

13.4. Change control

In case of changes in the software, the verification status needs to be re-established. If re-verification analysis is needed, it should be based on risk assessment and conducted not only for verification of the individual change, but also to determine the extent and impact of that change on the entire computerised system.

Changes should be handled following the V diagram in Figure 13.2. Depending on the system, it may be desirable to have a test version of the computerised system containing the same data (mirrored).

13.5. Maintenance and scheduled operations

Data should be checked periodically and systematically by qualified IT personnel to identify and remove unwanted data (e.g. duplicate records) and to ensure that data entries are stored accurately and appropriately. Manual entry of critical data requires independent verification by a second authorised person.

Table 13.1. **An approach to verification and control of computerised systems by system category**

Category	Description	Typical examples	Typical approaches
1. Infrastructure software	• Software on which applications are built • Software used to manage the operating environment	• Operating systems • Database engines • Statistical packages • Spreadsheets • Network monitoring tools • Scheduling tools • Document version control tools	• Record version number and verify correct installation by following approved installation procedures
2. Non-configured	• Software cannot be configured to suit the specific process, but working parameters can be set to suit the intended use.	• Firmware-based application* • Commercial off-the-shelf software packages • Instrument software (e.g. software associated with machines used for testing bacteriology or serology, cell counters)	• Specify user requirements before selection • Risk-based approach to supplier assessment • Record version number and verify correct installation • Risk-based tests against requirements as dictated by use (for simple systems, regular calibration may substitute for testing) • Procedures in place for maintaining compliance and fitness for intended use
3. Configured	• Software, often very complex, that can be configured by the user to meet the specific needs of the user's business process; software code is not altered	• Management system for donation, processing, storage and distribution of tissues and cells • Building-management systems (monitoring air pressures in rooms, temperature and/or particles, temperatures of fridges, freezers and incubators) • Clinical trial monitoring Note: specific examples of the above system types may contain substantially customised elements.	• Risk-based approach to supplier assessment • Demonstrate supplier has adequate quality management system • Some life-cycle documentation retained only by supplier (e.g. design specifications) • Record version number and verify correct installation • Risk-based testing to demonstrate application works as designed in a test environment • Risk-based testing to demonstrate that the application works as designed within the routine environment • Procedures in place for maintaining compliance and fitness for intended use • Procedures in place for managing data
4. Custom	• Software custom-designed to suit business process	Varies, but may include: • Internally or externally developed management systems for donation, processing, storage and distribution of tissues and cells • Internally or externally developed process control applications • Spreadsheet macro (i.e. database spreadsheet for clinical trial monitoring)	Same as for 'Configured' above, but also: • More rigorous supplier assessment, with possible supplier audits • Possession of full life-cycle documentation (as indicated in Figure 13.1)

Source: International Society for Pharmaceutical Engineering. Good Automated Manufacturing Practice (GAMP) 5 [7].

* In GAMP4, firmware applications represented category 2, now integrated into category 3.

Please refer to ISO/IEC 14764 for further guidance on maintenance [10].

Security should be maintained by:

a. an adequate change history of the system, for both software and hardware (when necessary);

b. periodically altering electronic passwords and by removing unnecessary or outdated access;

c. creating records of all data changes (i.e. an audit trail), including a retained record of previous data and the reason for the change;

d. appropriate use of malware (e.g. computer virus) protection programs;

e. control of administrative security access to ensure that only authorised personnel can

make changes to the software, system configuration and data;

f. regular testing to verify the appropriate integrity and accuracy of backed-up data;

g. consider if a permanent storage (e-archive) of specific data is necessary.

13.6. Quality assurance

The data-processing system should be considered as critical equipment within the quality-assurance programme, which as a minimum should:

a. ensure the ongoing accuracy and completeness of all documentation on equipment, software maintenance and operator training;

b. undertake audits periodically to verify appropriate accomplishment of all performance tests, routine maintenance, change procedures, data-integrity checks (including audit-trail checks), error investigations and operator-competence evaluations.

Appendix 23 contains an example of a checklist that can be used for internal or external audits.

13.7. Industry guidance for verification of computerised systems

The most common industry guide used for verification of computerised systems is that from the International Society for Pharmaceutical Engineering (ISPE) [8]. More specific guidance related to blood and tissues is available from the British Committee for Standards in Haematology [11].

13.8. Regulations governing verification of computerised systems in EU Good Manufacturing Practice

Regulation of computerised systems is well established in the pharmaceutical industry, with EU Good Manufacturing Practices (GMP) [12] acting as the regulatory reference in the EU. The advice of the OECD Working Group on Good Laboratory Practice on applying these principles to computerised systems is also recommended [13]. Inspectors in the EU also use the Pharmaceutical Inspection Co-operation Scheme Guidance (PIC/S) [14]. The pharmaceutical industry operates on a global scale, so many European companies maintain compliance with the US Food and Drug Administration (FDA) [15]. These regulatory documents can be useful sources of reference for TEs.

If a computerised system replaces a manual operation, there should be no decrease in product quality, process control or quality assurance. There should be no increase in the overall risk of the process [12].

The PIC/S document also lists the critical items that an inspector should consider during inspection and is a valuable tool for TEs since it details the minimum requirements that should be in place [14]. Appendix 23 contains a checklist adapted from ISO/IEC 27007:2011 and the guidance document of the Swedish Board for Accreditation and Conformity Assessment (SWEDAC)

13.9. Infrastructure

Infrastructures are necessary in order to guarantee the correct data handling between work stations hosting the computer system and the relevant server(s), and they include but are not limited to communication physical lines (e.g. ethernet), switches and routers. A correct design of a computer system must consider the use of suitable tools (e.g. test suites, servers, version- and configuration-control systems, modelling and architecture tools, communication tools, traceability and behavioural-modelling tools).

13.10. Failure of the system

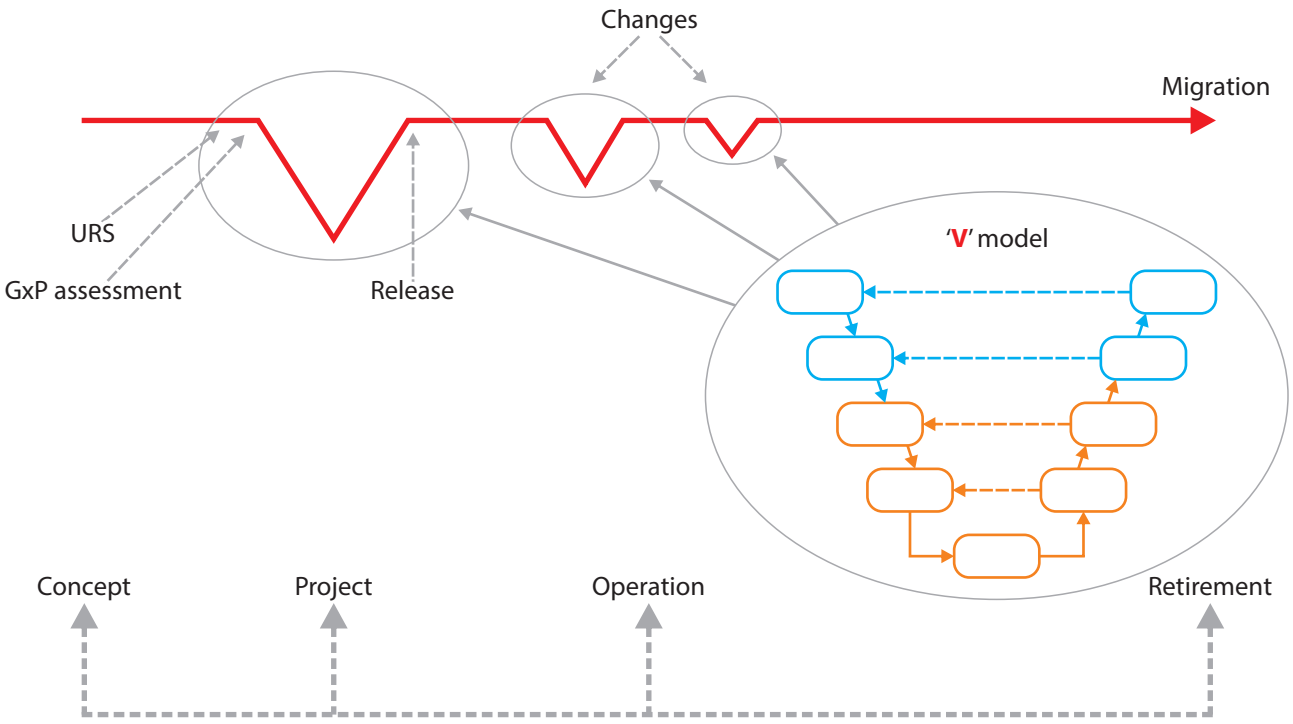
For computerised systems that support critical processes, provision (e.g. disaster recovery or business contingency plan/procedure) should be made to ensure continuity of support for those processes in the event of a system breakdown (e.g. a manual or alternative system). The time required to enact alternative arrangements should be based on risk assessment and should be appropriate for the particular system and the business process it supports. These arrangements should be documented and tested adequately [12]. Testing of these alternative systems and their ability to retrieve data should be assessed periodically (based on risk assessment).

13.11. Electronic signature

Records may be signed electronically. According to Annex 11 of EU GMP [12], all electronic signatures are expected to:

a. have the same impact as handwritten signatures within the boundaries of the organisation;

Figure 13.2. **Handling of software life-cycle activities**



URS: user requirements specifications. GxP: good [specialism] practice. Orange: user. Blue: supplier.
Source: modified from ISPE Good Automated Manufacturing Practice (GAMP) 5 [8].

b. be permanently linked to their respective record;
c. include the reason, the time and date that they were applied.

13.12. Data protection

Critical and sensitive data must be protected from unauthorised information modification and from unauthorised information access/release. Procedures for personal data protection must comply with national legal requirements or, for EU countries, with the requirements defined in Regulation EU 2016/679 and Directive 2010/45/EU on the Protection of natural persons with regard to the processing of personal data and on the free movement of such data.

Appropriate technical and organisational measures must be taken to guarantee a level of security appropriate to the risk, measured against the context and purposes of the processing. The factors which may be analysed to determine the appropriateness of the measures include degree of data sensitivity, the risks to data subjects in the event of a breach, and the costs involved in implementing specific types of security measures. The latter may include:

a. encryption or pseudonymisation;
b. measures to ensure the confidentiality, integrity and resilience of processing systems;
c. methods which enable the timely access to, restoration of or availability of personal data in the event of an incident;
d. regular tests and evaluation to ensure that the measures implemented meet their desired objective of maintaining security of data processing.

All personal data stored in computerised systems must be stored in a secure manner, with access available only to authorised personnel. The system should ensure data inalterability, and an audit trail with registration of data access and modifications, including date and identification of personnel executing modifications. For those applications in which all users should not have identical authority, some scheme is needed to ensure that the computer system implements the desired authority structure.

13.13. Archiving

Critical data must be archived in a long-term stable medium and placed 'off site' physically or as an e-archive backup at a location remote from the

hardware, to ensure secure storage. Archived critical data should be checked for accessibility, readability and integrity. If changes are made to the system (e.g. new computer equipment or software is installed), then the ability to retrieve archived data must be ensured and tested [12]. Archiving should be conducted using secure software methods such as databases compliant with ACID (atomicity, consistency, isolation and durability) requirements, that guarantee data integrity. Files should be stored in databases, if possible in a time-durable format. Among formats more commonly used are software-encrypted files and CRC (cyclic redundancy check)-secured files, that require dedicated software to be managed. As this characteristic may condition future retrieval, an ISO-standardised version of the Portable Document Format (PDF), called PDF/A [16], has been implemented to ensure document reproduction using any device in years to come, as the format is independent of hardware and software platforms.

13.14. References

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Related material

- Appendix 23. Checklist for revision of computerised systems

Chapter 14: **Coding, packaging and labelling**

14.1. Introduction

The quality and safety of tissues and cells is dependent not only on the way they are procured or processed, but on the way they are coded, packaged and labelled before being sent to an organisation responsible for human application [1, 2]. The World Health Organization (WHO) has published an *aide-mémoire* on the key safety requirements (including storage, packaging and labelling) for essential minimally processed human cells and tissues for transplantation, and some countries have adopted legal requirements to ensure that human tissues and cells are appropriately packaged, labelled and coded [3]. These steps are also addressed in the EU tissues and cells legislation. In this chapter, the coding of tissues and cells, their packaging and labelling requirements are discussed.

14.2. Coding

With increasing movement of tissues and cells across borders, the capacity to uniquely identify them is essential. This can be achieved by coding that facilitates tracing the tissues and cells from donor to recipient and vice versa while respecting data protection and confidentiality rules.

Coding started with the development of local coding systems applied in individual tissue establishments, but in the last two decades there has been significant movement towards the use of national and international coding standards, building on the longer and more consolidated experience in blood transfusion.

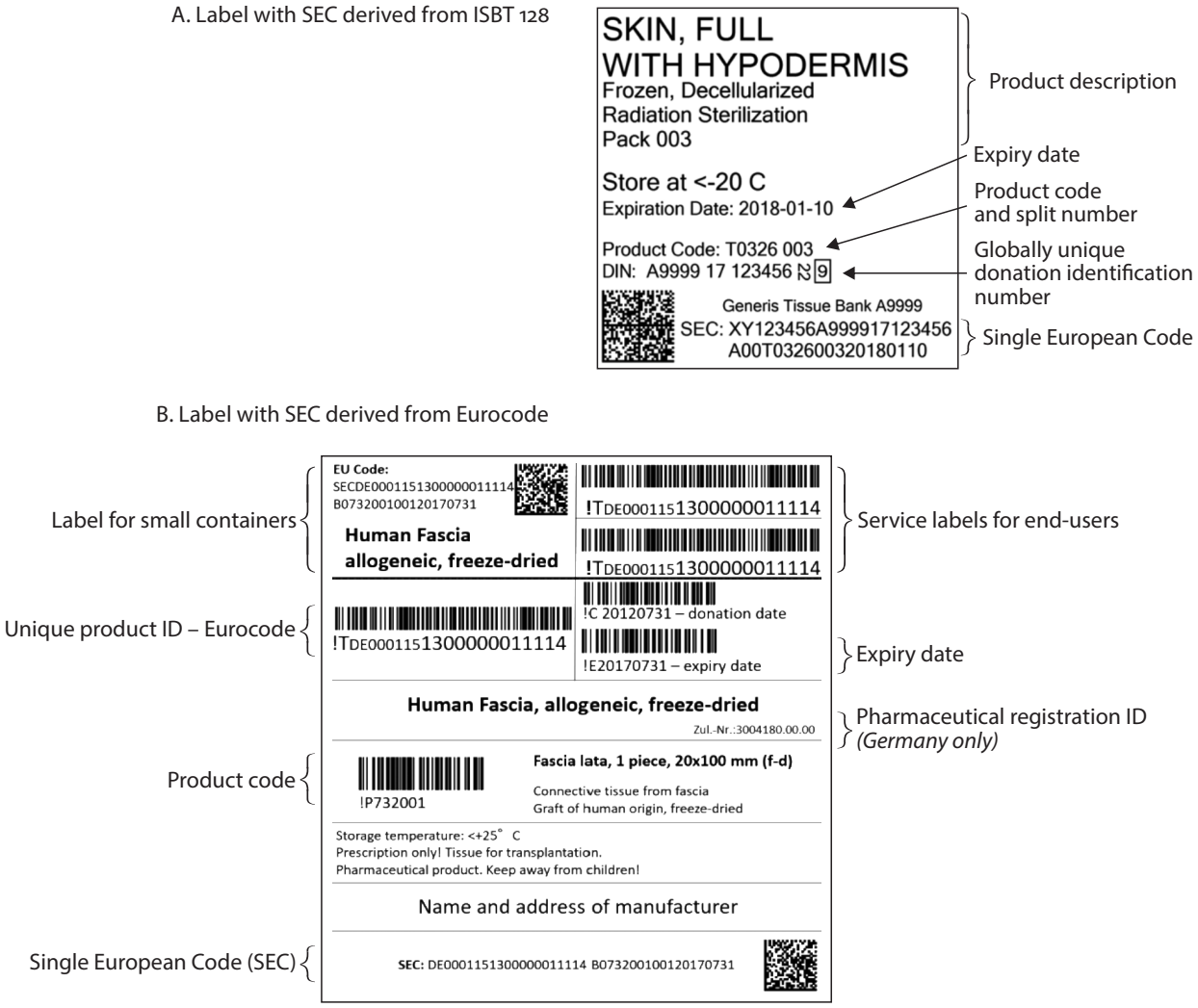
14.2.1. ISBT 128

ICCBBA (the International Council for Commonality in Blood Banking Automation) manages ISBT 128 [4, 5], which is the most widely used information standard for medical products of human origin, including tissues and cells. ICCBBA is a not-for-profit, non-governmental organisation in official relations with the WHO, and ISBT 128 is endorsed by 21 scientific and professional organisations. The standard is developed and maintained with input from more than 250 volunteer experts in the fields of transfusion and transplantation from around the world and provides a structured product terminology with more than 2 500 defined cell and tissue product codes.

14.2.2. Eurocode

Eurocode International Blood Labelling Systems e.V. (Eurocode IBLS) [6] is a not-for-profit association under German law. Eurocode IBLS manages the coding standard Eurocode, which is an ISO15418-listed information standard providing worldwide unique identifiers for labelling blood products, cells and tissues to enhance security in blood transfusion and cell and tissue transplantation. Today it is used in Germany and Austria.

Figure 14.1. **Examples of finished tissue product labels with the Single European Code**



14.2.3. Single European Code for tissues and cells

In 2015, the European Commission adopted Directive (EU) 2015/565, amending Directive 2006/86/EC [7] as regards certain technical requirements for the coding of human tissues and cells, which establishes the EU Coding Platform and the Single European Code. The EU Coding Platform (https://webgate.ec.europa.eu/eucoding) provides access to the EU Tissue Establishments Compendium, the EU Tissues and Cells Products Compendium (EUTC) and a code-translator application. The directive introduces the obligation on tissue establishments to affix a Single European Code (SEC) on tissues and cells distributed or imported for human application in the EU or exported from the EU [8]. The directive also sets out the requirements for its application (including exceptions) and the general obligations of tissue establishments, competent authorities and the European Commission.

The SEC provides for standardisation across the EU. The permitted product coding systems are ISBT 128, Eurocode and the EUTC (Figure 14.1). The ISBT 128 and Eurocode product descriptions are mapped to the high-level product description codes provided by the EUTC (see §14.2.3.3).

14.2.3.1. *Application of the Single European Code*

Except for the exemptions described later, application of the SEC [8, 9, 10] by EU tissue establishments is mandatory for all tissues and cells distributed for human application. When tissues and cells are released for circulation (e.g. to other tissue establishments, third parties, manufacturers of advanced therapy medicinal products), the primary container must include a unique identification number or code and the donation identification sequence (DIS) (see Table 14.1). If the container is too small to include the DIS on the label, the DIS must be included in the accompanying documentation.

There are some general exemptions to the requirement for application of the SEC code. These include partner donation of reproductive cells, tissues and cells distributed directly for immediate transplantation to the recipient (e.g. HPC), and tissues and cells imported from non-EU countries into the EU in cases of emergency that are authorised directly by the Health Authorities. EU member states may also allow exemptions for tissue and cells other than partner gamete donation, when these tissues and cells remain in the same centre or when tissues and cells that are imported from non-EU countries into the EU remain within the same healthcare facility from importation to application (provided that the healthcare facility is a tissue establishment authorised to import tissues and cells).

Application of the SEC does not preclude additional application of other codes in accordance with the national requirements of EU member states.

Countries already using existing coding systems compatible with the SEC requirements (i.e. ISBT 128, Eurocode) with a standard for barcoding and other forms of machine readability can continue using those systems while incorporating the new legal requirements. There will also be the potential of making the SEC machine-readable in the future. The use of machine-readable barcode labels will ensure the accuracy of records, as manual transcription errors will not occur, and the machine output can easily be entered into electronic databases.

Tissues or cells imported from third countries for distribution in the EU must also be labelled with the SEC (unless the EU member state applies the exemption above). The importing tissue establishment is responsible for the application of the SEC on the product and in the accompanying documentation (double coding/labelling with both the original code and the SEC).

The DIS (see Table 14.1) must use the tissue establishment number allocated to the importing tissue establishment in the EU Tissue Establishment Compendium. Imported tissues or cells that are already labelled with a globally unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS) must use this as the unique donation number. If the imported tissues or cells do not carry an identifier from one of these systems, the importing tissue establishment must assign its own unique number. The importing tissue establishment must retain traceability mapping between the identifier they have assigned and the original identification of the imported tissues or cells. Consideration must be given to the possibility that the original identifier may not be unique if products are received from more than one source where the suppliers have used local donation numbering systems; it is quite possible that the same identifier may be used by different suppliers to identify completely different donations. The traceability mapping must therefore include both the original identifier and the supplier identification.

If the imported tissues or cells are already labelled using ISBT 128 or Eurocode, the product code from the original label may be used in the product identification sequence (see Table 14.1) provided that this code is listed in the EU Product Compendium. In all other cases, the importing tissue establishment must assign a product code from one of the three product coding systems (EUTC, ISBT 128 or Eurocode) that is listed in the EU Product Compendium and most accurately describes the imported tissues or cells.

Split numbers carried by imported tissues or cells that do not exceed three alphanumeric characters can be used directly in the SEC. If the imported tissues or cells carry a longer split number, or where no split number is provided, the importing tissue establishment must assign a new split number with a maximum of three alphanumeric characters to ensure uniqueness of the SEC. Particular care needs to be taken where the product code being assigned to the imported tissues or cells is more generic than the original product; for example, bone rings and bone dowels imported with the same donation number where each product is identified by a product code assigned by the supplier and with a split number of 001. The importing tissue establishment applies the SEC using the EUTC of MUSCULOSKELETAL, BONE, SHAPED GRAFT, which means that the two different original product codes have now been mapped to one EUTC code. It is, therefore, no longer possible to use the allocated split numbers as this would result in duplication and the importing tissue establishment must assign a new split number and retain records to map back to the original identifiers.

14.2.3.2. *Structure of the Single European Code*

The SEC is a unique identifier that consists of two elements: a donation identification sequence that indicates the origin of the tissue or cells, and a product identification sequence that describes the type of tissue or cells. Further details are specified in Annex VII to the directive (see Table 14.1).

14.2.3.2.1. Donation identification sequence

The coding system must identify each donation event because donors can potentially donate tissues

and cells on several occasions (e.g. an individual may donate gametes and HPC when alive and corneal tissue after death). Each tissue establishment authorised in an EU member state must use the tissue establishment number allocated in the EU Tissue Establishment Compendium which, in combination with the International Organization for Standardization (ISO) country code, will create the tissue establishment code.

Each tissue establishment must assign a unique number for the donation based on the donation identification system in place in their country. Donation numbers with fewer than 13 characters will be padded with leading zeros in the SEC. The unique donation number may be created locally by the tissue establishment, centrally (by a Health Authority) or globally as a unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS).

Taken together, these codes will ensure that each donation event will have a unique donation identification number that can be used to label each tissue product. In the case of pooling of tissues and cells, a new donation identification number must be allocated to the final product.

14.2.3.2.2. Product identification sequence

The product identification sequence consists of the assigned product code, a split number (if applicable) and the expiry date of the product (if applicable) in ISO standard format (yyyymmdd). For tissues and cells without a defined expiry date, the expiry date must be 00000000. The product code includes an identifier of the coding system used ('E' for EUTC, 'A' for ISBT 128 and 'B' for Eurocode) followed by the appropriate product number corresponding to the tissue/cell type.

As explained above, and also taking into account the coding practices used by the EU member states, the SEC provides a flexible solution by allowing tissue establishments to use one of three product coding systems (EUTC, ISBT 128, Eurocode) for which all tissue and cell codes have been included in the EU Tissue and Cell Product Compendium. Tissues and cells in the three product coding systems are mapped to each other to ensure that a tissue or cell product code in the SEC can be 'translated' irrespective of the system used. EUTC provides only the basic nomenclature, but ISBT 128 and Eurocode include more detailed product information (e.g., EUTC may represent a product type such as a tendon, whereas the other two systems may specify whether the tendon is whole, shaped or irradiated).

EU member states may decide to permit tissue establishments to use only one product coding system (EUTC, ISBT 128 or Eurocode), or more than one in parallel.

The SEC on the label attached to each product will be in eye-readable format and preceded by the abbreviation 'SEC'. The DIS and product identification sequence must be separated by a single space or as two successive lines. Using ISBT 128, a data structure is available to allow the SEC to be machine-readable.

14.2.3.3. *EU Coding Platform*

The EU Coding Platform introduced by Directive 2006/86/EC (as amended by Directive 2015/565/EC) is the major tool for implementing the SEC requirements. It is an IT platform hosted by the Commission and it contains the EU Tissue Establishment Compendium and the EU Tissue and Cell Product Compendium [11].

a. The EU Tissue Establishment Compendium is the register of all tissue establishments that are authorised, licensed, designated or accredited by each EU member state's competent authority or authorities; it contains the information about these tissue establishments along with their corresponding tissue establishment codes. The EU Tissue Establishment Compendium is hosted by the European Commission and maintained by the member states' competent authorities. Each competent authority is responsible for the accuracy of the entries for the tissue establishments that they have licensed or authorised and for keeping these entries up to date.

Table 14.1. **Single European Code for tissues and cells**

Donation Identification Sequence			**Product Identification Sequence**			
Tissue establishment code		Unique donation number	Product code		Split number	Expiry date (yyyymmdd)
ISO country code	Tissue establishment number		Product Coding System identifier	Product number		
2 alphabetic characters	6 alpha-numeric characters	13 alpha-numeric characters	1 alphabetic character	7 alpha-numeric characters	3 alpha-numeric characters	8 numeric characters

Source: Annex VII of EU Directive 2015/565.

b. The EU Tissue and Cell Product Compendium is the register of all types of tissues and cells circulating in the Union and the respective product codes under the three permitted coding systems (EUTC, ISBT 128 and Eurocode IBLS).

Acknowledging the existence of product coding systems already in use in the EU, Directive 2015/565/EC allows the use of ISBT 128 [12] and Eurocode [13] coding systems, and has put in place bilateral agreements with their managing organisations (i.e. ICCBBA and Eurocode IBLS) to ensure that updated product codes are regularly made available and included in the EU Tissue and Cell Product Compendium.

The EUTC tissue and cell product coding system was developed by the European Commission for tissue establishments not using the other two coding systems. The EUTC covers all types of tissues and cells along with high-level terminology and their corresponding product codes. A mapping of the more detailed ISBT 128 and Eurocode product codes to the generic EUTC codes is also provided on the EU Coding Platform.

Each tissue and cell product must be assigned a specific code, which identifies and describes that product. The information in the SEC can be decoded by the code-translator application in the EU Coding Platform to obtain text that describes the tissues or cells and their origin.

These tools are publicly available and free of charge. Therefore, the EU tissues and cells product coding system used by EU member states may also be used by other countries. Further information on the SEC and its application can be found on the European Commission's website [8, 9, 10].

14.3. Packaging and labelling

Packaging of tissues and cells has an important role during all procedures, starting from procurement, through the processing and storage steps, to distribution and human application. Adequate packaging minimises the risk of contamination of tissues and cells, protects the persons involved in transportation and aids retention of required characteristics and biological functions.

Ensuring the traceability of all tissues and cells from the donor to the recipient is a responsibility shared by procurement centres, tissue establishments and organisations responsible for human application. All of these participate and contribute actively to safeguarding, in a continuous manner, the tracking of the tissues and cells through from procurement to human application. Accurate tracking of tissues and cells allows reliable data to be scientifically assessed for potential risks to the donor, to the procurement and processing operations, and to the storage, transport and clinical use of donated material. Traceability is addressed in depth in Chapter 15. An essential aspect of ensuring accurate traceability is clear and complete labelling of tissues and cells at all stages. The system of identification for donors and recipients must be aligned with the packaging and labelling system of tissues and cells in such a way that a connection between tissues and cells, the source and the recipients exists at all times.

Labels must be attached to packaging that has been validated to demonstrate that it maintains the required properties of the tissues and cells and ensures integrity. This part of the chapter addresses good practice in packaging and labelling at all stages from donation to implantation.

14.3.1. General concepts

Packaging and labelling operations must be considered an integral part of the activities of procurement organisations and tissue establishments. They must be included in the training of personnel and specified in all relevant procedures. Although this chapter establishes specific recommendations for packaging and labelling for the procurement and processing phases, they should equally apply to intermediate phases, such as in-process steps, in which all materials, containers, equipment and unfinished tissues and cells must be adequately identified at all times. In addition, tissues and cells procured or processed for research purposes should be clearly identified as such on their packages and labels (e.g., 'FOR RESEARCH USE ONLY' or 'NOT FOR CLINICAL USE').

There should be written procedures describing the receipt, identification, quarantine, sampling, examination, testing and release of packaging and labelling materials, as well as the handling of such materials.

Premises and procedures for the packaging and labelling of tissues and cells must be designed to prevent cross-contamination or mix-ups. Simultaneous operations should be avoided or, where unavoidable, adequate additional safeguards should be put in place.

Primary packaging and labelling of tissues or cells must be done in an environment specified in standard operating procedures (SOPs).

For EU member states, the requirements for packaging and labelling of tissues and cells are de-

tailed in Annex IV of Directive 2006/17/EC, Annex II of Commission Directive 2006/86/EC and Commission Directive (EU) 2015/565.

14.3.2. Packaging of tissues and cells

Packaging includes all operations, including primary and secondary packaging, which procured or processed tissues and cells undergo from the start, during processing or as final packaging. Packaging aims to protect tissues and cells, and to present them to the operator (in initial or in-process packaging) or to the clinical end user (in final packaging) in a suitable manner. The type of substance of human origin and its intended use will determine the requirements needed to carry out a packaging operation in a safe manner.

Special consideration must be given to the primary packaging that will be in direct contact with tissues and cells. Containers intended to be used as primary packaging should be submitted to visual inspection before use and, if single-use containers are unavailable, the need for applying an adequate cleaning process should be assessed along with suitable sterilisation methods such as irradiation or autoclaving of materials and containers. If the cells/tissues are stored in liquid nitrogen, they must be double-bagged to prevent cross-contamination during storage [14] or stored in validated high-security packaging especially designed for liquid nitrogen. The packaging materials should be stored in a clean area. In this case, the materials and the conditions under which packaging takes place must be carefully specified, assessed and approved before use. Processing facilities must establish and document validated packaging protocols.

Packaging must ensure the integrity and maintain the sterility of the contents of the primary container. Storage containers must be appropriate for the type of tissue or cells, the temperature and method of storage, and the intended application. They must withstand sterilisation (where this is to be applied), not produce toxic residues during storage and be adequately robust to remain intact when handled during transport. Each tissue or cell container must be examined visually for damage or evidence of contamination before distribution for clinical use and by the end user.

14.3.3. Labelling of tissues and cells

Written procedures must be established and followed to ensure correct labelling. Each labelling phase for all tissues or cells must be documented. Tissues and cells must be labelled during all phases of procurement, processing, storage and distribution. Labelling must be clear, legible, indelible and unique.

Before labelling a unit of donated or processed tissues and cells, the container must be inspected for evidence of impurities, defects, broken seals or contamination that could compromise the quality, integrity or safety of the product.

Labels attached to the containers should identify and describe the contents. The description should characterise the tissues and cells, and reflect key aspects of their maintenance and use. Standard nomenclature and standard international units of measurement must be used to describe the tissues and cells, and the processing they have undergone (see §14.2 on coding).

Identification should provide information on traceability that links the tissues and cells to the tissue establishment of origin and, ultimately, the donor. When tissues or cells are to be distributed internationally, language barriers should be considered, and information translated or coded to ensure understanding.

For autologous or directed donations, the name or identifier of the intended recipient must be included in the label. Further guidance on traceability is provided in Chapter 15.

The production of labels must be controlled. When applicable, reconciliation of labels that have been edited, used or returned/rejected must be undertaken according to written procedures. All excess labels containing quality or traceability information must be destroyed or maintained in a secure manner, when necessary, to prevent mix-ups. Obsolete, unused labels must be destroyed according to written procedures.

It is highly recommended to undertake labelling and packaging simultaneously, in a continuous process, to reduce the risk of mix-ups or cross-contamination. Before application to the container, printed labels must be carefully examined to ensure that the information they contain conforms to the corresponding tissues or cells. The results of this examination should be documented at identified critical stages. Labels must be designed to adhere firmly to the container under all anticipated storage and transport conditions. The label applied must not be removed, altered or obscured. A sufficient area of the container must remain uncovered to permit inspection of the contents, whenever possible.

Where additional labels are applied to packaging, an automated verification step to ensure the correct match between container label and package label is recommended.

For processing of batches that include large numbers of individual final units, a representative printed label should be included in the processing batch record.

In the European Union, the requirements for final labelling of tissues and cells for distribution are detailed in Annex II.E of Directive 2006/86/EC. Following the adoption of the Directive (EU) 2015/565, the label also needs to include the SEC and, for imported tissues and cells, the country of procurement and the exporting country (if different from the procurement country).

14.4. Sample and documentation labelling

All key cell and tissue samples for testing or archiving and all related documents must be labelled in a legible, indelible and unique manner that ensures traceability to the donor and the associated donations. A record of the time and place the sample was taken must be included on the label or in accompanying documentation.

14.5. Management of packaging and labelling materials

Selected packaging material must be able to withstand the requirements of the storage temperature (ambient temperature, refrigeration, freezing, cryopreservation) and sterilisation procedure (if this is to be applied) needed to preserve the required characteristics of the tissues or cells and, if applicable, biological function. Additionally, the shipping container must be able to maintain this environment for an appropriate amount of time during transport. Primary packaging and transport containers used for tissues and cells should be validated for this purpose, and they must be suitable for use with human materials (see Chapter 2). Selection of packaging, or a combination of packaging systems, should result in a sealed environment that prevents leaks.

As a general rule, labels should be machine-printed for clarity. They should be printed with ink that does not run or otherwise become unreadable when exposed to water or other liquids. Labels must maintain integrity and remain attached to primary packages and transport containers at the storage temperatures.

All printed labels for primary packaging, secondary packaging and for documentation intended to accompany the tissue or cell product should be stored in access-controlled areas.

Management of packaging and labelling materials must include the following elements:

a. there must be written specifications for all packages and labels used for tissues and cells;

b. there must be documented procedures describing the receipt, identification, quarantine, sampling, examination, testing, release and handling of both packaging and labelling materials;

c. a version control system should be in place to guarantee use of the current approved version. If a change of version occurs with regard to labels, inserts or packages, the actions needed to ensure that only the latest version is attached to the tissue or cells should be described in a written manner;

d. the suitability of packaging material, containers and labels for their intended purpose must be documented.

14.6. Primary packaging and labelling for procurement operations

'Primary packaging' refers to the materials that will come into direct contact with the tissues and cells and are, therefore, considered to be 'critical'. The selected materials should not leach harmful chemicals, they should be capable of being sterilised by a safe method (if required) and they should be sealable, leakproof and traceable.

After procurement, all tissues and cells must be packaged in a manner that minimises the risk of contamination and must be stored at temperatures that preserve the required characteristics and biological functions of the tissues and cells.

Packaging must also prevent contamination through exposure to those persons responsible for handling and transportation of the tissues and cells.

Procured tissue must be inspected and recognised appropriately before packaging and labelling to avoid mix-ups. Each tissue must be packed separately in sterile packaging as soon as possible after recovery. Double or triple wrapping may be necessary, depending on the tissue-specific requirements. Musculoskeletal tissues and skin may be packed in sterile, transparent polymer foil (though additional cotton wrapping can be used) or in containers with or without transport medium. Corneas must be placed in sterile transparent containers with medium, whereas heart-for-heart valves, amniotic membrane, skin, or cartilage for cell cultures must be packed in

sterile containers with transport medium. Whole eyes must be stored separately in moist chambers. Composition of the transport medium for a particular type of tissue must maintain the biological properties of tissues and may include antibiotics and antimycotics validated by type and concentration.

Procured cell products are mostly packaged in disposable bags. These bags are also double wrapped before the product is transported. Reproductive tissues and cells are mainly packed and transported in straws or tubes, either in culture medium or cryopreserved.

A unique identification number or code must be allocated to the donation and to donated tissues and cells during procurement, or at the end of the recovery process, to ensure appropriate identification of the donor and traceability of all donated material.

Table 14.2. **Labelling of the primary container**

As a minimum, the primary container must include a unique donation identification number or code. The information listed in this table in **bold print** must be included on the label if space permits or, if there is insufficient space on the primary container label, the information must be included in a separate sheet accompanying the primary container. Information listed in normal print must be included either on the label or in accompanying documentation. Accompanying sheets must include the unique donation identification number or code specified on the primary container.

Labelling of procured tissues and cells

- **unique donation number or code**
- **type of tissues or cells**
- **date (and, where possible, time) of procurement**
- identification of the procurement organisation

Any accompanying tissue or blood samples for testing must be accurately labelled to ensure identification with the donor and must include a record of the time and place the specimens were taken.

Labelling of tissues and cells from a tissue establishment released for circulation to another operator for further processing

- **unique donation number or code and, for tissue establishments in the EU, the donation identification sequence (DIS) from the Single European Code (SEC). For tissues or cells imported from outside the EU, the DIS must be applied by the tissue establishment responsible for import.**
- **identification of the originating tissue establishment**
- **type of tissues or cells**
- **expiry date and, where relevant, time (in UTC if the tissues or cells are to be shipped to another time zone). If an expiry date has not been defined, the expiry date must be recorded in the SEC as '00000000'**
- date of circulation (in accompanying documentation to avoid having to re-label the primary container)
- biological tests/assessments carried out on the donor and the results
- presence of potential harmful residues (e.g., antibiotics, ethylene oxide, etc.)

Final labelling of tissues and cells released for distribution to an organisation responsible for human application

- **unique donation number or code and, for tissue establishments in the EU, the SEC. For tissues or cells imported from outside the EU, the SEC must be applied by the tissue establishment responsible for import**
- **types of tissues or cells and lot or batch number where applicable**
- **expiry date and, where relevant, time (in UTC if the tissues or cells are to be shipped to another time zone). If an expiry date has not been defined, the expiry date must be recorded in the SEC as '00000000'**
- description (definition) and, if relevant, dimensions/volume of the tissue or cell product
- date of distribution (in accompanying documentation to avoid having to re-label the primary container)
- biological tests/assessments carried out on the donor and the results
- presence of potential harmful residues (e.g., antibiotics, ethylene oxide, etc.)
- morphological and functional data, where relevant
- a statement that the tissues or cells are suitable for human application according to relevant medical selection criteria and testing for markers of transmissible disease
- a statement limiting use of the tissues or cells to specific health professionals
- a statement, as applicable, that the tissues or cells may not be sterilised or re-sterilised
- a statement that it is the responsibility of the organisation responsible for human application to maintain the tissues or cells according to specified storage conditions and to follow instructions for opening the container, package and, where relevant, any required manipulation/reconstruction
- instructions for reporting serious adverse reactions and/or events

Information to be included on all labels

- **for autologous donations, the label must state FOR AUTOLOGOUS USE ONLY**
- **for directed donations, the label must identify the intended recipient**
- **when tissues or cells are from a donor known to be positive for a relevant infectious disease marker, the warning BIOLOGICAL HAZARD must be included**
- for imported tissues or cells, the country of procurement and, if different from the country of procurement, the exporting country
- nature of additives (if used)
- storage conditions required to maintain the quality and safety of the tissues or cells
- instructions for opening the container, package and, where relevant, any required manipulation/reconstitution
- expiry date after opening/manipulation

The minimum information that should be present in a primary label is described in Table 14.2. If any of the information listed in Table 14.2 cannot be included on the primary package label, it must be provided in accompanying documentation inside the transport container. Small containers, such as straws, must be labelled at least with a unique identification number or code (e.g., treatment code, donation number, or similar) and this identifier must

be provided on the accompanying documentation. Table 14.2 lists the required information that should be provided either on the label or in accompanying documentation.

14.7. Secondary packaging and labelling for procurement operations

If secondary packaging is used after procurement, it should adhere to the same requirements as those established for primary packaging. If labels with all the required information are not attached to the primary packaging they should be attached to the secondary packaging, which should be closed and sealed, ensuring that any unique identification number on the primary label is present on the label for the secondary pack and on accompanying documentation.

14.8. Outer container packaging and labelling for procurement operations

Packaged tissues and cells must be shipped in a container that is suitable for the transport of biological materials and maintains the safety and quality of the tissues or cells. Temperature conditions between recovery and processing must be appropriate for the type of tissue or cell to preserve the required characteristics and biological functions (i.e. temperature and duration of transport to the tissue establishment where the tissue processing will take place). The container must be closed fully with a tamper-evident seal and not opened until the procured tissues or cells are received by the tissue establishment.

When tissues or cells are shipped from the procurement site to the tissue establishment, the transport container must be labelled with the information described in Table 14.3.

14.9. Procurement package insert

It is recommended that the documentation accompanying the procured tissues or cells indicates, where applicable, that they are in a state of 'quarantine' to ensure that it is clear that a final review regarding their release for distribution and use has not been completed. See Chapter 6 for full guidance on the requirements for procurement documentation.

Table 14.3. **External labelling of the shipping container**

For transfer of procured tissues or cells from the procurement organisation to a tissue establishment

- identification of the originating procurement organisation, including name, address and telephone number of a contact person
- identification of the tissue establishment destination, including name, address and telephone number of a contact person

For transfer of tissues or cells from a tissue establishment to another operator for further processing

- identification of the originating tissue establishment, including name, address and telephone number of a contact person
- identification of the other operator destination, including name, address and telephone number of a contact person

For transfer of finished tissues or cells from a tissue establishment to an organisation responsible for human application

- identification of the originating tissue establishment, including name, address and telephone number of a contact person
- identification of the organisation responsible for human application destination including name, address and telephone number of a contact person

Information to be included on all shipping labels

- a statement that the package contains **HUMAN TISSUES/CELLS** and the warning **HANDLE WITH CARE**
- where living cells are essential for successful human application, the warning **DO NOT IRRADIATE** must be added
- when tissues or cells are from a donor known to be positive for a relevant infectious disease marker, the warning **BIOLOGICAL HAZARD** must be added
- date and time at the start of shipping
- shipping conditions relevant to the quality and safety of the tissues or cells (e.g., **DO NOT DELAY**, **KEEP COOL**, **KEEP IN UPRIGHT POSITION**, **DO NOT FREEZE**)
- when shipping by air, it is mandatory under International Air Transport Association (IATA) regulations that an IATA Time- and Temperature-sensitive Label is attached to the outside of the shipping container. The lower half of the label must indicate the permitted external temperature range in degrees Celsius (see §14.12)

14.10. Packaging and labelling during processing

Labelling of unfinished tissues or cells during intermediate phases of processing must be applied to all packaging materials and containers to assure identification at all times.

14.11. Packaging and labelling for finished tissues and cells

14.11.1. Primary packaging and labelling for finished tissues and cells

Primary packaging and labelling refers to the materials that will come into direct contact with tissues and cells, and the requirements in this regard are described in section 14.6, with a special focus on the radiation-resistance of packaging material for tissue that will be sterilised by irradiation. The expiry date will be determined not only by the properties of the tissues and cells but also by the integrity and stability of the packaging and labelling materials, among other factors.

Packaging and labelling procedures must be done to prevent cross-contamination or mix-ups. Simultaneous operations should be avoided or adequate measures should be taken to ensure that no cross-contamination or mix-ups occur [15].

Facilities where packaging or labelling operations have taken place should be checked before starting any other operation to guarantee that all previous materials have been removed.

Printed labels should be examined carefully to ensure that the information contained conforms to the corresponding tissues or cells. Results of this examination should be documented. A printed label, representative of those used, should be included in the processing records.

Unused and already printed labels must be destroyed according to written procedures.

The information that needs to be on the primary package label of the finished product is detailed in Table 14.2.

If the primary container is too small to host a label with all the required information (as may be the case with, e.g., gametes and embryos), the minimum information on the primary container needs to be a unique identification number or code. This unique identification number or code and the other required information must be included in an accompanying document.

The additional information that must be provided either on the label or in accompanying documentation is described in Table 14.2.

14.11.2. Secondary packaging and labelling for finished tissues and cells

'Secondary packaging' and labelling refers to materials that are not intended to come into direct contact with the tissues and cells. Special consideration must be given when primary and secondary packaging and labelling are designed to be kept together until the moment of use. If secondary packaging is not sterile, it should be clarified in the package instructions that the outside of the primary package is also not sterile and should not be placed within the sterile field during clinical application.

14.11.3. Outer container packaging and labelling for finished tissues and cells

When tissues or cells are shipped for distribution, every transport container must be guaranteed to maintain the conditions needed for the specific tissue or cell type. Containers must provide adequate protection against deterioration or contamination of tissues and cells that may occur during storage and transportation. Containers should be cleaned before use to ensure that they are suitable for their intended use. These containers should not alter the quality, safety or efficacy of the tissues or cells. Records should be maintained for each shipment of labels and packaging materials showing receipt, examination or testing, and whether accepted or rejected. For transport, the shipping container must be labelled with all the same information as specified in Table 14.3.

14.11.4. Package insert for finished tissues and cells

A 'package insert' refers to the supplementary information associated with tissues and cells that cannot be placed on labels. Critical information for the clinical user must be provided.

14.12. Customs clearance

For clearance of customs, all tissues and cells crossing borders require a clear description of the content of the consignment, its destination and intended use. The paperwork sent with the consignment should include the World Customs Organization Tariff Number for Human Tissue for Transplantation, which at present is 30029010, but there is a request that this should be altered to base code 082 with subcodes. It is important that the transport of frozen or cryopreserved products packed in dry ice or stored in a dry-shipper, must not be delayed at border crossings. If the goods are being transported by air, packages must be labelled with the appropriate International Air Transport Association (IATA) codes: UN1845 for dry ice or UN1977 for liquid nitrogen in a dry-shipper, and UN3373 for shipment of biological substances by air [16, 17]. Therefore, it may be expedient

for the importer to inform customs of a prospective consignment, and any enquiries by customs should always be answered promptly (see Chapter 10). For tissue or cell transport, the agreement with the shipping tissue establishment should define responsibilities for meeting the cost of transport and storage under appropriate conditions at a receiving facility for any items that may be detained pending customs enquiries.

14.13. References

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Chapter 15: **Traceability**

15.1. Introduction

Clinical application of tissues and cells brings great benefits for patients. There are, however, rare (but important) risks associated with such clinical use, including graft/application failure, donor-transmitted infections, malignancies and genetic conditions. The concept of traceability is the means to link a donor with recipients, or with offspring born through medically assisted reproduction (MAR), and all information about the transferred tissues and cells from donation to clinical outcome and follow-up.

Traceability means the ability to locate and identify the tissue/cell during any step from procurement, through processing, testing and storage, to distribution to the recipient or disposal, which also implies the ability to identify the donor and the tissue establishment receiving, processing or storing the tissue/cells, and the ability to identify the clinicians at the medical facility applying the tissue/cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues/cells [1].

The increased transportation of grafts across national boundaries has made traceability difficult and sometimes impossible [1]. It is therefore essential to facilitate rapid action to prevent harm when links in the safety and quality chain are found to have been compromised. Apart from quality and safety, traceability is also crucial for ethical reasons, as it allows legitimate donation with proper consent to be verified for every tissue or cell product.

The system of traceability is inseparable from, and in practice dependent on, the coding system (see Chapter 14). Effective traceability and biovigilance in the global context depend upon the use of globally unique identification for all donated biologic products [2].

The need to comply with traceability requirements should not compromise the need to guarantee anonymity between donor and recipient (or newborn), depending on the type of donation and the national legislation enforced. Records should be kept by the entities involved in the donation, procurement, processing, storage, distribution and application of tissues and cells to ensure compliance with safety requirements, but records should never allow the disclosure of confidential information to unauthorised persons.

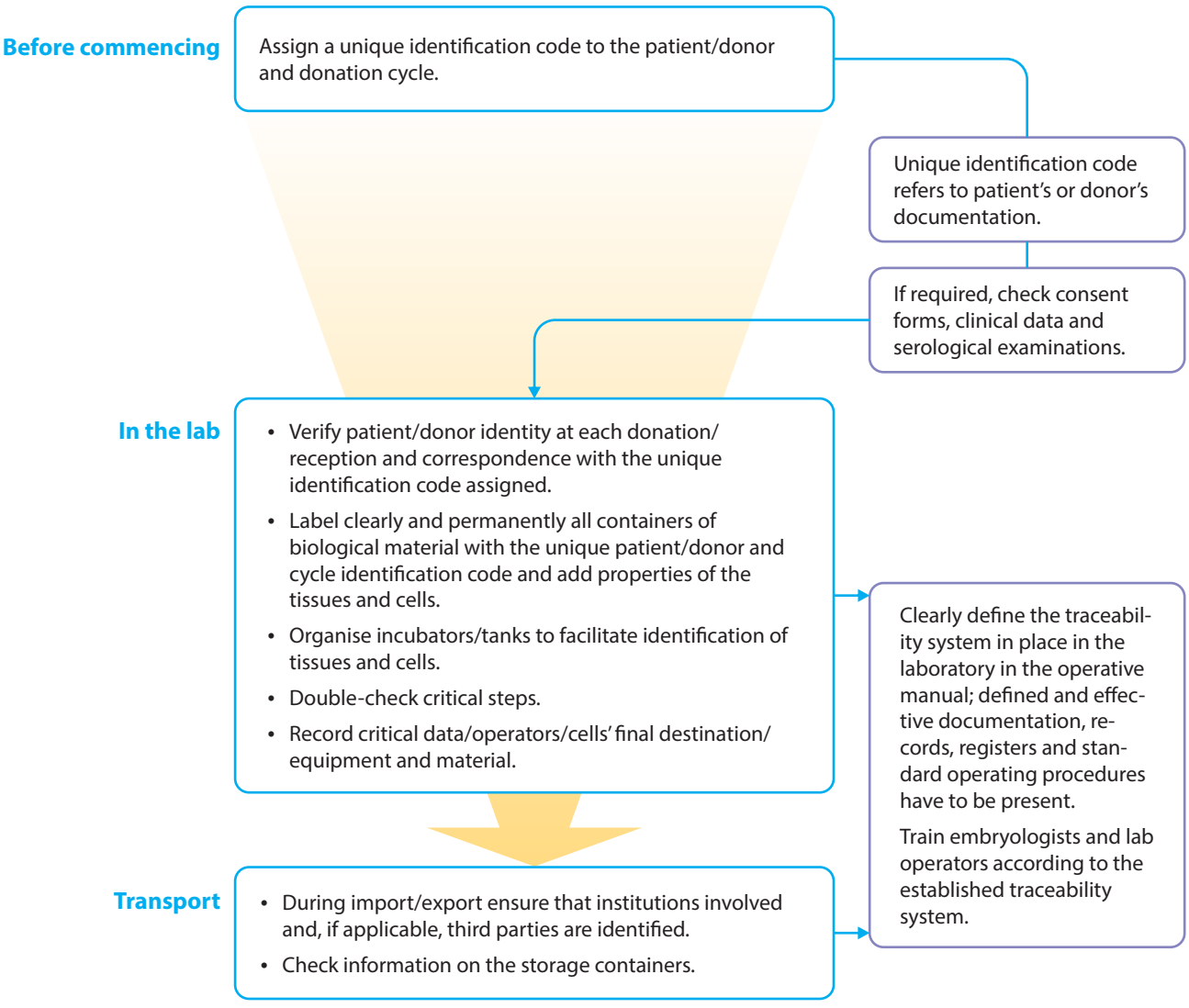
Human error, equipment failure, use of inadequate written procedures or new risks that cannot be predicted may affect quality, safety or effective use of tissues and cells at any stage, potentially increasing the risk to recipients and offspring. In the case of deceased donors, procurement teams are provided with a medical history at short notice, and additional information about the donor at a later stage may have implications for the safety and quality of tissues procured from those donors. Use of defective equipment, poor-quality consumables, contaminated solutions or defective testing kits may only come to light after the tissues and cells have been processed and transplanted. This means that traceability, from donation through to end use, is essential to determine which tissues or cells could potentially be affected by addi-

tional information or adverse incidents. For MAR, traceability does not stop when the tissues and cells reach the recipient. The health of the children born as a result of MAR treatment must be followed up, so that data on children's health and follow-up of pregnancies are included in the chain of traceability.

Tissue establishments play a special role in assuring traceability, collecting the data that guarantee the ability to locate and recall tissues and cells or inform the applying clinicians and recipients, once the establishment becomes aware of information that may have implications for their quality and safety. Tissue establishments are responsible for communication with other entities, such as organ transplant units, and other tissue establishments (including MAR centres) involved in the procurement/collection or processing of additional tissues and cells, or cells from shared donors.

The time interval between detecting risks to the quality and safety of tissues and cells, and preventing them from being used in patients, has been referred to as the 'traceability window period' [3]. Recalls can be due to inappropriate evaluation of donors, positive serology tests in the donor, contamination of tissues or cells, infection in recipients of other tissues donated by an individual donor and other risks introduced during the processing or storage of tissues or cells. The increasing global circulation of tissues and cells for clinical use, the fact that several tissue products can originate from one donor who may also donate organs, or that many children may be born from one sperm donor, and the existence of international markets for equipment, consumables and additives all add to the need for robust systems of traceability.

Figure 15.1. **Example of traceability system in medically assisted reproduction**



Source: ESHRE revised guidelines for good practices in IVF laboratories (2015) [4].

Records and procedures required to maintain traceability must be kept long after the clinical use of products (see §15.2.e), allowing personnel to track and trace all steps associated with the tissues and cells long after their clinical application, making adequate biovigilance and follow-up procedures possible. Traceability underpins biovigilance (see Chapter 16). Within each tissue establishment, investigation of adverse events and adverse reactions, and of deviations from standard procedures, can be carried out only if a system of traceability is in place. Many establishments share practices and standards, and effective investigations can help to improve them. Hence, in addition to biovigilance, ongoing quality improvement of procedures relating to procurement, processing, donor testing, storage and distribution of tissues and cells also benefits from good systems of traceability.

Traceability requirements are often defined in legal obligations, and may include the ability to report the precise number of units and recipients, for use as denominator data in the evaluation of adverse occurrence frequency at national and international level.

Traceability must encompass all the data associated with the final destination of tissues and cells distributed by third parties, including records of the final distribution of imported and/or exported units.

15.2. How traceability works

Traceability is the 'thread' that joins all the pieces of critical information together, from the moment that a potential donor is identified until the moment when the tissues or cells are applied to the recipient or discarded. This means traceability is a concept allowing (i) tracing of procedures when recipients show any adverse reaction that could be linked with the quality of the tissues or cells distributed, (ii) tracking the fate of recipients and (iii) follow-up of the health of MAR children, and (iv) tracking units associated with incidents detected after distribution or clinical application.

Tissue establishments must ensure that data protection and confidentiality measures are in place, in accordance with the local national data protection laws. Many organ donors are also tissue and cell donors, so it is important that effective links are in place between organ-procurement organisations and tissue establishments.

All records must be legible and indelible, protected from unauthorised amendments, stored securely and readily retrievable. Establishments should conduct regular audits of records to ensure that they are accurate and comprehensive. Good practice requires that amendments to written records are signed and dated. Computer records should be maintained in a validated system (see Chapter 13) and there must be procedures to back up electronic records to prevent loss, corruption and unauthorised access or amendment. Records must be shown to be reliable and a true representation of the events. Records may be handwritten or transferred to another validated system, such as a computer or microfilm. Records should be maintained of equipment and consumables, including the lot numbers and expiry dates of additives, cryoprotectants and packaging materials used during procurement and processing. The tissue establishment should also retain temperature records, analyser printouts and relevant environmental monitoring records for viable and non-viable particles. If tissues and cells have been imported, it is important that tissue establishments ensure that the traceability chain is retained and that the records for traceability (see §15.3) are accessible.

The following are the key requirements of an effective traceability system:

a. Unique identification

At each stage in the pathway, from donor to recipient or child conceived as a result of MAR treatment, each tissue establishment must have records of the donor, the donation and donation samples, and must ensure that they are identified and labelled uniquely within their own organisation (see Figure 15.1 for an example). While uniqueness can be ensured without difficulty within one organisation, the risks of duplication are increased when tissues, cells, samples or records move from one organisation to another. For example, duplicate identifiers may result when samples are sent to a testing laboratory or when tissues or cells are sent to a hospital because each receiving establishment may assign its own identifier. This risk can be eliminated if a global standard is used to identify samples or tissue products. Within the European Union (EU), the Single European Code (SEC) will help to address this need (see Chapter 14). The SEC allows, within its structure, incorporation of the international coding systems ISBT 128 and Eurocode.

b. Safe transfer of critical information

The traceability trail depends on the accurate transcription of critical identification information. Manual transcription errors can cause breaks in the traceability trail. Use

of electronic transfer of critical information (bar codes or other machine-readable codes) is recommended. If manual transcription is used, double checking of data must be implemented. Electronic storage of data – preferably in well-protected databases for easy and quick access by authorised personnel – is preferred to paper-based information collections.

c. Timeliness
If a risk is identified, it must be possible to rapidly trace all implicated products or all potentially affected recipients and children conceived through MAR. A delay could result in harm to patients or children conceived through MAR. Systems need to be quickly accessible, with efficient links between organisations to reduce the 'traceability window period'.

d. Clarity of responsibilities at interfaces between organisations
To guarantee traceability, tissue establishments should distribute tissues and cells to other tissue establishments, to organisations responsible for human application (ORHA) or to healthcare professionals who have responsibility for clinical application (and not directly to recipients). It is essential that each organisation in the chain clearly understands its responsibilities for traceability. It is notable that in the published high-profile cases of viral transmission during transplantation, hospitals were often not able to trace all recipients [5]. Maintaining traceability is one of the key legal and technical responsibilities of an ORHA (see Chapter 12). Tissue establishments must define responsibilities and procedures prior to the distribution of tissues, cells, gametes or embryos to those organisations.

e. Long-term storage of secure records
For effective reviews, traceability data need to be maintained for long periods of time. For example, in the EU, all information related to traceability must be maintained for 30 years after application or the expiry date of the tissues and cells. Data that are critical to the safety and quality of tissues and cells, including records of equipment used and materials such as consumables coming into contact with those tissues or cells, should be kept so as to ensure access to the data for at least 10 years after clinical use of the product, its expiry date or disposal. Organisations need to consider the impact of the obsolescence of technology and to ensure that records remain quickly accessible. There is a need for regular management review of data storage, with a proactive approach to prevent obsolescence.

f. Traceability provisions
The location of traceability records may change when organisations are closed or merged, or if they cease activities relating to donor selection, donor testing, procurement, processing, distribution or transplantation. In such cases, there must be an effective link between the new location of the data and the previous location, and provision must be made to prevent loss of traceability information, for example by signing contracts with other tissue establishments for taking care of these data in critical situations.

g. Traceability audits
Organisations must include audits of traceability from donor to recipient and vice versa as part of ongoing quality management. The traceability trail may encompass data stored in several organisations.

The EU definition of traceability is provided in the Glossary (Appendix 3) of this Guide.

15.3. Which records must be traceable?

There must be a system of record keeping for all activities associated with tissues and cells. Records should describe donation procurement, donor testing, processing, storage, distribution and end use. Records should include details of equipment used, materials such as consumables that have come into contact with those tissues and cells and the identity of the members of staff who were responsible for all critical activities from procurement until implantation or disposal. These robust systems must ensure secure identification of:

a. the donor and all records associated with the donor and their medical and behavioural history;

b. the donation (tissues or cells procured/collected from the donor);

c. all records associated with processing, storage and distribution of the final products, and related events;

d. all samples taken from the donor or from the tissues/cells for the purposes of testing for quality and safety;

e. the clinical application and recipient(s) of the tissues or cells;

f. the health of the resulting child(ren) and any adverse data on pregnancies (for MAR treatment).

15.3.1. Records of identification, donor tests and clinical evaluation of the donor

Besides the information defined in Chapter 4: Donor evaluation, tissue establishments must keep in their records at least the following data:

a. donor identity;
b. age, sex, medical and behavioural history of the donor;
c. outcome of physical examination for deceased donors;
d. completed haemodilution algorithm (where applicable);
e. consent/authorisation form;
f. relevant clinical data, laboratory test results and the results of any other tests carried out;
g. for deceased donors, results of the autopsy (if carried out) or preliminary verbal report;
h. for haematopoietic progenitor cell (HPC) donors, the donor's suitability for the chosen recipient (see Table 15.1 for an example);
i. for unrelated HPC donations, where the organisation responsible for procurement has limited access to recipient data, the ORHA or the physician should be provided with the relevant donor data to confirm suitability.

In addition, the donor testing records must be accessible at the laboratory (in-house or at a contracted laboratory) and contain at least:

a. date and time donor blood samples were taken;
b. date of receipt of the blood sample at the testing facility;
c. record of each test kit used to test donor blood sample (i.e. manufacturer, lot number, expiry date);
d. results of donor testing, including repeat testing (if applicable).

Accessibility authorisations and the responsibilities associated with record keeping and reporting, of both tissue establishments and testing laboratories, should be properly defined through a technical and legal written agreement (for technical agreements with testing laboratories, see §5.4).

15.3.2. Records of procurement of tissues and cells

Besides the information defined in Chapter 6: Procurement, the organisation undertaking procurement should produce procurement reports and provide them to the tissue establishment. The procurement report should contain at least:

a. the identification data of the tissue establishment receiving the tissues or cells;
b. donor identification data (including how and by whom the donor was identified) and records of testing of the donor;
c. description and identification of procured tissues and cells (including samples for testing);
d. identification of the person who was responsible for the procurement session, including his/her signature;
e. date, time (start and end, if relevant) and location of the procurement and standard operating procedure used;
f. description of the physical area where procurement took place, including environmental conditions at the procurement site (where necessary);
g. for deceased donors, storage conditions of the deceased donor, i.e. refrigerated (or not) and time of start and end of refrigeration;
h. manufacturers and lot numbers of reagents and transport solutions used;
i. any incidents that occurred during procurement.

15.3.3. Records of processing of tissues and cells

Besides the information defined in Chapter 8: Processing and Chapter 9: Storage and release, the organisation undertaking processing should keep at least the following records:

a. tissues and cells received and evaluation of their suitability;
b. standard operating procedures used to process the tissues and cells;
c. equipment used during processing;
d. records of consumables used during processing (manufacturer, lot number, storage conditions of consumables – if appropriate – and expiry date);
e. records of sterilisation or decontamination, if applicable;
f. records of cryopreservation and freezing protocols, if applicable;
g. records of environmental monitoring (temperature monitoring, microbial monitoring and particle counts as appropriate);
h. records of product testing, including microbial testing;
i. any incidents that occurred during processing.

Table 15.1. **Traceability of unrelated haematopoietic progenitor cells donor and recipient data**

	Donor centre	National registry	WMDA	Collection centre	Tissue establishment	Transplant centre patient
Activities	Consent Testing Donor follow-up	Listing Donor and patient follow-up	Listing	HPC collection	Product labelling, processing and release	Infusion Patient follow-up
Donor data*	ID code Identity	ID code Identity of National Registry donors only	ID code only	ID code Identity	ID code Product code (e.g., SEC)	ID code only
Patient data*	ID code Identity	ID code Identity	NA	ID code Identity	ID code Identity	ID code Identity

WMDA: World Marrow Donor Association.

The identity and privacy of all patients and donors are protected throughout the process of HPC donation and transplantation (Identity=name).

* Anonymous contact between patient and donor allowed post-transplantation only through Registry.

15.3.4. Records of storage and distribution of tissues and cells

Besides the information defined in Chapter 11: Distribution and import/export, organisations undertaking storage of tissues or cells should keep at least the following records:

a. storage location and a transfer record if storage locations change;
b. date placed in storage;
c. date removed from storage;
d. records of storage temperature including details of nitrogen storage (e.g. vapour phase above liquid nitrogen);
e. any incidents that occurred during storage.

In addition, when the tissues or cells are transported or distributed to hospitals or clinics for application, tissue establishments should keep the following records:

a. name of party responsible for distribution;
b. identification of the establishment, courier or individual who transported the tissues and cells at any stage between procurement and end use (clinical application);
c. packaging records (e.g. records of the dry-shipper used);
d. time and date of distribution of tissues and cells;
e. time and date of delivery of tissues and cells;
f. identification of the receiving establishment, clinician or ORHA;
g. any incidents that occurred during distribution.

15.3.5. Records of clinical application of tissues and cells

Besides the information defined in Chapter 12, the ORHA should keep at least the following records:

a. identification of the supplier tissue establishment;
b. identification of the clinician or ORHA;
c. type(s) of tissues and cells;
d. product identification;
e. identification of the recipient;
f. date of clinical application;
g. any incidents that occurred during clinical application;
h. any adverse reactions or adverse events in the recipient;
i. health outcomes of children born following MAR.

Systems must be in place to assure the follow-up of tissue and/or cell recipients and children conceived after medically assisted reproduction (MAR) treatment. Such follow-up can be achieved only if a close working relationship exists between all stakeholders: that is, the tissue establishment, ORHA, MAR centre and parent(s) involved.

Some national standards require the ORHA to provide the supplying tissue establishment with details of the patient to whom the tissues or cells were clinically applied. Whether this information is sent to the tissue establishment or not, it is essential that the end user maintain these records because ultimately they are responsible for recording the fate of the tissues or cells.

15.4. References

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Chapter 16: **Biovigilance**

16.1. Introduction

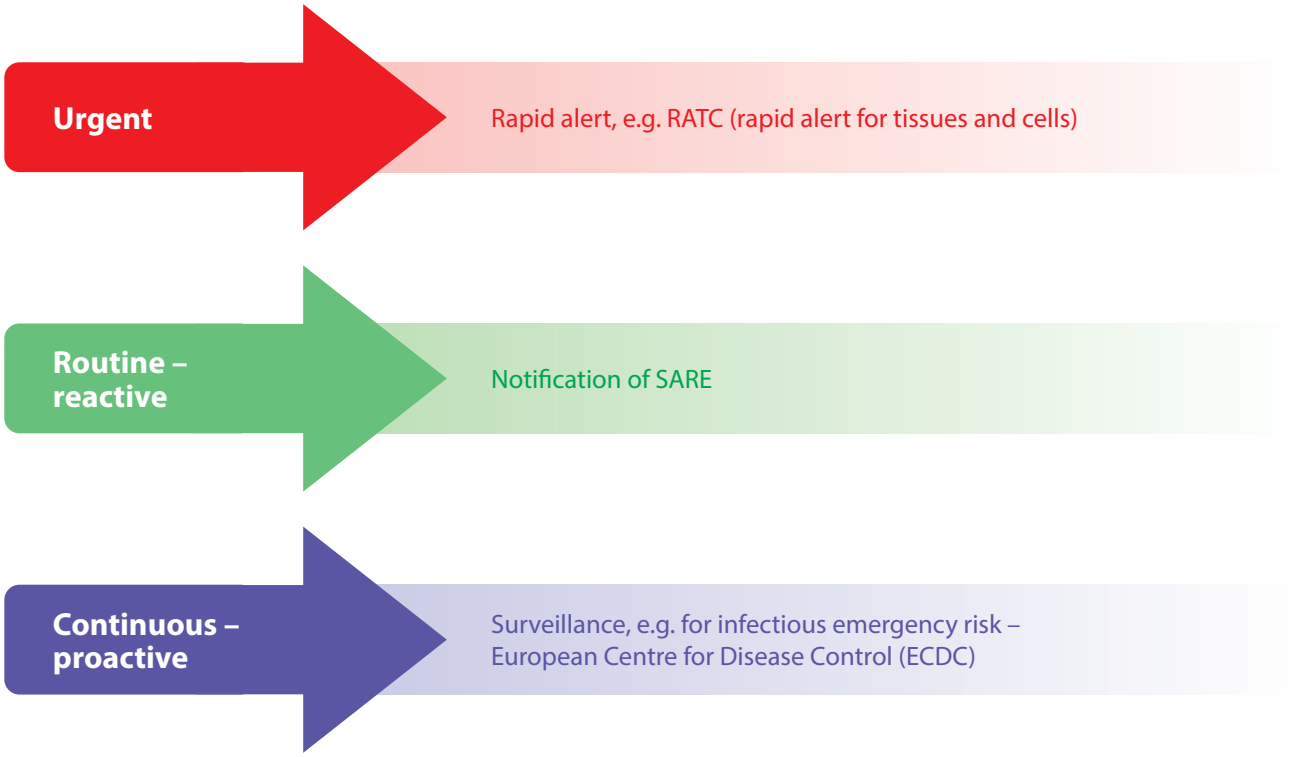
This chapter provides general guidance on the implementation of good vigilance and surveillance (V&S) practice by all those (including regulators and Health Authorities) involved in the processes of transplantation, which includes medically assisted reproduction (MAR), from donation through banking to clinical use until the donated tissue or cell functions in the recipient. The tissue- and cell-specific chapters in Part B provide additional specific guidance on vigilance in those fields; in particular, Chapter 27 details several specificities within MAR/ART (assisted reproductive technology) vigilance.

A programme of V&S is essential for ensuring the quality and safety of tissues and cells for human application. The quality system focuses on preventing errors and maintaining a consistent standard of agreed specification for tissues and cells released for clinical application. However, occasionally, residual risks or procedural errors result in failures, disease transmissions or situations in which donors or patients are exposed to risk, even if not harmed. Reporting of these incidents presents important learning opportunities that can help all procurement organisations, tissue establishments (TEs), cell therapy and MAR facilities, and clinical users (not only those involved in the incident in question) to improve their processes and to achieve higher standards of safety and quality at all levels: from TEs to donors and recipients [1, 2].

Biovigilance is the systematic monitoring of serious adverse reactions and events (SAREs) from the selection of the donor to the follow-up of the recipient, with the objective of making the application of tissues, cells, MAR and organs safer and more effective. There are several stages (phases) in a biovigilance system. The first one is to detect and identify a biovigilance case that could be described as an adverse event or reaction. Depending on the case and the system in place at national level, the following steps can be done in parallel. After identifying the case, it must be reported or notified to the Health Authority even if the investigation is not concluded. If there is a suspicion that other centres, TEs, suppliers or donors could be affected or involved, they have to be alerted rapidly by the TE or by the Health Authority to prevent further complications. Every single case (at least every serious case) must be investigated and evaluated by the TE and Health Authority, with the collaboration of all centres involved and also a group of professionals with experience in infectious and malignant or genetic diseases, quality control and quality management, as well as professionals with experience in the use of the tissue involved in the case.

Once the investigation is finished, it is important to decide how its findings should be managed, depending upon what kinds of actions have been decided on. Finally, the case will be closed and the final report must include both corrective and preventive measures. This final report should detail how to act on similar occasions in the future. It is worth noting that learning is an important benefit derived from biovigilance.

Figure 16.1. **Levels of biovigilance**



16.2. Definitions

These adverse occurrences can be classified into 'adverse events' (AEs), which are process failures that might lead to harm in a recipient or living donor or to a loss of any irreplaceable autologous tissues or cells or to a loss of any highly matched allogeneic tissues or cells, and 'adverse reactions' (ARs), which are adverse outcomes that have indeed occurred with harm to a donor, a recipient or a child born through MAR procedures related to *in vitro* fertilisation (IVF) with gamete or embryo donation. An adverse event may or may not cause an adverse reaction. Similarly, an adverse reaction may or may not be related to an adverse event.

According to European Union (EU) definitions, a 'serious adverse event' (SAE) in the present context is any untoward occurrence associated with the procurement (including donor selection), testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to a life-threatening, disabling or incapacitating condition for the patient or that might result in prolonged hospitalisation, morbidity or death. According to EU definitions, a 'serious adverse reaction' (SAR) is an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling or incapacitating, or which results in, or prolongs hospitalisation or results in morbidity.

These definitions are reflected in the World Health Organization (WHO) Notify Library for V&S of medical products of human origin (MPHO). Adverse outcomes are categorised in the library as follows:

a. Adverse reaction
- i. harm to a donor
- ii. harm to a recipient
- iii. harm to a foetus or offspring

b. Adverse event
- iv. risk of harm

In summary, an adverse reaction is an incident whereby a living donor, a recipient or a foetus or child created by IVF or intra-uterine insemination with donor gametes has been harmed, whereas an adverse event is an incident that results in a risk of harm, although no harm may actually occur. Those that are classified as 'serious' should be notified to Health Authorities, in accordance with national or regional (e.g. EU) requirements.

Although adverse events may occur at all stages from donor selection to distribution of tissues and cells, many of them are not severe and may be managed through the quality management system (QMS) of the TE. Conversely, SAREs are rare. Therefore, there are significant benefits associated with

consolidating V&S data on regional, national or international scales and on an integrated system for the different substances of human origin (SoHO), because they share exposure to risks from donation to transplantation (from breaches of ethical, legal and safety standards).

The follow-up of living donors after donation should ensure that, if a condition not known at the time of donation occurs to the donor, and it may have an impact on the recipient, it is clearly identified. In such cases there should be a documented procedure to notify the recipient's physician of this condition. This is not necessarily an adverse event. Conversely, when the recipient's physician detects an impact on the recipient, this must be reported to the TE. The same also applies to the potential long-term influence of any treatment provided for the procurement (e.g. mobilisation with cytokines or hormonal stimulation), in which case pharmacovigilance should also be involved.

If products containing tissues or cells are classified as advanced therapy medicinal products (ATMPs) in the EU, the regulatory framework of pharmacovigilance must be applied. The relevant legal texts and guidelines are described on the pharmacovigilance web page of the European Commission [3]. Donation, procurement and testing of tissues and cells used to prepare an ATMP are regulated in the EU by directives on tissues and cells. Consequently, good communication between biovigilance and pharmacovigilance systems is essential to facilitate effective investigation and corrective/preventive actions if ATMPs are associated with adverse outcomes.

16.3. Management and quality of vigilance

16.3.1. Vigilance

As for other vigilance systems, vigilance activities in the field of tissues and cells should be considered and recognised at all levels of TEs that are authorised for tissue and cell activities. The organisation of the vigilance system, as well as the role of the various parties involved, should be defined and broadly communicated within the TE.

Three levels of biovigilance can be described, depending on the types of measures and actions that can be taken into consideration: urgent communication, routine notification and proactive monitoring of possible SARs/SAEs (see Figure 16.1). These levels will be explained further in this chapter.

Health Authorities are encouraged to draw up guidelines for vigilance systems, notification forms, surveillance methods, acceptable risk criteria and examples of SAREs for tissues and cells that should be reported to them. Appropriate communication and co-ordination between procurement organisations, TEs and organisations for human application (OHRA) are essential for an efficient vigilance system. Organisations or bodies involved in activities based on tissues and cells (including clinical users) should have standard operating procedures (SOPs) in place that describe how to collect, report, investigate and communicate notifications for adverse reactions and events (AREs). Identification of a local co-ordinator, who has responsibility for V&S specified in their job description, is an effective measure. It is recommended that the QMS and V&S systems, both of which contribute to risk-management policy, should be co-ordinated at TE level according to guidelines established by the Health Authority and under the direct responsibility of the Responsible Person (RP). Implementation of computerised and integrated systems for collection and management of ARE data is encouraged.

This chapter focuses on the procedures for detection, reporting, alert, investigation, management, evaluation and closure of AREs that may occur from donation until clinical application and follow-up. All AREs and non-compliances involving any party (clinical users, donors, patients or third parties) and including those with minor consequences, should be documented and reviewed regularly within the QMS of the TE. Each report or communication should be considered for classification as an SARE and should be managed as such if it meets the criteria described in this chapter. This allows trends to be monitored and actions to be taken to continually improve quality and safety.

16.3.2. Surveillance

The term 'surveillance' denotes the follow-up of organs, cells or tissue recipients or living donors, with or without SARE, to provide indicators and information on stratification of risks. Furthermore, an active surveillance system should also monitor some specific expected serious side reactions or events. When a surveillance system is implemented, periodic analyses can show if there is an upward trend of SAREs, AEs or ARs, more or less systematically occurring and expected. These should be reported to the Health

Authority, a root cause analysis should be initiated, and corrective measures should be implemented.

Routine monitoring of clinical outcomes is part of the surveillance system. Clinical teams have to set up registries with follow-up on grafts and recipients post-transplant, as well as living donor outcomes, in order to monitor the results and to identify currently unknown risk factors. This monitoring should be complemented by an active surveillance system for well-known adverse reactions. Unfortunately, in the case of tissues there are very few registries at European level; existing ones are mostly at national level, e.g. cornea registry (see Figure 16.2). Although they are outside the scope of the vigilance system – because this focuses only on the 'undesired and unexpected SAR' – 'known ARs' should be evaluated further in order to exclude their occurrence being caused by a systematic error (e.g. incorrect handling of heart-for-heart valves during procurement).

The expected frequency of these 'known SARs' can be defined by the relevant experts or professional bodies, based on local experience and evidence based on literature data (e.g. expected rate of arterial thrombosis after the use of cryopreserved arteries). When the frequency of these complications increases in one centre beyond a threshold of pre-defined rates, a notification of 'suspected SAR' must be made to the Health Authority. The root cause analysis has to be performed by the local investigation team in order to determine the reasons for this deviation from the acceptable rate. This investigation falls within the framework of the vigilance system, in order to trigger corrective measures and to improve quality and safety of care to patients. But such surveillance should also identify whether good results are achieved by risk-avoiding behaviour of an institution or by true best clinical practice applied.

The widespread use of active surveillance systems will be a step-by-step process that still requires healthcare professionals to obtain consensus views on some important points, including issues like the definition of serious adverse reactions and events, as well as the description of their appropriate monitoring.

16.4. Adverse reactions

Adverse reactions must be detected, reported, investigated and assessed in terms of severity, imputability, probability of recurrence or frequency, and consequences. Efficient systems for rapid quarantine or recall of unsafe tissues or cells must be in place, along with procedures for look-back where donors or recipients are found to have been exposed to a risk. Important learning outcomes from each adverse reaction should be communicated appropriately to all professionals involved.

Several symptoms or situations can suggest that an adverse reaction might have occurred in a recipient of a tissue or cells and should, therefore, be seen as 'triggers' for an adverse reaction report. Note that, in certain circumstances, clinicians may knowingly transplant an infective donation (e.g. *Cytomegalovirus*-positive bone marrow); in such cases, patients should be informed about the benefits and the additional risks, and there should be specific follow-up. Clinical and biological monitoring, as well as prophylactic or pre-emptive treatment, should comply with existing recommendations or regulatory requirements, where they exist. Below are examples of reportable adverse reactions (for more information, see the chapters in Part B on each specific tissue) [with abbreviated descriptions in square brackets]:

a. suspected harm in living donor related to procurement [Donor harm];

b. unexpected primary infections possibly transferred from donor to recipient (e.g. viral, bacterial, parasitic, fungal, prion) [Infection from donor];

c. suspected transmitted infection (viral, bacterial, parasitic, fungal, prion) possibly due to contamination or cross-contamination by an infectious agent in the procured tissues, cells or associated materials, between procurement and their clinical application [Infection from infected/contaminated tissues and cells];

d. immunological reactions, including allergic reactions, graft-*versus*-host disease, rejection, haemolytic reactions or other immunological reactions [Immunology];

e. malignant disease possibly transferred by the tissues or cells (donor-derived, process-associated or other) [Malignancy];

f. unexpectedly delayed or absent engraftment, or graft failure (including mechanical failure) [Failure];

g. toxic effects to tissues and cells or associated materials [Toxicity];

h. unexpected immunological reactions due to tissue or cell mismatch or, in the case of ART, mismatch between oocytes and spermatozoa in a partner donation [Mismatch];

i. aborted procedure involving unnecessary exposure to risk, e.g. wrong tissue supplied, discovered after patient is anaesthetised and the surgical procedure has begun [Undue risk];

j. suspected transmission of genetic disease by transplantation or gamete/embryo donation [Genetic abnormality];
k. suspected transmission of other (non-infectious) illness [Other transmission];
l. transfusion-associated circulatory overload in haematopoietic progenitor cell (HPC) transplantation [Volume overload];
m. neurological reaction [Insult];
n. severe febrile reaction [Fever];
o. other [Other].

16.4.1. Detection of adverse reactions

Effective V&S relies heavily on all health professionals involved, from procurement to clinical application, namely:

a. medical staff (including surgeons) involved in tissue- and cell-procurement activities who might become aware or informed of additional safety information on donors during their follow-up;
b. staff and personnel carrying out procurement of tissues and cells;
c. clinical users who should pay attention to adverse outcomes and be aware when such outcomes might be associated with the clinical use of tissues or cells;
d. physicians caring for children born after non-partner MAR/ART treatment who may detect a genetic abnormality and, by reporting it, prevent further distribution of gametes/embryos from that donor;
e. any other TE staff involved in any procurement and transplant activities;
f. other vigilance systems (e.g. haemovigilance, vigilance of organs, material/device vigilance, pharmacovigilance) when issues of concern are detected that might affect the safety of tissues or cells for transplantation.

Adverse outcomes might result from many diverse factors associated with the surgical procedure or the patient's underlying condition. Hence, clinicians might not consider the tissues or cells that were applied to be a possible source of the adverse outcome. TEs that supply tissues and cells should encourage procurement organisations and clinical users of tissues and cells to always consider whether adverse outcomes might have been associated with the donation process or caused by the tissues or cells applied, so that similar occurrences are prevented in the future.

For most types of well-established clinical application of tissues and cells, detailed reporting of clinical outcome by the clinical user to the TE is required only in those exceptional circumstances in which there is suspicion of an untoward adverse reaction. However, reporting of the clinical progress of tissue and cell recipients to the TE might also be required for all highly matched, life-saving transplants such as HPC infusions, or when novel tissue or cell processes have been applied or new types of tissues or cells are being transplanted. This routine clinical follow-up is not considered as part of vigilance.

An important part of vigilance is detecting donation complications (also considered to be adverse reactions) in living donors that might be associated with the donation process in some way. For example, adverse reactions may be detected after stimulation treatment in living donors and recipients (see Chapter 22: Haematopoietic progenitor cells from bone marrow and peripheral blood and Chapter 27: Medically assisted reproduction).

16.4.1.1. *Surveillance for new risks*

Surveillance programmes should include an activity of scanning for new risks that have not been recognised previously. New risks may be related to new donors, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing. Newly emerging infectious diseases, for which targeted testing can be carried out or which might imply the need to exclude certain donors, represent an example of one type of new risk. The European Centre for Disease Prevention and Control (ECDC) monitors the epidemiology of diseases in Europe and publishes a weekly Eurosurveillance report that provides useful data to support the development of donor-selection policy. Moreover, the ECDC has recently been mandated to initiate risk assessment on particular epidemic agents, infectious diseases or new *in vitro* diagnostic techniques in the field of tissues and cells.

16.4.2. Reporting adverse reactions

16.4.2.1. *Clinicians to tissue establishments*

TEs that supply tissues and cells should provide organisations representing clinical users with clear instructions on how to report adverse reactions, preferably using standardised documentation. In general, suspected adverse reactions should be reported immediately by the clinical users to the TE that supplied the tissues or cells before investigation or confirmation. This approach allows the TE to take appropriate

precautionary actions to prevent harm to other patients, and start the investigation process. Clinical users should be encouraged to report all types of suspected adverse reactions (serious and non-serious) which might be related to the tissues and cells from the supplying TE, to allow filtering of those considered to be serious and reportable to an authority at a later stage.

Specifically, in MAR, couples undergoing non-partner donations are important stakeholders when reporting of SARs is concerned. Clinicians treating patients with donor gametes should inform and encourage patients that, if any disease is detected in their donor child, they must report back to the MAR/ART centre. Patients should be clearly informed of their registration obligations concerning diseases in donor children. It is in the interest of all patients using donor gametes that SARs in donor children are quickly notified in order to be able to quarantine straws from these specific donors and prevent further spread of a particular disease. It is imperative to note that not all diseases in donor children are directly related to the donor. Therefore, a careful risk assessment is needed that takes into account the type of disease (chromosomal, multifactorial, single-gene or mitochondrial disorders, communicable and non-communicable diseases) and the possibility of (genetic) testing of the child, biological parent and/or donor, as well as global prevalence and genetic predisposition. A similar approach applies to HPC transplantation (see §22.9.1.1.12).

16.4.2.2. *Procurement organisations to tissue establishments*

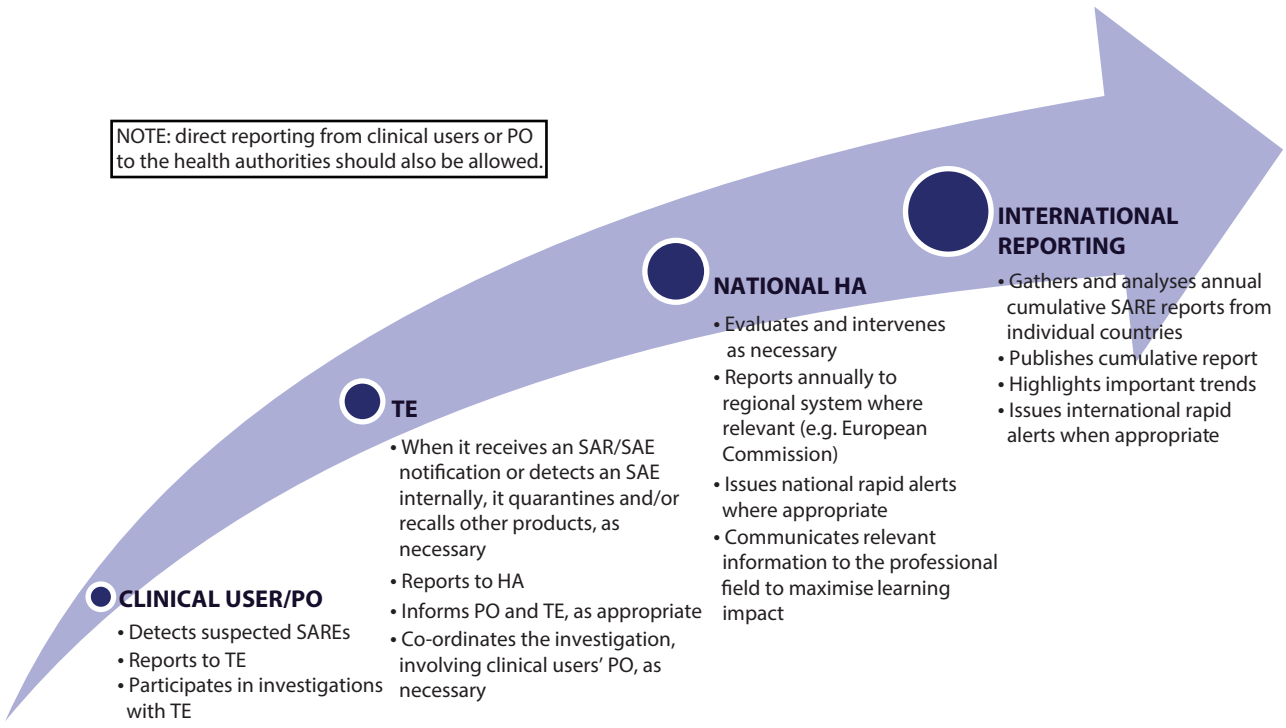
Similarly, health professionals and procurement organisations should report adverse reactions in living donors and recipients to the TE, even if the adverse reaction is only suspected to be donation-derived, so that the broader implications for other centres and donors can be considered without delay.

16.4.2.3. *Reporting to regional/national programmes*

TEs must report information on SARs to Health Authorities (see Figure 16.2). In the EU, all SARs related to quality or safety that meet the descriptions of 'serious', 'life-threatening' or 'death' must be reported to the Health Authorities.

The TE is responsible for providing clinical-user entities, procurement organisations and critical third parties with clear instructions, forms and guidance on how to notify adverse reactions in accordance with national or local requirements. Reporting and management of adverse reactions should be incorporated in the quality system of the TE, with one or more SOPs that describe the process for acknowledgement of notifications, investigation, follow-up on corrective and preventive actions and reporting to the Health Authorities if criteria are met. More-

Figure 16.2. **Reporting flow for serious adverse events/reactions**



HA: Health authority; EU: European Union; PO: Procurement organisation; SAE: Serious Adverse Event; SAR: Serious Adverse Reaction; SARE: Serious Adverse Reactions and Events; TE: Tissue establishment.

over, a specific procedure should enable rapid action, if needed, to be taken by all affected organisations to protect the safety of recipients.

This may involve tissue and cell quarantine, recall and look-back in patients who have already had implicated tissues or cells applied. These actions may need to be taken by organisations other than the one that received the original notification. For example, the organ procurement organisation will play a central part when the donor was an organ and tissue donor.

Figure 16.3 shows a series of actions that might need to be taken in a report of suspected transmission from a deceased donor of organs and tissues. It makes clear that communication with other organisations that might need to quarantine implicated tissues or cells, or conduct recalls or look-backs, should be quick and effective.

Although reporting of SARs should, in general, be co-ordinated and centrally reported by TEs at a national level, it is recommended that national V&S programmes allow direct reporting from clinical users or even patients to Health Authorities. This might occur where a clinician or a patient suspects that a TE is not working correctly or where they do not have confidence, for whatever reason, that the report will be fully investigated.

16.4.2.4. *International reporting*

If SARs are detected in relation to tissues or cells that have entered international distribution channels, appropriate international collaboration should ensure that all the stakeholders involved (clinicians, TEs and Health Authorities) in each of the countries concerned are informed and participate, as necessary, in the investigation and follow-up actions.

EU member states are obliged to send an annual report of the SARE notifications that they have received to the European Commission [4]. Such international reporting allows for trend analyses on the basis of consolidated data and for sharing of the lessons learned.

At national level, the Health Authority or regulatory agency mandates SARE reporting for donors (as described above for the EU). However, WMDA and national HPC registries have implemented a global reporting system because HPC products have specific characteristics (see Chapter 22).

16.4.2.5. *Follow-up of the donors of tissue and cells*

The follow-up of donors of tissue and cells is important for the early detection and reporting of SAREs. Short-term follow-up is essential to ensure recovery from the donation procedure. Long-term follow-up is desirable to enable any long-term effects of the donation to be identified. The nature and duration of this follow-up depend on the type of donation, the nature of the intervention and its potential impact on the individual's health [5].

Figure 16.3. **Example of an adverse reaction involving multiple parties**

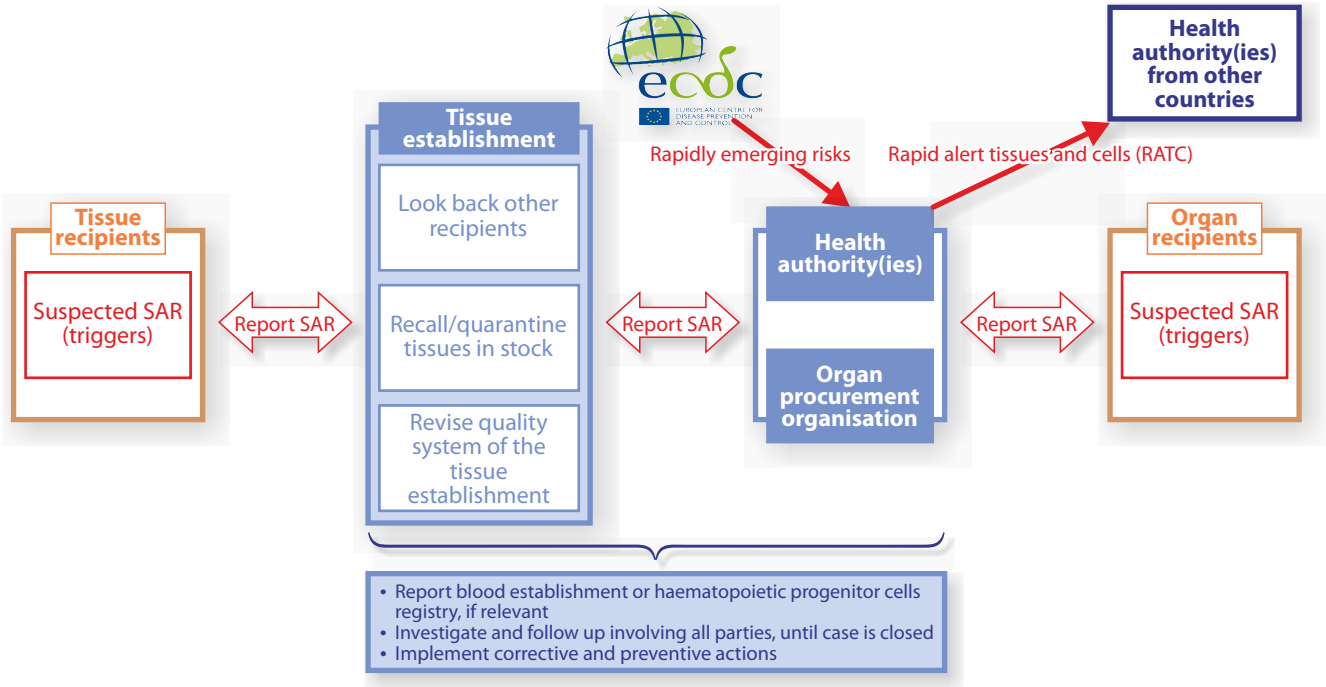


Table 16.1. **Severity scale for serious adverse reactions**

Not reportable	Insignificant	No harm to the recipient or living donor, and considered to be reportable as an event rather than a reaction according to EU directives
	Non-serious	Mild clinical consequences that do not necessitate hospitalisation and/or do not result in long-term disability or consequences for the recipient or living donor
To be reported	Serious	Adverse reaction resulted in: • hospitalisation or prolongation of hospitalisation and/or • persistent or significant disability or incapacity and/or • medical or surgical intervention to preclude permanent damage or impairment of a body function and/or • evidence of a serious transmissible infection and/or • birth of a child with a serious genetic disease after ART with non-partner gametes or donated embryos
	Life-threatening	The living donor or recipient required major intervention after procurement or application of tissues or cells (vasopressors, intubation, transfer to the intensive care unit) to prevent death and/or There is evidence of a life-threatening transmissible infection
	Fatal	Death in a living donor or a recipient of tissues or cells

Completed donor follow-up has been defined as physical, phone or laboratory contact at a given time point [6]. There should be written SOPs for follow-up of donors. For related donors, the responsible person for the follow-up should be the physician undertaking the assessment of the donor for the donation. For unrelated donors, the follow-up should be the responsibility of the relevant donor registry, if present.

After donation, the living donor should also be followed up, by documented procedures. This follow-up will depend on the type of donation, and the length of follow-up should reflect the guidance of the professional body. This follow-up ensures that, if a new condition occurs that may have an impact on the recipient, proper action can be taken. This should clearly be documented. No matter how extensive the testing that is performed prior to donation, the donor can develop diseases not known at the time of donation. These can be newly discovered infectious diseases or malignancies. These conditions may have been transmitted to the recipient as well. There is no clear evidence regarding malignancies, but haematological malignancies within one year of donation may have been present in the graft and transmitted to the recipient. In these cases, the recipient should be specifically monitored for the presence of the disease and if possible, preventive steps should be taken.

16.4.3. Investigation and assessment of adverse reactions

Depending on the level at which the adverse reactions occurred, certain measures have to be taken before starting the investigation. The first precautionary measure is to quarantine any other tissues or cells from the same donor if there are any in storage. If tissues or cells have already been distributed, it must be determined where they are allocated and consider the possibility of recalling. These measures are intended to minimise the number of recipients exposed to the same reaction.

a. The first step in the investigation is to determine the severity. A 'severity scale' can be used to decide whether a particular adverse reaction is an SAR that needs to be reported to the Health Authorities. The scale shown in Table 16.1 is used in the EU. It was proposed by the project European Union Standards and Training for the Inspection of Tissue Establishments (EUSTITE) [7] for vigilance for tissues and cells and is based on the scale used for haemovigilance.

Adverse reactions in recipients of tissues or cells should be investigated by a team that can carry out an independent investigation; it should include the clinician who transplanted the tissues or cells, the TE that provided them and, in more serious cases, the Health Authority in that country. Efficient co-ordination of the investigation is critical to rapid implementation of effective corrective actions. If relevant, experts in particular fields (e.g. viral transmission) should also be invited to participate in the investigation of the adverse reaction.

b. The second step is to assess imputability. The investigation should focus on establishing the level of imputability (i.e. the extent to which the tissues or cells used clinically can be considered to have caused the adverse reaction). The scale provided in Table 16.2, developed by EUSTITE, can be applied to describe the outcome of an imputability investigation. It proposes that all adverse reactions be graded

in terms of imputability. Table 16.2 also recommends specific approaches to the establishment of imputability for suspected infectious or malignant transmissions, as proposed by Garzoni and Ison in the context of transplantation [8]. Imputability grades might change during an investigation and should, in general, be assigned at the point of initial notification and again at the completion of the adverse reaction investigation. The evaluation of imputability should be based on scientific or clinical data. The ECDC, the WHO or other sources of epidemiological and risk information may be useful to support the process.

Consideration should also be given to the practice of keeping pre-transplant serum archives for transplant recipients to support imputability investigations.

TEs have to review:

1. all the reagents, substances etc. that were in contact with the tissues and cells during processing, checking the expiry date and the sterility;
2. the tissues processed in the same room on the same day, the day before and the day after;
3. all the microbiological results of the donor and of each specific graft;
4. the microbiological checks of the processing rooms.

The ORHA that uses the tissues or cells needs to determine if other patients were operated on in the same operating room and their clinical and microbiological conditions. This approach is not advised for recipients of gametes or embryos (see Chapter 27).

Table 16.2. **Scale describing possible outcomes of an imputability investigation**

	Criteria adapted from EUSTITE-SoHO V&S [9, 10]	Criteria for infectious and malignant transmissions, adapted from the Disease Transmission Advisory Committee [11]
Not assessable	Insufficient data for imputability assessment	Insufficient data for imputability assessment
0. Excluded	Conclusive evidence beyond reasonable doubt for attributing an adverse reaction to alternative causes	Suspected transmission and fulfilment of at least one of the following conditions: • clear evidence of an alternative cause • the appropriate diagnostic tests carried out have failed to document infection by the same pathogen in any recipient from the same donor • laboratory evidence that the recipient was infected with the same pathogen or had a tumour before the application of organs, tissues or cells
1. Possible	The evidence is indeterminate for attributing an adverse reaction to the quality/safety of tissues and cells, to the donation process or to alternative causes	Either • suspected transmission and • laboratory evidence of the pathogen or tumour in a single recipient or • data suggest a transmission but are not sufficient to confirm it
2. Probable	The evidence is clearly in favour of attributing the adverse reaction to the quality/safety of tissues and cells (for recipients) or to the donation process (for donors)	Not only are the following two conditions met: • suspected transmission and • laboratory evidence of the pathogen or tumour in a recipient but also at least one of the following conditions is met: • laboratory evidence of the same pathogen or tumour in other recipients • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, such evidence must indicate if the same recipient was negative for the pathogen involved before transplantation
3. Definite; certain	The evidence is conclusive beyond reasonable doubt for attributing the adverse reaction to the quality/safety of tissues and cells (for recipients) or to the donation process (for donors)	All the following conditions are met: • suspected transmission • laboratory evidence of the pathogen or the tumour in a recipient • laboratory evidence of the same pathogen or tumour in other recipients (if multiple recipients) • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, it should be noted that the same recipient was negative for the pathogen before transplantation

This table can be adapted to other tissues or cells and for MAR in order to take into account the specificities of each type of product.

16.5. Adverse events

Adverse events can occur at any moment from donor selection to clinical application. However it should be emphasised that an adverse event may not always produce a subsequent reaction in the recipient.

Non-compliances with the quality system should be documented and investigated as part of the internal QMS. On occasions, however, a particular non-compliance may be of such importance that it should be considered an SAE and reported through the vigilance system. Some examples of SAEs are (for more information, check the specific tissue chapter in Part B):

a. Final result of a negative-to-date release with a cultured cornea was reported as positive; no adverse reaction detected in the cornea recipient.
b. Aspergillus was detected in an incubator used for storing tissues and cells that have since been distributed.
c. Loss of an embryo during manipulation of the culture dish; the patient requires new cycle of IVF.
d. Skin donor not tested for malaria although donor resided in a malaria-endemic area.
e. Lack of liquid nitrogen in a liquid nitrogen refrigerator containing several tissues, resulting in thawing of the tissues.
f. When a frozen bone arrives at the hospital and the bag is broken.

16.5.1. Detection of serious adverse events

For effective detection of adverse events, all relevant stakeholders must be aware of their responsibilities for identifying errors or unexpected results. This includes all staff in TEs and procurement organisations, those working in organisations such as testing laboratories that provide 'third party' services to TEs, and clinical users who may also detect errors at the point of clinical use. In EU Directive 2006/86/EC, the definition of an SAE includes those incidents often referred to as 'near misses', i.e. where an error or fault is detected and corrected without causing harm.

16.5.2. Serious adverse event reporting

According to instructions from the European Commission to EU member states for annual vigilance reporting, deviations from SOPs in TEs (or other adverse events) that have implications for the quality and safety of non-reproductive tissues and cells should result in an SAE report to the Health Authority if one or more of the following criteria [12] apply (see also Figure 16.1):

a. Inappropriate tissues/cells have been distributed for clinical use, even if not used;
b. The event could have implications for other patients or donors because of shared practices, services, supplies or donors;
c. The event resulted in loss of any irreplaceable autologous tissues or cells or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;
d. The event resulted in the loss of a significant quantity of unmatched allogeneic tissues or cells.

The criteria adopted for reproductive tissues and cells are:

a. Inappropriate gametes, embryos or germinal tissues have been released for clinical use, even if not used;
b. The event could have implications for other patients or donors because of shared practices, services, supplies, critical equipment or donors;
c. The event resulted in a mix-up of gametes or embryos;
d. The event resulted in a loss of traceability of gametes or embryos;
e. Contamination or cross-contamination;
f. Accidental loss of gametes, embryos, germinal tissues (e.g. breakdown of incubators, accidental discard, manipulation errors) resulting in a total loss of chance of pregnancy for one cycle.

16.5.3. Investigation and assessment of serious adverse events

Despite the fact that SAEs, by definition, have not (or not yet) involved harm to recipients or donors, the impact of an SAE can be significant if considered in a broader way. The impact assessment tool given in Appendix 24 can also be applied to SAEs to help reach a decision on the response required.

16.6. Vigilance co-ordination

Co-ordination between various systems of vigilance (e.g. organ and haemovigilance, material/device vigilance, pharmacovigilance) should be in place at the local level (TE) and at the Health Authority level.

The lack or omission of the exchange of information can put more patients or the same recipients at risk. Sometimes it is someone within the system who

discovers a problem and needs to inform the Health Authority. For example, if there is problem with a bag used for cryopreserved tissues or cells and that problem altered the characteristics of the product, or if such a bag was stored at −196 °C and after some time this bag was broken, then the medical device vigilance system should be informed. The same caution should apply when faced with an SAR or SAE with organs if it involves tissues which were retrieved, or vice versa; in either case, all the corresponding vigilance systems should be informed.

16.6.1. Rapid alerts

In some circumstances, a particular event or reaction requires rapid communication nationally or internationally to facilitate urgent action, such as a recall of products or critical materials or the quarantine of tissues or cells. In that case a communication system must be available at all times. Rapid alerts should only be issued in exceptional circumstances. The following criteria have been identified in the SoHO V&S project [13] as triggers for rapid alerts within or between EU member states:

a. an ARE of a serious or potentially serious nature;
b. potential risk to other individuals or other TEs;
c. wider public health implications;
d. rapid intervention needed (preventive or corrective measures, urgent communication).

Within the EU, a system for rapid alerts – called Rapid Alerts for Tissues and Cells (RATC) – is hosted by the European Commission and enables the competent authorities of EU member states to rapidly share urgent information on risks to patients where that information has consequences in more than one EU member state [14]. In February 2013, this system was moved to a new secure internet platform where all rapid alerts are generated and shared, with access restricted to Competent Authorities.

16.7. Vigilance communication: education and training

16.7.1. 'No blame' culture

Effective communication of the results of vigilance systems is fundamental to ensure that the benefits of these programmes are realised in practice. Regular feedback to health professionals is critical to support continued notification of AREs. All stakeholders, Health Authorities, TEs and clinicians should promote a culture that encourages reporting in a non-punitive context for the benefit of patients and donors. It should be accepted that mistakes happen and that no programme of transplantation or MAR is risk-free. Programmes of training and awareness should be organised to encourage reporting. The message should be promoted that reporting and disseminating V&S information can result in positive improvements for donors and patients, as well as feedback to health professionals.

16.7.2. Vigilance experience and feedback

Health Authorities and professional societies should publish the results of their programmes without identifying individual centres, hospitals or individual people. Those TEs or hospitals directly involved in specific incidents should also consider publishing their experience to alert others to the means by which they detected and confirmed the event or reaction.

The Notify Library is an initiative launched by the WHO and supported by the Italian National Transplant Centre (CNT) that has gathered information on documented adverse occurrences in transplantation and assisted reproduction. It has reviewed cases to identify general principles supporting detection and investigation. The database has been constructed from the information gathered and is accessible on a dedicated website [15, 16]. The database is maintained and updated on this platform and is intended as a communication hub for institutions and organisations worldwide collaborating in the facilitation of access to V&S information to improve safety and efficacy.

16.7.3. Educational training and workshops

When SoHO are used, there is always a risk that needs to be considered if something happens afterwards that can be related to the quality of the tissue or cells used and needs to be communicated. Health Authorities should encourage biovigilance awareness in all professionals involved in tissues and cells at any step (from donation to implant). To achieve this awareness, it is necessary to educate and train the professionals about the benefits of implementing a biovigilance system. This can be done with a high-quality educational programme and with well-organised workshops, disseminating the message in meetings, publishing reports with anonymised data, etc.

The purpose is to stimulate reporting in an appropriate manner, but avoiding over-reporting,

which can collapse the system. Professionals need guidance about what to communicate, when, and to whom. Healthcare providers need to have confidence in this system, which is why it is important that the reporting system is non-punitive and confidential. Reporting and further analysis are very useful tools for learning how to avoid mistakes and other errors; in the end, the resulting analysis is beneficial for the safety of donor and patients.

Workshops – using real cases for discussion, describing how to investigate them and defining the possible causes of SAREs summarised in the final reports – can help in professionals' daily work.

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Related material

- Appendix 24. Serious adverse reaction or event: impact assessment form

Part B. Tissue-specific requirements

Chapter 17: **Ocular tissue**

17.1. Introduction

Ocular tissues procured from deceased donors are used for treating loss of vision caused by corneal disease or trauma, as well as for reconstructive and glaucoma surgery. The cornea is the principal refractive component of the eye. Good vision depends on corneal transparency and a smooth, spherical ocular surface. The cornea is also part of the outer coat of the eye and must therefore be strong enough to withstand the intraocular pressure and help protect the delicate inner structures of the eye.

17.1.1. Corneal transplantation

A corneal transplant (keratoplasty) is an operation to remove all or part of a diseased or damaged cornea and replace it with healthy donor tissue. In Europe, the main indications for corneal transplantation include:

- Fuchs endothelial dystrophy (FED) – failure of the corneal endothelium, the monolayer of non-dividing cells lining the inner surface of the cornea that controls stromal hydration and thus maintains corneal transparency.
- Keratoconus – a connective tissue disorder resulting in thinning of the corneal stroma, which normally accounts for 90 % of corneal thickness, and distortion of corneal shape.
- Pseudophakic bullous keratopathy (PBK) – failure of the corneal endothelium as a consequence of previous cataract or other intraocular surgery.
- Infection, such as *Herpes simplex* keratitis (HSK) – mainly treatment of post-infectious scars, but acute infection may require a therapeutic keratoplasty.
- Regraft for a failed corneal transplant.

These conditions may all be treated with a full-thickness corneal transplant (penetrating keratoplasty, PK); however, current practice is to replace, where possible, only the dysfunctional part of the cornea [1, 2, 3] as illustrated in Figure 17.1.

Endothelial keratoplasty (EK) is the method of choice for endothelial dysfunction such as FED or PBK. The graft lamella, which consists of endothelium on its basement membrane (Descemet membrane), with or without a thin supporting layer of stroma, is inserted through a small incision into the anterior chamber of the eye and attaches to the posterior surface of the patient's cornea. The initial attachment is ensured by air or sulphur hexafluoride gas tamponade of the anterior chamber. The affected patients are mostly elderly and the advantages of EK over PK include much faster visual rehabilitation, lower rejection rates and, since there are no sutures required to hold the graft in place, negligible surgically induced astigmatism. There are currently four techniques for preparing tissue for EK:

- Descemet stripping endothelial keratoplasty (DSEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared by manual dissection.
- Descemet stripping automated endothelial keratoplasty (DSAEK) – endothelium on De-

scemet membrane with a thin layer of stroma, prepared using a microkeratome.
- Descemet membrane endothelial keratoplasty (DMEK) – endothelium on Descemet membrane without any stroma, prepared by manually separating Descemet membrane from the stroma.
- Pre-Descemet membrane endothelial keratoplasty (PDEK) – endothelium on Descemet membrane with a thin layer of pre-Descemet stroma, prepared by injecting air to create a 'big bubble', which separates the graft from the bulk of the stroma.

For keratoconus, which typically affects younger patients, deep anterior lamellar keratoplasty (DALK) is an alternative to PK that replaces the full thickness of the stroma, leaving the recipient's endothelium intact. As immunological rejection directed against the endothelium is one of the main reasons for PK failure, the advantage of DALK is that the patient's endothelium cannot be rejected. Superficial stromal defects and scars can also be treated by anterior lamellar keratoplasty (ALK) but without the need to replace the full thickness of the stroma.

Tissue for PK and EK requires a corneoscleral disc with a viable endothelium. Tissue for DALK and ALK does not require a viable endothelium; however, corneoscleral discs with an endothelium suitable for PK may be requested for DALK owing to the occasional need to switch procedure in the operating room from DALK to PK. Tissue for EK may be prepared in a tissue establishment (TE), which saves time for the surgeon and avoids the risk of damage to the tissue when prepared in the operating room.

Corneal transplant outcome – in terms of both graft survival and visual outcome – depends on the indication and reason for transplant, the presence of pre-operative risk factors, such as vascularised cornea, glaucoma and inflammation, and post-operative complications such as allograft rejection. In the absence of other risk factors, keratoconus and FED are considered to be low-risk grafts with 5-year survivals of, respectively, 95 % and 82 % [4]. Regrafts and PBK are more likely both to suffer allograft rejection and to fail and are therefore considered higher-risk grafts with 5-year survival of, respectively, 56 % and 54 % [4]. A major cause of graft failure is allograft rejection. Most rejection episodes can be successfully treated with topical (sometimes intraocular, subconjunctival, peribulbar or systemic) corticosteroids. In some cases, use of systemic immuno-suppression (e.g. cyclosporine, tacrolimus, mycophenolate) is considered necessary to reduce the risk of immune reactions. The results from studies of human leukocyte antigen (HLA) matching to reduce the risk of rejection are not as conclusive as for organ transplantation, but matching may be beneficial for high-risk corneal grafts [5].

17.1.2. Further use of ocular tissue

If, for example, a patient has suffered multiple failed corneal grafts, a keratoprosthesis may be an appropriate alternative to a corneal graft [6]. Keratoprostheses are attached to a ring of corneal tissue from a donor, which is then sutured to the recipient's cornea after removal of the failed graft. Sclera and cornea can also be used in glaucoma surgery and for reconstructive surgery of the ocular surface. Limbal tissue, which contains a population of corneal epithelial progenitor cells, may be transplanted as a keratolimbal allograft (KLAL) to treat ocular surface disease caused by failure of the corneal epithelium (limbal stem cell deficiency) [7].

There are other tissues and cells that are used in ocular surgery but not covered in this chapter:
- Limbal progenitor cells may be isolated from a corneoscleral disc and expanded *ex vivo* for treating ocular surface disease (see Part C, Chapter 29 and Chapter 30) [8];
- Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease (see Part C, Chapter 29 and Chapter 30) [9];
- Amnion is used for treating ocular surface conditions and as a support for limbal progenitor cells (see Chapter 18) [10];
- Autologous and allogeneic serum eye drops may be used for treating dry eye (see Part C, Chapter 35) and/or persistent epithelial defects [11].

The following generic chapters of this Guide (see Part A) all apply to ocular tissue banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,

h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines the specific requirements for ocular tissues that vary from the generic chapters in Part A of this Guide. Where differences are not specified, the requirements of the relevant generic chapter should be followed.

17.2. Donor evaluation

17.2.1. Tissue-specific exclusion criteria for ocular tissue donation

Acceptance and exclusion criteria for cornea donation that differ from the criteria for other tissues are based on the avascularity of the cornea and ocular-specific conditions that may affect the cornea.

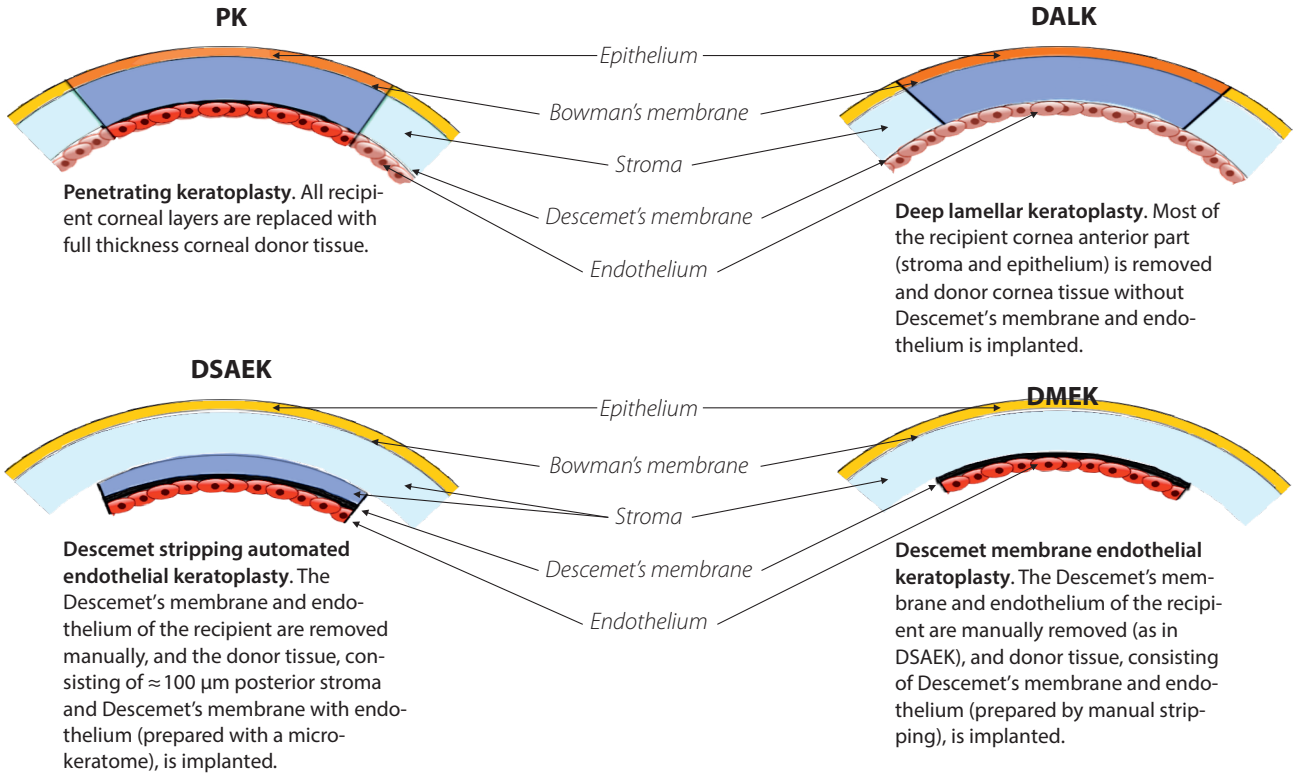
17.2.1.1. *Selection criteria for ocular tissue other than cornea*

For any tissue or cells that are not derived from the avascular cornea, such as sclera, limbal tissue and limbal cells, the exclusion criteria for non-ocular tissues must be followed (see Chapter 4).

17.2.1.2. *Donor age*

Provided that corneas are examined to exclude those unsuitable for transplantation based on endothelial cell density and/or stromal abnormalities (see §17.7), the upper age limit for eye donors may be determined by the TE. The likelihood that corneas will be suitable for PK or EK does decline with increasing donor age but, where the endothelial cell density is considered sufficient, graft survival is little affected by donor age [12, 13]. The minimum donor age is more uncertain and should be determined by the TE because corneas from young children lack rigidity and have a high radius of curvature. There is likely, therefore, to be little demand for corneas from such young donors for transplantation; however, corneas from these donors may be important as a source of limbal grafts or limbal progenitor cells. Older donors (over 65 years) are often preferred for DMEK surgery as the graft in older donors is easier to unfold during surgery [14], although a clinical study does not suggest any clinical disadvantage of using donors younger than 55 years [15].

Figure 17.1. **Different types of keratoplasties**



17.2.1.3. *Malignancies*

Haematological neoplasms, retinoblastoma and malignant tumours of the anterior segment are obligatory contraindications to cornea donation. Donors with certain malignant diseases may be evaluated and considered for avascular cornea donation but not for vascularised ocular tissues (i.e. limbal tissue, limbal stem cells or sclera). A report of metastatic cholangiocarcinoma cells found in the sclera and sclerocornea interface, but not avascular cornea, supports this [16]; but there has been a more recent report of malignant cutaneous melanoma (MCM) metastases in peripheral, but not central, avascular cornea [17]. The Medical Advisory Board of the Eye Bank Association of America has issued an amendment to their donor-selection criteria excluding donors with a history of melanoma with known metastatic disease [18]. The incidence of metastases from non-ocular tumours to the anterior segment of the eye is reportedly extremely low; however, corneas must be excluded where there is evidence of anterior segment metastases from the slitlamp examination of the eye or the corneoscleral disc [19, 20, 21]. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

17.2.1.4. *Infections*

Individuals with localised ocular infection (bacterial, viral, fungal, protozoal, parasitic) are excluded from donation of ocular tissues. This exclusion includes those with a known history of past ocular *Herpes* infection. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

Individuals suffering from bacterial septicaemia may be considered for cornea donation, provided that the corneas are stored by organ culture and the medium tested for microbial contamination before transplantation. Donors colonised with multidrug-resistant bacteria need a thorough risk assessment before they may be accepted as donors.

17.2.1.5. *Diabetes mellitus*

Diabetes mellitus may exclude cornea donation for EK, but not donation for DALK or PK. It increases the risk of unsuccessful DMEK graft preparation [22]. However, the risk of unsuccessful graft preparation may be related to the severity of the diabetes and an algorithm has been proposed to allow grafts to be prepared from corneas from donors with less severe disease [23].

17.2.1.6. *Eye diseases*

The following exclude cornea donation:

a. ocular inflammation and infection (see 17.2.1.4);

b. autoimmune disease, e.g. sarcoidosis, rheumatoid arthritis, but only where there is ocular involvement.

The following exclude cornea donation for PK or DALK, but not necessarily for EK:

a. corneal disorders including keratoconus, keratoglobus and epithelial and stromal dystrophies. As these diseases typically are bilateral, both eyes should be excluded;

b. corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central area of the cornea. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

17.2.1.7. *Previous intraocular or anterior segment surgery*

The following exclude cornea donation:

a. previous ocular surgery that would prejudice graft outcome;

b. receipt of a corneal, sclera or limbal allograft.

The following exclude cornea donation for PK or DALK, but not necessarily for EK:

a. refractive corneal surgical procedures, including radial keratotomy, lamellar inserts and laser refractive surgery. As these procedures typically are performed bilaterally, both eyes should be excluded.

17.3. Procurement

17.3.1. ***Post mortem* time**

Ocular tissues should be procured from donors as soon as possible after cardiac arrest, preferably within 24 h; however, Health Authorities or local practice may allow procurement up to 72 h after cardiac arrest. For EU member states, a blood sample for the mandatory tests for transmissible disease must be obtained from the donor within 24 h of death (see Chapter 5).

17.3.2. Procurement team

Ocular procurement personnel must be appropriately clothed and apply aseptic technique to minimise the risk of contamination of the tissue to be removed and also to protect personnel. Usually, this requires hand disinfection, the wearing of sterile gowns and gloves and the use of face masks or protective masks.

17.3.3. Procurement procedures

Since the ocular surface is exposed to the environment and, after death, there is no blinking and no tear film, the ocular surface is highly likely to be contaminated by environmental micro-organisms before procurement. Therefore, a classified area with a specified air quality is not typically required for ocular tissue procurement but other guidance given in Chapter 6 does apply. In case of concomitant skin donation, procurement of skin before ocular tissue is recommended (as described in §19.3.1).

The donor's eyelids and skin should be cleaned with an antiseptic solution and a sterile drape (eye sheet) placed over the face leaving the eyes exposed. The donor's eyes should be flushed with an appropriate sterile solution to remove debris, mucus and foreign matter from the cornea and conjunctival sac. An antiseptic solution suitable for cleaning the ocular surface prior to intraocular surgery may be applied to the ocular surface. A broad-spectrum antibiotic solution may also be used. After insertion of a lid speculum, peritomy is performed, preferably leaving a frill of conjunctiva at the limbus to avoid damage to the limbal progenitor cell niche.

The subsequent procedure depends on whether the eye is to be enucleated or just the corneoscleral disc procured by *in situ* excision. Advantages of *in situ* excision include: reduced death-to-preservation time since the corneoscleral disc is placed in storage medium immediately following procurement; potentially improved cosmetic reconstruction of the donor; and it may be more acceptable to some relatives or next of kin than enucleation. However, procurement by enucleation is simpler, with less risk of harm to the corneal endothelium; and enucleated eyes provide sclera, for glaucoma or reconstructive surgery, and retina, optic nerve, lens and iris for research. There is no reported evidence of a difference in corneal quality or clinical outcome between these two procurement methods.

17.3.3.1. *Procurement of the whole eye*

The lateral extraocular muscle is located and lifted with a muscle hook and clamped with artery forceps close to its point of attachment to the sclera. The muscle is divided distally, leaving the artery forceps in place to stabilise and steady the eye. The remaining rectus muscles are then lifted in turn with the muscle hook and divided close to the sclera. It is not necessary to divide the oblique muscles. The eye is then gently lifted and the optic nerve severed using curved enucleation scissors. After enucleation, the eye should be placed, cornea uppermost, in a fixed position in a moist chamber and transported to the TE refrigerated in ice. Broad-spectrum antibiotics may be used to further minimise the risk of bacterial contamination.

17.3.3.2. *Procurement of corneoscleral discs*

After peritomy, sclerotomy is performed, maintaining a wide scleral rim (*circa* 4 mm) around the cornea. The corneoscleral disc is then gently lifted away from the eye without folding, to avoid damage to the endothelium. After excision, the corneoscleral disc should be immersed, endothelium uppermost to avoid the risk of damage, in an appropriate corneal storage solution that may contain antibiotics and antimycotics. Unless the cornea is to be transferred directly to organ culture at the TE, it is recommended that, when possible, the container be a corneal viewing chamber or have a flat bottom and adequate optical properties to facilitate subsequent assessment by slit lamp and specular microscopy.

For light microscopy evaluation, other relevant containers and evaluation methods may be used.

17.3.3.3. *Procurement of scleral tissue*

Scleral tissue is prepared in the TE from the whole eye after excision of the corneoscleral disc.

17.3.4. Reconstruction of the donor

The aim is to mimic as closely as possible the original profile of the donor's closed eyes. After enucleation, the orbit should be filled with an appropriate prosthesis or other suitable material. The eye lids are then closed to restore the appearance of the donor.

17.4. Temporary storage and transportation to the tissue establishment

Whole eyes should be stored and transported in a moist chamber at 2 to 8 °C. The time from procurement to processing at the TE should not exceed 24-48 h.

Table 17.1. **Factors influencing the air quality for processing ocular tissue**

Criterion	Ocular tissue-specific
Risk of contamination of tissues or cells during processing	Corneoscleral discs procured by in situ excision are placed in a storage medium in an environment where air quality typically is not controlled. Careful cleaning of the ocular surface before excision, aseptic technique and use of antimicrobials in the storage medium help to minimise the risk of contamination. Processing whole eyes in a tissue establishment allows control of air quality (e.g. laminar flow cabinet in a room with HEPA-filtered air). Cleaning of the eyes before processing is important because it has to be assumed that bacteria and fungi will be present on the ocular surface owing to lack of blinking and tear film after death of the donor. Organ-cultured corneas may be removed from their storage medium just prior to surgery to examine the endothelium by light microscopy and for further processing for EK. They are therefore re-exposed to the environment and an appropriate air quality must be applied. The EU Tissues and Cells Directive requires the equivalent of Grade A air quality with at least a Grade D background for such purposes.
Use of antimicrobials during processing	Corneoscleral discs may be stored in media containing antibiotics and antimycotics. The medium may also contain a marker (e.g. phenol red) that changes colour with a fall in pH caused by growth of micro-organisms. Turbidity of the storage medium is also an indication of contamination. Storage of corneas in organ culture not only allows the testing of samples of medium for microbial growth during storage but also ensures that any antimicrobials in the medium will be more effective, owing to the higher storage temperature than that used for hypothermic storage.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	There is typically no microbiological testing of hypothermic corneal storage media. Even if a sample of hypothermic medium is taken, the time available before transplant is limited to just a few days, which reduces the chance of detecting contaminants. Some eye banks recommend that surgeons send the remaining corneoscleral rim and storage medium for microbiological testing after preparation and transplantation of the corneal graft. For organ-cultured corneas, there is a greater chance of detecting contamination because of the extended, albeit still limited, storage period. A second sample of storage medium may be taken after transfer of an organ-cultured cornea to medium, to reverse stromal oedema and for transport to the recipient hospital, but the time before transplantation is only a few days and a negative-to-date release will apply. Therefore, there is a risk that contamination may not be detected until after transplantation.
Risk of transfer of contaminants at transplantation	Corneal tissue for the great majority of transplant procedures cannot be sterilised because living cells are required for a successful graft outcome. Post-operative endophthalmitis caused by micro-organisms transferred with the graft is therefore a risk and is a defined serious adverse reaction. It is considered to be rare. Attributing a cause is not always straightforward owing to the, albeit slight, risk of post-operative infection associated with any intraocular surgical procedure.

HEPA filter: high-efficiency particulate air filter.

Corneoscleral discs procured by *in situ* excision may be placed in a hypothermic storage solution or in a medium designated by the manufacturer for room temperature storage. In both cases, the manufacturer's recommendations for storage temperature should be followed or the temperature conditions during transport should be validated.

17.5. Processing, preservation and storage

17.5.1. Processing facilities

The requirements of Chapter 8 and Chapter 9 on processing, preservation and storage should be applied when selecting an appropriate air-quality specification for ocular tissue processing and for environmental monitoring and quality control.

17.5.2. Cornea processing methods

When corneoscleral discs have been procured by *in situ* excision, they will already be in a storage medium on arrival at a TE and may not require further processing unless they are to be transferred to organ culture. When whole eyes are received by a TE, they should be subjected to a cleaning protocol to reduce the bioburden on the ocular surface before excision of the corneoscleral disc; for example, rinsing in sterile saline and immersion in a disinfectant such as povidone-iodine or chlorhexidine [24]. Further processing of corneoscleral discs to prepare grafts for EK may be undertaken in the TE [25, 26].

17.5.3. Cornea storage methods

a. Hypothermic storage at 2 to 8 °C

i. For whole eyes in moist chambers, storage times of < 48 h are recommended for procedures where a viable corneal endothelium is required. This may be extended to 72 h for other purposes.

ii. For corneoscleral discs in storage medium, the manufacturer's recommendations should be followed for storage temperature and for maximum storage time, which can vary up to 21 days [27, 28, 29]. Corneas prepared in a TE for DSAEK or for DMEK can be shipped to hospitals in hypothermic storage media.

b. Organ culture at 28 to 37 °C

iii. A storage time of up to 4-5 weeks is typical for organ culture, although successful transplants after 7 weeks have been reported [30]. It is at the discretion of the Responsible Person (RP) or medical director to approve prolonged storage times, provided that the procedure has been validated. An inspection of the endothelium is mandatory at the end of the storage period and then the transplant can be assigned to the proper kind of surgery based on the cell density. Renewal of the storage medium using aseptic procedures during the storage period is at the discretion of the RP/medical director and may depend on the manufacturer's recommendations.

iv. To reverse the stromal oedema that occurs during organ culture, corneas are transferred to a medium, the transport or 'deswelling' medium, containing a macromolecule to increase oncotic pressure and induce an efflux of water from the stroma. The cornea may be kept at 28 to 37 °C for up to 4-6 days, at the discretion of the RP and depending on the medium used [31, 32].

v. Organ-cultured corneas can be prepared in the eye bank for DSAEK after pre-thinning in deswelling medium, or for DMEK with or without pre-thinning [33]. The DSAEK grafts may be laid back on the anterior stroma to provide additional support during transport in deswelling medium [34]. DSAEK grafts are prepared after mounting the corneoscleral disc in a pressurised artificial anterior chamber followed by cutting away the anterior stroma using a microkeratome. Clinical results after graft preparation using a femtosecond laser have been found inferior when compared with microkeratome-cut DSAEK grafts [35, 36]. Clinical quality-control studies comparing DSAEK grafts prepared in an eye bank with DSAEK grafts prepared by the surgeon immediately before surgery have not identified differences in early complications (graft detachment), primary graft failure or endothelial cell density two years after surgery [37]. DSAEK grafts possibly should be cut as thin as possible because visual acuity is better after grafting with a thin graft compared with a thick graft [38].

vi. Of relevance for eye banks, DMEK grafts can be prepared by manual dissection, pneumatic dissection, or hydrodissection. A no-touch technique, without direct physical tissue manipulation during tissue preparation, may be an ideal approach to minimise graft damage [39]. Clinical results after eye-bank- and surgeon-prepared DMEK grafts seem similar [40]. For DMEK, the graft may be supplied rolled in the final diameter direct into medium or attached, either in the centre or at the periphery, and laid back on the stroma [26, 41]. Pre-prepared grafts for DSAEK and DMEK may be shipped to hospitals in medium at room temperature [26]. Pre-loaded graft for both DSAEK and DMEK can also be provided in order to minimise the time and efforts needed for tissue preparation in the theatre [42, 43].

c. Storage of non-viable corneal tissue

vii. Corneoscleral discs or pieces of cornea for glaucoma or reconstructive surgery may be stored in ethanol (≥ 70 % v/v) or glycerol for extended periods. Corneal tissue may also be irradiated and stored in albumin, cryopreserved or frozen [44].

17.5.4. Sclera processing and storage

After excision of the corneoscleral disc from the eye, sclera is prepared using aseptic techniques by removing the intraocular contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). Sclera may be stored – whole, or divided into smaller, individually packaged pieces – in ethanol (≥ 70 % v/v) or glycerol, or fixed in formalin, freeze-dried, frozen or kept in physiological solution or medium with antibiotics. Sclera stored in saline with antibiotics in a refrigerator should only be kept for short periods (≤ 7 days).

17.6. Microbiological testing

Testing may be carried out before processing by swabbing the eye before excision of the corneoscleral disc; however, there appears to be little predictive value in this procedure [45, 46]. At the discretion of the transplanting surgeon, any corneoscleral tissue and storage medium remaining after preparation of the graft may be sent for microbiological testing;

although there appears to be little predictive value from this [47], it can be helpful for the investigation of post-operative endophthalmitis. For further information refer to Chapter 10 on the principles of microbiological testing.

a. Organ-culture storage of corneas

Since corneoscleral discs intended for transplants requiring viable cells cannot be sterilised, microbiological testing of samples of organ-culture medium taken during corneal storage must be undertaken to test for microbial contamination. Microbiological media for bacteria and fungi should be inoculated and incubated at appropriate temperatures. A minimum corneal storage period of at least 3 days is required before taking samples for microbiological testing. In addition to microbiological testing, the culture medium should be inspected regularly for turbidity and change in pH (e.g. change in colour of phenol red in the medium), which may indicate microbial contamination. It is recommended to keep the organ-culture medium for at least a week after transfer of the corneoscleral disc to transport medium to allow additional monitoring for signs of contamination.

Further microbiological testing should, if possible, be carried out whenever a cornea is re-exposed to the environment, for example after endothelial assessment and transfer of the cornea into the transport medium or after preparation of corneas for DSAEK or DMEK in a TE. However, given the restricted time a cornea may remain in this medium, it is possible that growth of micro-organisms may not be detected before the cornea is transplanted. A negative-to-date release is possible, as described in Chapter 10. If growth is detected, the surgeon must be informed immediately to prevent transplantation of the tissue. If the transplantation has taken place, the identification and sensitivities of the contaminating micro-organisms must be established as soon as possible in order to help the surgeon's post-operative management of the recipient. The fellow cornea should be discarded. If the fellow cornea has already been transplanted, the transplanting surgeon should be informed and the patient monitored.

b. Hypothermic storage of corneas

Taking a sample of medium for microbiological testing during hypothermic storage of corneas is not standard practice, but may be required by national guidelines. Testing a sample of medium is a surrogate for direct testing of tissue. The short storage period and low temperature, which would suppress microbial growth, greatly reduce the likelihood of detection of contamination in the medium.

c. Sclera

Depending on the method of storage, for example refrigerated in saline, microbiological testing should be carried out after processing. Storage in ethanol (≥ 70 % v/v), glycerol (≥ 85 % v/v) or gamma irradiation of the tissue may render microbiological testing unnecessary unless required by local or national guidelines.

17.7. Quality control and cornea evaluation

Quality-control tests on corneal grafts should consider at least the following minimum quality criteria:

a. no evidence of microbiological growth (aerobic or anaerobic bacteria, yeast or fungi);
b. endothelial characteristics;
c. morphology and integrity of the cornea layers;
d. diameter of clear central area of cornea.

Depending on the specific use of the cornea, it is necessary to document the appearance of:

a. epithelium, taking into account that the epithelium may partially detach or reduce in thickness during storage;
b. stroma, which should have no central opacities or scars; the stroma of organ-cultured corneas may be hazy but should be transparent after reversal of stromal oedema in transport medium;
c. endothelium.

The quality-control tests to be carried out include the following:

a. Gross examination

i. abnormalities of the external globe;
ii. signs of previous surgery of the anterior segment;
iii. epithelial abrasions, retention of excessive orbital tissue or laceration of the globe;
iv. epithelial defects;
v. stromal opacities – a mild arcus senilis with a defined clear central zone may be acceptable; the minimal diameter of the clear zone is at the discretion of the RP/medical director;
vi. abnormal corneal shape (keratoconus, micro- or megalocornea);

vii. condition of the anterior chamber (shape, evidence of blood);
viii. abnormalities, such as the pterygium extending over the optical zone of the cornea.

b. Slit-lamp evaluation
i. Slit-lamp examination of whole eyes and corneoscleral discs is recommended by the European Eye Bank Association [21].
ii. It facilitates exclusion of pathological changes to the epithelium or stroma, such as scars, oedema, significant arcus, striae, epithelial defects, endothelial guttae or disease, infiltrates or foreign bodies, and anterior segment tumours or metastases.

c. Microscopic evaluation of corneal endothelium
i. The aim is to provide an estimate of endothelial cell density and a qualitative assessment of the appearance of the endothelium.
ii. This evaluation must be applied to all corneas intended for PK or EK in order to minimise the risk that factors such as low endothelial cell density may have a negative influence on graft survival [12].
iii. For corneas stored by hypothermia, this assessment is typically at the start of storage.
iv. If the corneoscleral disc is not in a corneal viewing chamber, it needs to be turned over so that the endothelium is facing downwards to allow observation by specular microscopy through the base of the container.
v. It should then be returned to the endothelium-uppermost position to avoid the risk of subsequent damage.
vi. For organ-cultured corneas, this endothelial assessment can be both at the start and at the end of the storage period; assessment at the end of storage, shortly before the cornea is transferred to the transport medium, is considered to be essential, whereas assessment at both the start and end of storage allows endothelial cell loss during storage to be determined.

There are two main methods used for endothelial evaluation by microscopy:
i. Specular microscopy. This method allows direct examination of the endothelium without staining; however, the appearance of the endothelial cells varies with temperature, type and time of preservation and the storage medium used. It is recommended that cold-stored corneas are warmed to room temperature to enhance the quality of the endothelial image.
ii. Transmitted light microscopy (bright field or phase contrast). If necessary to enable cell counting, brief exposure to hypotonic sucrose solution (1.8 % w/v) or short exposure (few seconds, max. 4 minutes) to either balanced salt solution (BSS) or 0.9 % (w/v) NaCl is possible to make endothelial cell borders visible. The exposure time to these solutions must be limited. Prior use of a stain such as trypan blue (0.06-0.4 %) will help to identify dead cells and areas of denuded Descemet membrane.

Contraindications to use of corneas for PK or EK include:
i. low endothelial cell density – the minimum endothelial cell density is set at the discretion of the RP/medical director but is typically 2 000 to 2 500 cells/mm^2;
ii. moderate to severe signs of polymegathism and pleomorphism;
iii. significant (> 25 %) endothelial cell loss during organ culture;
iv. abnormalities such as guttae;
v. central stromal scars or opacities (may be acceptable for EK depending on cause and depth);
vi. presence of dead endothelial cells – corneas with scattered, isolated dead cells are acceptable, whereas corneas with larger areas of dead or missing cells are not.

For eye-bank-prepared tissues for DSAEK or DMEK, quality control also includes:
i. gross inspection for larger variations in the thickness of DSAEK grafts and edge ruptures in DMEK grafts;
ii. measurement of the overall diameter of the prepared graft;
iii. for DSAEK grafts, measurement of the central thickness of the graft by indirect ultrasound or optical coherence tomography.

17.8. Corneal transplant registries

Corneal transplant registries, such as those in Australia, the Netherlands, Sweden and the UK, provide an invaluable resource to validate the quality and safety of transplanted corneas. Registries also allow investigation of donor and recipient factors influencing graft survival, post-operative complications (including immunological rejection and serious adverse reactions) and visual outcome [4, 12, 48]. Although randomised clinical trials (RCT) are considered to provide the highest level of evidence,

they are costly and complicated to set up, they can be undermined by changes in clinical and surgical practice during the course of the study, and it is not always straightforward to generalise beyond the specific inclusion/exclusion criteria of an RCT, especially when corneal transplantation outcomes and risk of post-operative complications are influenced by many factors.

Registries, while not without pitfalls, rely on large datasets to reduce selection bias. They provide a broad overview across multiple transplant units and an evidence base that does not always reflect the optimism generated by the excellent results from single-centre studies [49, 50]. In addition to evaluating the outcome of established techniques and monitoring the uptake and success of new processing and surgical techniques, such as endothelial keratoplasty, registry data can also be used for validating eye-bank processes and storage methods in terms of clinical outcome measures rather than simply relying on *in vitro* laboratory measures of quality and safety [12, 51, 52]. A project, part funded by the EU and led by the European Society for Cataract and Refractive Surgery, aims to establish a European Cornea and Cell Transplant Registry (ECCTR), building on the existing registries in the Netherlands, Sweden and the UK (see www.ecctr.org).

17.9. Biovigilance

Serious adverse reactions (SARs) for corneal transplants include:

a. primary graft failure (corneal transplant never cleared);
b. local infection (endophthalmitis or other serious ophthalmic infection);
c. graft failure due to a defect in the donor tissue, which was out of date, scarred or marked by incisions from previous surgery;
d. transmission of malignancy (possibly attributable to the transplanted tissue);
e. transmission of systemic infection (possibly attributable to the transplanted tissue).

Serious adverse events (SAEs) include:

a. wrong tissue supplied for the intended surgical procedure;
b. tissue supplied was damaged or showed signs of unacceptable previous surgery;
c. tissue supplied beyond its expiry date;
d. infection detected in organ-culture medium after cornea supplied to surgeon.

Partial or complete graft detachment after EK is not uncommon; although the reported incidences vary, they are often in the range of 5-10 % for DSAEK procedures and 10-30 % for DMEK procedures [53]. Fortunately, graft detachment is most often successfully treated by re-bubbling (repeated air tamponade of the anterior chamber of the recipient). It has not been possible to ascribe this serious adverse event to donor factors such as donor endothelial cell density, donor age or method of preservation [54, 55]. Consequently, graft detachment is mainly considered a surgical complication.

The Notify Library includes some well-documented cases of adverse reactions and adverse events in transplantation of ocular tissue; for example:

- A case of donor-to-recipient transmission of the *Herpes simplex* virus (HSV) by cornea transplantation was confirmed by polymerase chain reaction-based DNA fingerprinting of donor and recipient HSV strains (Record Number 429);
- A case of a transplant-acquired diagnosis of rabies is supported by temporal association of the recipient's illness, lack of other exposure to rabies and the retro-orbital pain of the recipient of the corneal transplant (Record Number 20);
- A case of transmission of T-cell lymphoma is described, whereby molecular analyses were used to detect the same alleles in HLA-DQα testing of the recipient and donor of the graft (Record Number 338);
- A case of donor ocular tissue being examined and then shipped to the eye bank with a contact lens on the cornea, thereby highlighting the importance of *in situ* inspection before recovery (Record Number 720);
- A case of metastases from a cholangiocarcinoma in the vascularised limbal region of a corneoscleral disc. There was no evidence of transmission to the recipient of the avascular corneal graft. The authors recommended that tissue from donors with a history of malignancy should not be used for limbal allografting (Record Number 1663) [16].

Further cases of adverse outcomes associated with ocular tissue can be found in the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by the substance type, adverse occurrence type and record number. A recent report, not yet in the Notify Library, concerns the identification of metastases in the peripheral, but not central, avascular cornea from a donor with ma-

lignant cutaneous melanoma (see §17.2.1.3) [17]. The implications for donor-selection criteria have been considered by the Eye Bank Association of America Medical Advisory Board, resulting in the exclusion of donors with a history of melanoma with known metastatic disease [18].

Examples of two different SAR notification reports for ocular tissues used in France and by the NHS (UK) are included as Appendix 25 and Appendix 26.

17.10. Developing applications for patient treatment

The Bowman Layer lies between the epithelial basement membrane and the stroma. It can be dissected from donor corneas and inserted into the mid stroma of corneas with advanced keratoconus to help strengthen and flatten the patient's cornea [56].

Decellularised stroma can be used as a scaffold or for transplantation for corneal scars/ulcers. Decellularised porcine stroma is being used clinically, and development of human decellularised stroma is in progress [57]. Retinal pigment epithelial cells derived from human embryonic stem cells, induced pluripotent stem cells, umbilical cord, fetal brain or bone marrow are being investigated for the treatment of age-related macular degeneration (see Part C) [58].

Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease (see Part C) [9].

17.11. References

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Related material

- Appendix 25. Serious adverse reaction: notification form – for ocular tissues (Agence de la Biomédecine, France)
- Appendix 26. Serious adverse reaction or event: notification form – for ocular tissues (NHS, UK)

Chapter 18: **Amniotic membrane**

18.1. Introduction

Amniotic membrane (AM) allografts have been used in different medical specialties since the early 20th century. Histologically, AM is the innermost, semi-transparent layer of the fetal membranes (amnion and chorion), formed by a single layer of cuboidal epithelial cells (epidermis-like cells), that is attached to a thick basement membrane and an avascular stromal matrix consisting of scattered mesenchymal stromal cells in a collagen scaffold. The amnion contains no blood vessels, lymphatic vessels or nerves. It has some unique properties. A number of mechanisms have been suggested to explain the beneficial effects of AM, on the basis of its biological composition. Overall, AM is mainly formed by three types of components: structural collagen and extracellular matrix, biologically active cells and a large number of important regenerative molecules [1].

Clinical and experimental data have shown [2, 3, 4, 5, 6] that AM provides a compatible substrate for cell growth, facilitating migration and differentiation of epithelial cells, supporting maintenance of the original epithelial phenotype and having low or no antigenicity.

18.1.1. Properties of amniotic membrane

Collagen types I, III, IV, V and VII and specialised proteins (including laminin and fibronectin) have been identified in the amniotic basement membrane and stroma. Laminin and fibronectin are particularly effective in facilitating epithelial cell adhesion. The presence of a rich extracellular matrix and collagen endows the stroma with anti-inflammatory properties, which arise from the entrapment of inflammatory cells, the presence of various growth factors and the inhibition of protease activity and decreased lipid peroxidation. In addition, AM has other biological properties that include anti-adhesive, antibacterial and anti-fibrosis effects, along with the ability to decrease scarring and neovascularisation, enhance wound healing and reduce pain [7, 8, 9]. Moreover, AM contains various growth factors (e.g. epidermal growth factor, basic fibroblast growth factor, hepatocyte growth factor, keratinocyte growth factor and transforming growth factors) and cytokines (e.g. interleukin 6 and 8) [10, 11, 12].

These characteristics have led to the use of AM for a wide range of ophthalmic indications (e. g. corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, chemical or thermal burns) and in the treatment of a broad variety of pathological conditions including management of burns (as a temporary or permanent wound dressing), repair of skin lesions of different aetiologies (e.g. vascular ulcers, epidermolysis bullosa, radiation burns), in arthroplasty and in intra-abdominal and reconstructive as well as oral and maxillofacial surgery [13, 14, 15, 16, 17].

In addition the special structure and biological properties of AM make it an important potential source for scaffold material [18].

Stem cells derived from AM have been demonstrated to display multilineage potential and immuno-modulatory properties [19, 20].

Foetal membranes can be donated either separately or together with placenta. In this chapter, when discussing donation, the term 'placenta/foetal membranes' is used to mean 'not processed', whereas 'AM' here means 'processed in a tissue establishment'.

The following generic chapters (Part A) of this Guide apply to AM banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines the additional specific requirements for AM.

18.2. Donor recruitment and evaluation

Prior to full-term delivery, potential donors are approached to ascertain whether they would be willing to donate their placenta/fetal membranes (see Chapter 3). A trained nurse or healthcare professional will discuss the donation process and complete the consent and medical and behavioural lifestyle assessment. General criteria for donor evaluation are described in Chapter 4. The potential donor should be evaluated before giving birth and after full consent, also having been informed that donation will take place only if the delivery is without any complications.

Placenta/fetal membranes should be collected only from living donors, after a full-term pregnancy.

18.2.1. Specific exclusion criteria

In addition to the general exclusion criteria described in Chapter 4, there are some specific conditions that exclude placenta/fetal membranes donation. The diseases of the female genital tract or other diseases of the donor or unborn child that present a risk to the recipient include but are not limited to:

a. significant local bacterial, viral, parasitic or mycotic infection of the genital tract, especially amniotic infection syndrome;
b. (known) malformation of the unborn/newborn;
c. premature rupturing of membranes;
d. endometritis;
e. meconium ileus.

Individual tissue establishments (TEs) may have additional exclusionary criteria.

18.3. Procurement

18.3.1. Procurement facility and procurement team

Donor placenta/foetal membranes are procured by medical staff at obstetrics units after caesarean section. AM could be contaminated by normal vaginal flora during vaginal delivery; therefore, procurement under aseptic conditions after elective caesarean section is to be preferred. If placenta/foetal membranes are procured during vaginal delivery, different sterilisation procedures [21, 22, 23] should be applied to the processed AM (e.g. sterilisation by gamma irradiation). Staff undertaking procurement must be dressed appropriately for the procedure so as to minimise the risk of contamination of the procured tissue and any hazard to themselves. Further details can be found in Chapter 6.

18.3.2. Temporary storage and transportation to the tissue establishment

Placenta/foetal membranes should be stored at appropriate temperatures so as to maintain the structural and biological properties of AM.

The storage and transport time of procured placenta/foetal membranes should be kept as short as possible (the recommended maximum time is 24 h) and a temperature of 2-8 °C should not be exceeded. If AM is processed within less than 2 h after the delivery, the placenta/foetal membranes may be transported at room temperature [24].

Procured placenta/foetal membranes should be placed in a sterile receptacle containing a suitable transport medium (or decontamination solution) if transport time exceeds 2 h [25]. The sterile packaging should then be placed inside an adequately labelled sterile container to be transported to the TE. Indi-

vidual TEs should validate the composition of the transport medium and determine if antibiotics are required.

The temperature during transport to the TE must be maintained. Temperature stability should be guaranteed by the container, conditions of transport used and for the time interval before processing. In cases of unexpectedly high or low environmental temperatures, a temperature-recording unit (data logger) should be enclosed in the container to record temperature at ≤30-minute intervals unless the transport system has been previously validated to maintain the temperature within the required limits for the required transport time.

18.4. Processing and storage

18.4.1. Receipt of placenta/foetal membranes at the tissue establishment

Upon receipt, the procured tissue should be stored in a temperature-controlled refrigerator at 2-8 °C to ensure that the refrigeration process is not interrupted. Processing should be carried out within 24 h after procurement [25].

18.4.2. Processing facilities

In selecting an appropriate air-quality specification for AM processing, the criteria identified in Chapter 7, Chapter 8 and Chapter 10 should be considered. Table 18.1 outlines factors to be considered for AM processing.

Taking the factors from Table 18.1 into consideration, it is appropriate that processing of AM should take place in a controlled environment with defined air quality (see Chapter 7), especially for cryoprotected AM where there is less opportunity for microbial inactivation.

Within the EU, tissues exposed to the environment without subsequent microbial inactivation should be processed in environments with an air quality equivalent to GMP Grade A, with a background environment of at least Grade D.

18.4.3. Processing and preservation methods

Processing must not change the physical and biological properties of AM so as to make it unacceptable for clinical use. TEs may use different processing and preservation methods, according to their own standard operating procedures (SOPs) and mandatory regulations. The methods used must be in line with current best practice and must be validated in accordance with the guidance given in Chapter 2 and Chapter 8.

Processing of AM generally includes the mechanical detachment of fetal membranes (after being previously separated from placenta, when applicable), according to a documented SOP. Fetal membranes easily split into an amnion and a chorion leaflet, separated by a jelly-like, intermediate layer. The chorion is discarded and then the amnion should be rinsed several times in sterile saline until blood residues are removed completely. During processing, AM may be decontaminated by soaking in antibiotic/antimycotic solution. The incubation temperature and the

Table 18.1. **Factors influencing the air-quality specification for processing human amniotic membrane**

Criterion	Amnion-specific
Risk of contamination of tissues or cells during processing	During processing, AM is necessarily exposed to the processing environment for extended periods during dissection, sizing and evaluation of its characteristics.
Use of antimicrobials during processing	Soaking in antibiotic/antimycotic solution is an antimicrobial step that can be used when processing AMs. It is important to validate the decontamination solution and to list the micro-organisms that are acceptable pre-decontamination. Since glycerolised, lyophilised and frozen AM can be exposed to sterilisation processes, the processing environment may not be as critical as for tissue that cannot be sterilised. However, the process should be validated, and maximal acceptable bioburden defined.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of AM for microbiological testing following decontamination step is not extensive; typically, only a small amount is sampled, but the storage medium can also be sampled.
Risk of transfer of contaminants at transplantation	Although not vascularised, AM can support microbiological contaminants and has transmitted bacteria and viruses. AM is mostly used in ophthalmology. AM is also used for other indications, such as burns, skin ulcers and arthroplasty, and intra-abdominal and reconstructive as well as oral and maxillofacial surgery. Immuno-compromised patients, despite recent advances in therapy, are at a substantially higher risk of transmission of infection and even death from infections.

composition of decontamination cocktails should be defined, after validation, by each TE. Following the decontamination step and rinsing procedure, AM should be spread on a suitable carrier membrane (e.g. nitrocellulose), or fine mesh gauze for easier handling and if it needs to be cut into multiple pieces. Depending on the intended clinical use, both sides of amnion (epithelial and mesenchymal) can be placed directly on the selected carrier.

Consistent with the defined preservation method, AM grafts may be decontaminated or sterilised by irradiation. Sterilisation methods should be validated for the initial estimated level of bioburden [21, 22, 23]. AM grafts should be packaged in sterile containers and labelled as advised in Chapter 14. Maximum storage time will depend on the preservation method and should be defined and validated [24, 26]. There are several methods of AM preparation and preservation, as below.

18.4.3.1. *Cryoprotected amniotic membrane*

AM can be preserved in culture medium containing glycerol or dimethyl sulphoxide (used to protect cells against freezing injury). AM intended to be cryoprotected may be decontaminated by soaking in antibiotic/antimycotic solution.

Following package, AM grafts are stored at – 80 °C (deep frozen) or in liquid or vapour phase of nitrogen at temperatures below – 140 °C (cryopreserved) [27, 28, 29] but, in the case of cryopreservation, after being previously submitted to a controlled-rate freezing procedure.

18.4.3.2. *Frozen amniotic membrane*

If cell viability is not to be maintained, the processed AM can be frozen without addition of a cryoprotective agent.

AM intended to be frozen may be decontaminated by antimicrobial solution or sterilised by irradiation [24]. Following package, AM grafts should be stored between – 15 °C and – 80 °C.

18.4.3.3. *Heat-dried amniotic membrane*

The processed AM is dried overnight in an oven at 40 ± 2 °C, then packed and sterilised by irradiation. Storage should be at room temperature [25].

18.4.3.4. *Air-dried amniotic membrane*

The processed AM is air-dried overnight in a laminar-flow hood. It can then be packed and sterilised by irradiation. Although high temperatures are not applied using this method, some properties of the amnion are lost or altered due to dehydration. Air-dried irradiated AM grafts should be stored at room temperature [30, 31].

18.4.3.5. *Lyophilised (freeze-dried) amniotic membrane*

The processed AM is rapidly frozen at – 50 °C to – 80 °C. Then it is vacuum-dried using a freeze-drying device. Water from the tissue is extracted through sublimation until a final water content of 5-10 % is attained. Following package, AM grafts may be sterilised by irradiation [32]. This preservation method induces minimal changes in the properties of the AM and the product can be stored at room temperature [33].

18.4.3.6. *Glycerolised amniotic membrane*

Glycerolisation is a preservation method combined with the antimicrobial properties of high concentrations of glycerol. Since glycerol permeates more slowly than water, there will be an initial efflux of water when the glycerol is added. However, as glycerol begins to permeate the tissue, water will re-enter. At the end of the glycerolisation process, the final water activity (a_w) is *circa* 0.3, which is known to minimise lipid peroxidation and reduce other degradation reaction rates to very low levels. Rather than dehydrating the tissue, as is commonly assumed, it has been demonstrated with skin that glycerolisation results in the effective sequestration of water [34, 35]. Typically, 85 % (v/v) glycerol is used to preserved AM, which can then be stored at 2-8 °C for up to two years, although it does lose some of its biological properties [36].

AM intended to be glycerolised may be decontaminated by soaking in antimicrobial solution or sterilised by irradiation.

18.4.3.7. *Antibiotic-soaked amniotic membrane*

The processed AM is placed overnight in a decontamination solution composed of a range of wide-spectrum antibiotics and an anti-fungal agent and then deep-frozen at – 80 °C. The resultant AM is particularly suitable for wound healing.

18.5. Quality control

During procurement and processing of AM, reliable macroscopic examination of the donor foetal membranes should be undertaken to exclude visible pathological changes and ensure structural integrity of the tissue (to provide barrier function). Samples for detecting aerobic and anaerobic bacteria and fungi should be obtained from the transport/storage medium or from the initial washings of

the AM, and from pieces of the tissue obtained both before and after antibiotic decontamination step. Microbiological testing for the detection of bacteria and fungi should be carried out according to the procedures described in Chapter 10.

These approaches cover the minimum standards to control microbiological safety (see Table 10.2). Deviations from such standards should be justified, and the suitability of the intended test method must be demonstrated. Factors such as samples containing antibiotics or very small sample amounts may affect the sensitivity of tests leading, in the worst case, to false-negative results. Where samples taken before antibiotic/antimycotic decontamination yield micro-organisms that are considered pathogenic and highly virulent (see Table 18.2), the tissue cannot be approved for clinical use. Tissue showing heavy or confluent bioburden growth should also be rejected. After decontamination, tissue is not deemed suitable for transplantation if the samples taken for microbiological testing show signs of any microbial growth.

Table 18.2. **Microbial contaminants that should result in tissue discard if detected at any stage of processing**

Acinetobacter spp.
Aspergillus spp.
Bacillus spp.
Bacteroides spp.
Beta-haemolytic *Streptococci*
Burkholderia cepacia complex
Candida spp.
Clostridium spp. (notably *C. perfringens*)
Corynebacterium diphtheriae
Enterobacteriaceae (coliforms)
Enterococcus spp.
Fusobacterium spp.
Klebsiella rhinoscleromatis
Listeria monocytogenes
Mucor spp.
Mycobacteria spp. (for at-risk donors)
Neisseria gonorrhoea
Nocardia spp.
Penicillium spp.
Porphyromonas spp.
Prevotella spp.
Pseudomonas spp.
Salmonella spp.
Shigella spp.
Sphingomonas maltophilia
Staphylococcus aureus
Stenotrophomonas maltophilia
Streptococcus pyogenes (Group A)

Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

18.6. Distribution

The TE must ensure that distribution of AM grafts is carried out under controlled conditions. General considerations can be found in Chapter 11.

For cryopreserved AM, distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. For deep-frozen AM grafts, distribution should be in dry ice. Transport temperatures of cryoprotected AM above −60 °C must be avoided, to ensure the stability of the product and maximum safety for the recipient. Frozen AM grafts should be transported at or below −15 °C.

Heat-dried, air-dried and freeze-dried AMs can be distributed at room temperature, whereas glycerolised AM should be transported at 2-8 °C.

18.7. Biovigilance

Serious adverse events and reactions must be recorded, reported and investigated according to the relevant national regulations to Health Authorities for tissues and cells, as described in Chapter 16.

The Notify Library includes some documented cases of adverse occurrences. Examples of SARs include:

- Loss of significant quantity of AM grafts due to storage at inadequate temperature (e.g. in a case of equipment failure).
- High level of microbial contamination of procured AM (reflecting the hygienic conditions of the delivery room).

18.8. Developing applications for patient treatment

18.8.3.1. *Amniotic drops/suspension/extract*

The use of drops/extracts described in the literature [37-48] shows that this form of application also has a good effect and can be used in many therapeutic fields as an alternative to transplantation.

18.8.3.2. *Chorion*

So far, literature hardly differentiates between amnion and chorion grafts. However, based on the finding that amnion and chorion present different content of growth factors and cytokines [49], different effects from amniotic and chorionic grafts can be expected [50].

18.9. References

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Chapter 19: **Skin**

19.1. Introduction

Autologous skin is considered the gold standard for wound treatment and final wound closure. For large wounds, autologous thin split skin can be widely expanded (meshed) or transplanted as island grafts (Meek wall technique). The autologous skin grafts will grow out to close the wound.

In case of shortage of autologous skin sources (especially in burns), donor skin is used to treat patients with extensive skin loss, for wounds with either split-thickness or full-thickness depth. Deep injuries lead to dermal damage, impairing the ability of the skin to heal and regenerate. Skin allograft basically acts as a temporary coverage, preparing the wound bed, reducing scarring, controlling pain, preventing infection and maintaining patient homoeostasis by reducing loss of fluids, proteins and heat through the burn wound. For these reasons, donor skin is critical (and often life-saving) in the treatment of severely burned patients, after escharectomy. In addition, allogeneic skin is considered to be an excellent biological dressing for the treatment of other types of skin defects such as venous ulcers, decubitus ulcers, diabetic foot, surgical wounds, exfoliative skin disorders such as toxic epidermal necrolysis (Lyell's syndrome) and congenital epidermolytic skin disease. In these cases, skin allografts promote re-epithelialisation and formation of granulation tissue, shorten healing time, control pain and protect important structures (e.g. tendons, bones, cartilage, nerves) [1]; they are also successfully used as skin substitutes that incorporate the dermal component into the wound bed, guiding a more physiological healing process, having the ability to be integrated into the wound bed of full-thickness burns or leg ulcers.

Allogeneic skin can also be placed on top of the autologous skin (the 'sandwich technique') to protect it from mechanical damage, dehydration and infection. After 7-10 days the allogeneic skin can be removed from the wound [2, 3, 4].

These factors explain the constant demand for skin allografts by burn centres and reconstructive surgery units, where the capacity of these bioproducts to 'take' and integrate into the wound bed is exploited. In the past, allogeneic skin was used sometimes to replace the lost dermis ('Cuono technique') [5], but the donor cells and hairs still present may cause inflammatory reactions with a negative effect on the final scar formation. Nowadays acellular dermis is available. Several tissue banks have developed this type of skin graft that is more suitable as a dermal equivalent.

The shortage of allogeneic skin grafts has promoted the development of skin-replacement products, and many research teams have focused on biomaterials for skin substitution in wound healing. In the past 30 years, a huge number of biological, semisynthetic and synthetic skin/dermal substitutes have been developed with the aim of producing an artificial skin that is able to replace human skin completely, but an ideal skin substitute has not yet been realised. A further logical development of this research involves the use of stem cells to re-populate the dermal matrix and reproduce 'physiological' skin,

but to date there is no ideal skin-replacement product available based on stem cells.

The following generic chapters of this Guide (see Part A) all apply to skin banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines the additional specific requirements for skin.

19.2. Skin-specific donor evaluation

19.2.1. Skin inspection and skin-specific contraindications

In addition to the standard physical examination described in Chapter 4, the donor's skin must be inspected in a particular manner before skin procurement. Skin should be visually checked for mechanical damage, open wounds, multiple (> 100) or dysplastic naevi (see Appendix 16), dermatitis, local infections, scars and ectoparasites. The results must be recorded and taken into account.

The list of selection criteria for donors is based on a risk analysis related to the use of the tissue on patients, i.e. to minimise the risk of transfer of diseases to the recipient and to ensure the appropriate quality of skin for optimal functional results. The following conditions contraindicate skin donation:

a. autoimmune diseases and systemic connective tissue diseases affecting skin;
b. diseases affecting the dermis (e.g. dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria, lupus erythematosus);
c. toxicity of the skin as a result of the presence of toxic agents or poisons;
d. systemic use of corticosteroids or Cushing disease, inducing severe skin atrophy.

The following relative contraindications for skin donation should be considered case-by-case, and eventually require risk assessment:

a. extensive lacerations, haematoma or scars;
b. skin diseases with extensive involvement (e.g. psoriasis, eczema);
c. relevant skin ulcers, pressure ulcers, stoma, pyoderma or mycoses;
d. skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos, jaundice);
e. diabetes mellitus with skin complications (e.g. ulcers, amputation, neuropathy);
f. pre-malignant conditions such as actinic keratoses and Bowen's disease;
g. mechanical, thermal or microbial damage where skin is to be procured;
h. extreme peripheral oedema, high body mass index (BMI) (≥ 40) or poor nutritional status affecting procurement or body reconstruction.

The common practice is not to procure skin from donors aged < 15 years but many tissue establishments (TEs) do not indicate any age limits, which are basically determined by the medical director of the TE, according to characteristics and quality of tissues.

19.3. Skin procurement

Skin can be obtained from deceased donors after brain death (DBD) or circulatory death (DCD). It is recommended to procure the skin within a period of 12 h, should the body not be refrigerated, or up to 24 h after death if the body has been cooled or refrigerated within 6 h of death. It is obvious that refrigeration of the DCD donor before procurement will reduce skin contamination and facilitate skin procurement due to the harder consistency of the subcutaneous tissue.

It may be possible to extend procurement times up to 48 h after death if skin processing has been validated to guarantee quality and microbiological safety; in these cases the blood samples for serological testing should be taken within 24 h after death

(to avoid extensive haemolysis) [6]. See Chapter 5 for details of sample collection.

Skin can also be obtained from living donors if there is a shortage of *post-mortem* donors, from patients having abdominoplasty or mammoplasty procedures who consent to tissue donation. Potential living donors are evaluated similarly to deceased donors to determine donor suitability according to standard exclusion criteria for skin donation and absence of adverse physical, psychological or emotional outcome before, during or following the donation (see Chapter 4). In these cases, the procurement area is prepared by depilation and disinfection and the tissue is processed to obtain full-thickness skin grafts.

19.3.1. Procurement teams and sequence

Skin-procurement teams should consist of at least two people operating under aseptic conditions and appropriately clothed for the type of procurement. In the case of multiple-tissue procurements, the order in which the tissues are removed should be standardised and predefined and, in the case of multiple procurement teams, should be agreed between the teams beforehand so that risks of cross-contamination between tissues are minimised (see Chapter 6). Studies show that, whether the skin procurement is done before or after bone procurement, the contamination rate of skin is not different if the procurement process is controlled and standardised [7, 8]; therefore, skin is usually retrieved in aseptic technique prior to bone tissue due to the difficulty in obtaining grafts of consistent quality in particular after extensive bone procurement.

Notably, procurement of skin before ocular tissue is recommended to avoid eye bleeding from the sockets if the donor has to be placed in a prone position following enucleation of the eyes.

19.3.2. Skin-procurement procedure

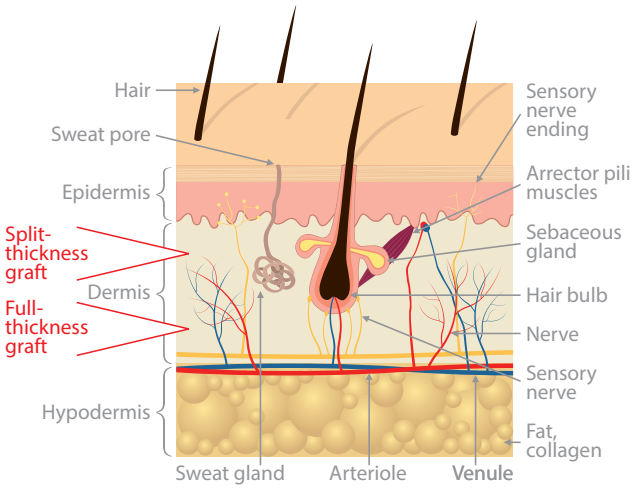
Skin is procured under aseptic conditions after adequate shaving of the donor areas and appropriate pre-operative scrubbing and disinfection of the donor skin to remove the transient, and reduce the resident, microbial flora. An effective and validated procedure for skin disinfection should be established by the TE and allocated to all procurement sites.

The procedure should aim to reduce the bioburden, which can significantly decrease the microbial positivity rate of processed skin samples. Therefore, suitable disinfectants, such as povidone iodine or chlorhexidine, should be chosen. Their concentrations and the durations of exposure should also be evaluated and validated.

A local sterile field using sterile drapes must be used prior to procurement to effectively prevent microbial contamination. Skin grafts can be procured by manually, electrically, compressed-air or battery-operated dermatomes from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs. Grafts should be cut as homogeneously as possible.

According to graft thickness, skin grafts can be divided into split-thickness and full-thickness grafts. They consist of the entire epidermis and a dermal component of variable thickness. If the entire thickness of the dermis and skin adnexal structures is included, the appropriate term is full-thickness skin graft (FTSG). This type of skin graft can be procured using a scalpel instead of a dermatome. If less than the entire thickness of the dermis is included, this graft is referred to as a split-thickness skin graft (STSG). STSGs are categorised further as thin (0.008-0.012 in/0.2-0.3 mm), medium (0.012-0.018 in/0.3-0.45 mm) or thick (0.018-0.030 in/0.45-0.75 mm). The choice between full- and split-thickness grafting (see Figure 19.1) depends on wound conditions, location, thickness, size and aesthetic concerns [9, 10].

Figure 19.1. **Differences between split- and full-thickness graft**



Staged separate procurement of particular body areas, with placement of procured material from each area into separate containers, is sometimes preferred in order to reduce eventual cross-contamination of the procured tissue. Containers and solutions for transportation of procured skin to processing TEs must be sterile and suitable for the intended use. Pre-labelling of the containers is impor-

tant to prevent mix-up of tissues and to ensure their full traceability (see Chapter 14).

19.3.3. Reconstruction of the skin donor

For aesthetic reasons and with a view to a respectful reconstruction of the donor, it is not acceptable to take skin from the neck, face and other typically exposed areas of the body that might be visible when people pay their last respects to the donor. Conditions which may affect body reconstruction after procurement such as extreme oedema or a high BMI (≥ 40) should be evaluated prior to procurement; body reconstruction should ensure that loss of fluid that accompanied skin procurement is not noticeable. Once the tissue has been procured, sealing agents (polymers) and appropriate garments (e.g. polyethylene overalls) should be used to prevent leaking and oozing from sites where tissue has been obtained. Effective communication with all parties involved can help to meet expectations in regard to delays, as well as aesthetic considerations in case of unexpected leaking.

19.3.4. Temporary storage and transportation to the tissue establishment

Immediately after procurement, skin samples for initial bioburden estimation should be obtained and the recovered tissue must be stored in a suitable transport medium in sterile, pre-labelled containers filled with an appropriate transport medium. The containers must be sealed securely, refrigerated to 2-8 °C and transported to the processing facility or TE. Transportation at low temperatures prevents proliferation of most bacteria and fungi, and maintains skin viability (if viable grafts are requested). Antibiotics can be added to the transport medium, but at 2-8 °C even the latest broad-spectrum antibiotic cocktails can fail to decontaminate skin grafts. According to Rooney *et al.* [11], approximately 22 % of skin allografts are not reliably decontaminated by antibiotic treatment.

There are three likely causes as to why antibiotic cocktails are not always fully efficient. First of all, bacteria can be 'hidden' in the procured skin (e.g. in the hair follicles) where the antibiotics cannot reach them. Secondly, the optimal operating temperature of most antibiotics is much closer to 37 °C than to 2-8 °C [12]. The inclusion of an additional short antibiotic incubation step at 37 °C could be considered during processing stages. Finally, diluted antibiotic suspensions are known to lose activity relatively quickly (even when frozen). It is thus important that concentrated antibiotic suspensions are kept at low temperatures (e.g. according to manufacturer instructions) and are not added to the transport media much in advance.

The container with procured skin must be cooled during transport to the TE. If skin grafts are to be glycerolised (i.e. they are non-viable grafts), refrigerated transportation may not be required and the recovered skin can be stored and transported at ambient temperature in 50 % glycerol solution.

After procurement, skin grafts should be transferred to the processing TE as soon as possible, and tissue processing should commence within 24-72 h of procurement having taken place. Before processing, the recovered skin should be kept in a temperature-controlled refrigerator at 2-8 °C, without interruption throughout the refrigeration process. It is recommended that the cell nutrient medium used for viable grafts be changed shortly after receipt of skin grafts, or that the medium be validated for 72 h storage (i.e. adequate buffering capacity). All the manipulations where the transport containers are going to be exposed and the media changed must be performed in a controlled-air-environment (safety cabinet in a clean room of at least Grade D).

19.3.5. Procurement documentation

The organisation responsible for the skin procurement must produce a procurement report to be provided to the TE. In addition to the generic requirements defined in Chapter 6, it must contain a description and identification of the procured skin (including samples for testing).

19.4. Skin processing

The recovered skin is processed to reduce microbial contamination and allow longer storage periods until transplantation. All human tissues intended for human application are processed into specimens appropriate for clinical use. Processing must not change the physical properties of the tissue, making it unacceptable for clinical use.The methods used must be in line with current state-of-the-art procedures and validated procedures (see Chapter 2 and Chapter 8). Different TEs apply specific preparation methods according to their own standard operating procedures (SOPs) and any applicable local authorisations. All processes must be validated in accordance with the guidance given in Chapter 2.

19.4.1. Skin processing methods

Depending on the intended clinical use and the quality requirements, skin grafts can be processed and preserved according to various methods (cryopreservation, glycerol preservation, lyophilisation, possibly followed by gamma-irradiation). These methods ascertain different grade of skin viability, integrity and immunogenicity. Skin grafts destined for cryopreservation should be processed immediately after receipt in order to maintain cell viability and structural integrity. Skin allografts can also be processed into de-epidermised skin and acellular dermis. Processing of skin grafts generally includes soaking in antibiotic and antimycotic cocktails; if skin viability is to be maintained, then this is the only decontamination step that can be included and it should always be applied. The incubation temperature and the composition of decontamination cocktails should be defined, after validation, in written procedures by each TE, assessing the initial tissue bioburden.

The usual width of the grafts procured depends on the width of the dermatome blades (usually 8 or 10 cm). The length of the grafts varies according to the size of the donor site and the final storage containers. Procured skin allografts can be cut into specific smaller sizes according to requirements of the end-user clinicians. The skin grafts may be provided as sheets or meshed (extended on a synthetic mesh to increase the surface area and to allow wound fluid drainage). The graft's irregular edges should be trimmed and, typically, a rectangular shape should be obtained. The final graft sizes are measured with a ruler or calipers. The dimensions and area of each graft must be recorded and displayed on the label. The grafts should then be packaged in validated sterile packages and labelled appropriately (see Chapter 14).

19.4.1.1. Glycerol-preserved skin allografts

Glycerol-preserved skin allografts (GPA) were developed [13] to maintain skin allograft at 2-8 °C using an increasing series of glycerol concentrations (50 %, 70 %, 85 %) for preservation and storage without freezing. Glycerol preservation is an excellent preservation method to obtain de-vitalised skin grafts characterised by reduced immunogenicity and low antibacterial/antiviral properties [14-24]. If there are positive microbiology results from cryopreserved skin, the skin can be processed in 85 % glycerol as a recovery procedure. The glycerol solutions used must be sterile and of high quality (e.g. see *European Pharmacopoeia* monograph 0497 – Glycerol 85 %). Most skin banks validate an expiry date of 5 years for GPA stored at 2-8°C.

19.4.1.2. Unprocessed skin allografts

The use of unprocessed skin allografts ('fresh skin allografts') is not the preferred option because it may not allow for complete donor screening, autopsy reports and/or extensive microbiological testing. However, some TEs use unprocessed skin allografts as it is possible to maintain structural integrity and cell viability for short periods of time (maximum 7-8 days) [11]. These allografts were initially preferred in burn centres due to their high cell viability [25].

19.4.1.3. Cryopreserved and deep-frozen skin allografts

These preservation methods aim to maintain cell viability and structural integrity of skin allografts. Biological and structural functions of skin tissue are preserved by cooling to subzero temperatures in a freezing medium with cryoprotectants, such as dimethylsulphoxide (DMSO) or glycerol [26, 27], to protect cells against freezing injury. Cryoprotectants can, however, adversely affect cell viability and graft efficacy. A controlled-rate freezing procedure is recommended to preserve cell viability. Cell viability is maintained by cryopreserved and, to a lesser degree, deep-frozen skin grafts. It favours the tissue engraftment to the wound bed, being a substrate for revascularisation and recolonisation by host cells [28-29, 12]. Skin-cell viability, referred to as the mean percentage of cell viability after 10-20 days of storage, is reported to be between 40 and 50 % that of the fresh skin [30-33]. When cell viability is required for clinical use, it should be validated and can be assessed by various methods, including vital dye staining, oxygen consumption, and enzymatic and metabolic assays (described in §19.5.2).

Cryopreservation is considered to be the best method for the long-term preservation of skin [12]. After cryopreservation, skin allografts can be stored in liquid or vapour nitrogen to a maximum of 5 years. Storage at higher temperature (– 60 °C/– 80 °C) is a method applied for medium-term (maximum of 2 years) preservation of viable skin allografts. Inappropriate storage compromises the potential to restore normal metabolic activity and, thus, physiological functioning after transplantation.

19.4.1.4. Lyophilised skin allografts

Processing of grafts by freeze-drying devitalises the grafts while maintaining their structure. A maximum limit for residual water content should be established and measured (ideally < 5 %). Lyophilised skin grafts can be stored at ambient temperature for 3-5 years.

19.4.1.5. De-epidermised skin and acellular dermis

De-epidermising or de-cellularising skin is a method to lower the antigenicity of the skin graft. Thicker skin obtained from deceased donors is processed aseptically to remove the epidermis and possibly the dermal cells that can accelerate tissue rejection and graft failure. Acellular dermis can permanently replace the lost dermis in patients with full-thickness wounds (burns). Various methods for separating the epidermis from dermis are reported such as chemical (sodium chloride, phosphate buffered saline, dispase), physical (heat; freezing and thawing) or mechanical (dermatome). These methods are frequently used in association to obtain optimal de-epidermisation. In cases of shortage of deceased skin donors, full-thickness skin can be obtained from living donors undergoing abdominoplasty or body-contouring procedures and is processed in a similar manner to produce thicker dermal allografts to be used in full-thickness skin loss if primary closure or donor-site availability of autografts is limited or suboptimal [34]. The result is an intact dermal matrix that can be cryopreserved, preserved in glycerol or lyophilised.

19.4.1.6. Skin tissue decellularisation

Tissue decellularisation is a technique that aims to remove all cells from a tissue, maintaining an intact extracellular matrix (ECM). In the last years several innovative biological products based on decellularisation of donor-derived skin tissue have been developed using biotechnological sciences.

Three methods are actually used to obtain tissue decellularisation: chemical, physical and biological (enzymatic). Each of these methods has a different mode of action and effect on the ECM. A combination of methods is recommended to ensure effectiveness [35]. Chemical methods comprise hyperosmotic/hyposmotic solutions, ionic detergents and non-ionic detergents; physical techniques are based on temperature (freeze–thaw cycles), hydrostatic pressure, mechanical agitation and sonication. Sterilisation techniques may be applied to provide a higher level of safety. There are significant advantages in combining decellularisation and sterilisation processes to ensure a clinically safe ECM, minimising the effect on its ultrastructure. Each method used for tissue decellularisation has to be standardised and validated. Then, standardisation and validation can be repeated in order to monitor the product obtained as well as to ensure maximum biological safety of decellularised tissues.

The common goal of all these methods is to obtain an acellular dermal matrix characterised by an intact fibrous and collagenous architecture, able to be repopulated by autologous cells of the patient after its engraftment. The absence of immune response and graft rejection in patients is ensured by removing the cellular components (fibroblasts and endothelial cells) as well as the donor DNA and hair remnants. From a functional point of view, these dermal matrices act as cell-free scaffolds able to permanently reconstruct and regenerate damaged and/or pathological skin tissue.

The main biological characteristics of an optimal dermal matrix are biocompatibility (the ability to take after engraftment and the absence of rejection/inflammatory reaction due to cytokine release), integrity of the matrix (the maintenance of integrity of elastic fibres and collagen physiologically identified in the tissue), sterility (absence of Gram+/– bacteria, fungi and bacterial endotoxins), malleability and suturability (handling; mechanical resistance with or without stitches) and storage options (the ability to be stored by different methods e.g. cryostorage in nitrogen vapours, storage at –80 °C, freeze-drying, dehydration, room-temperature storage, storage in high-percentage glycerol).

See Chapter 8 and Part C of this Guide for information on decellularisation of tissues as natural extracellular matrices.

Clinical indications of acellular dermal matrices are in the field of regenerative medicine and surgery and depend on wound thickness. Among them are:

- dermatology, plastic and reconstructive, general surgery and vascular surgery used for the treatment of acute (e.g. burns) and chronic (e.g. skin ulcers in various aetiology) skin wounds with extensive loss of substance: acellular dermal matrices with a thickness of 0.2-0.4 mm can be used in combination with a thin autologous split-skin graft to improve the scar quality of the wound; thicker acellular dermis is used for other indications such as reconstruction of the breast after mastectomy or hernia repair [36-46].
- orthopaedics for the repair of the rotator cuff of the shoulders as well as for the treatment of skin surgical wounds [47].
- maxillo-facial surgery, ENT (ear, nose and throat) surgery, dentistry for the sinus lift and implant dentistry for augmentation in gum reconstructions [48-49].

Table 19.1. **Factors influencing the air-quality specification for processing of skin**

Criterion	Skin-specific
Risk of contamination of tissues or cells during processing	During processing, skin is necessarily exposed to the processing environment for extended periods.
Use of antimicrobials during processing	Soaking in antibiotics is the only anti-microbial step possible for cryopreserved skin, with maintained cell viability. To minimise the risks of particulate or microbial contamination of the product or materials being handled, it is indispensable to process tissues in cleanrooms (with air-quality standards as specified in Chapter 7). High concentrations of glycerol (85 %) used in the glycerolisation process have been shown to achieve long-term anti-microbial effect, though it cannot be considered a sterilising agent [51].
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of skin for microbiological analysis following antibiotic soaking is not extensive (random sampling). Sterility testing has significant statistical limitations, and can be used to detect only large-scale contamination. Final sterility testing may even be unreliable, especially if antibiotics remain on tissues [52]. Due to these limitations, aseptic methods must be used at all times.
Risk of transfer of contaminants at transplantation	Although skin is placed on the external surface of the body, it is mostly used for severely burned patients whose own skin barrier is no longer functional. These patients usually develop immuno-suppression by various mechanisms and, despite recent advances in therapy, they have a significant risk of death from infection. As there is always a risk of transfer of contaminants by tissue transplantation, the demand for an aseptic method is obvious.

19.4.2. Processing facilities

In selecting an appropriate air-quality specification for skin processing, the criteria identified in Chapter 7 should be considered. Table 19.1 outlines the factors to be considered for skin processing.

Taking the factors from Table 19.1 into consideration, skin grafts should be processed in optimal environments with air quality equivalent to Grade A in EU Good Manufacturing Practice (GMP) Guidelines, with an adequate background environment. For EU countries, the background must be at least Grade D but, given the risks associated with the use of skin grafts which are not sterilised or treated with equivalent microbial reduction methods, more stringent requirements are recommended.

19.4.3. Sterilisation of skin allografts

When tissue viability is not required or when skin tests positive for microbiological contaminants, it can be sterilised by gamma irradiation or electron beam. Ionising radiation (in relation to its dose) can cause structural changes in the irradiated skin allografts, especially in the epidermis [53]. Research has shown that a maximum dose of 25-kGy irradiation of deep-frozen skin in radio-protective solutions sterilises tissue without relevant histomorphological or physical alterations (such as pliability) compared with normal cryopreserved skin [11]. Sterilisation methods should be validated for the initial estimated level of bioburden prior to application of the sterilisation method to skin allografts.

19.5. Quality control

19.5.1. Microbiological control

In addition to the standard microbiological controls described in Chapter 10, microbiological testing should be done before the start of processing and on post-processed samples of skin (without antibiotic) before the skin is approved for clinical use. These approaches are based on the microbiological test methods of *Ph. Eur.* and cover the minimum standards to control the microbiological safety of preparations of human tissues. Deviations from such standards should be justified and the suitability of the intended alternative test methods must be demonstrated and validated. Specimens of a representative sample of finished product (e.g. a predefined number of pieces of skin allografts that have undergone all stages of production) should be sent for microbiological testing to check for aerobic and anaerobic bacteria and fungi using appropriate culture media [27]. Acceptance criteria for microbial load and types of contaminant in processed tissues should be defined in advance and reported in written procedures.

If a positive microbiology result is obtained at the initial stage or at an intermediate stage of processing, a risk-assessment analysis should be conducted to assess the suitability of the skin tissue (and other tissues from the same donor), taking into account the micro-organism(s) detected. Basically, skin allografts may be accepted for clinical use, without sterilisation, when bacteriological and mycological assessment (refer to Chapter 10 for acceptable

microbiological examination techniques) reveal only low bioburdens of inherent inhabitants of the residential skin flora. The surgeon must be informed of the skin-graft bioburden before the use of the graft (see §19.9 for further information). Bioburden can be determined using quantitative cultures, but it is permitted to use more pragmatic validated approaches in which bacterial density is measured in terms of bacterial lawn confluency or the appearance of turbidity in periodically inspected liquid cultures. The presence of micro-organisms in finished product samples results in a definite rejection of the donor tissue if no validated sterilisation or decontamination method is applied (see Figure 19.2).

For terminally sterilised skin, an equivalent analysis should be carried out, taking into consideration the capacity of the sterilisation process as demonstrated through validation.

Specimens contaminated by (endo)spore-forming micro-organisms such as *Bacillus* and *Clostridium* spp. or any of the pathogens listed in Table 19.2, at any stage of the process (even if negative at the end of processing), should be discarded without corrective actions in order to remove potentially unsuitable tissue from the transplantation process. Table 19.2 is a suggested list of such micro-organisms that is non-exhaustive, can be updated and is subject to change, according to different geographical areas.

Table 19.2. **Contaminants that should result in tissue discard if detected at any stage of processing**

Acinetobacter baumanni
Actinomyces
Bacillus anthracis
Bacteroides spp.
Burkholderia cepacia complex
Carbapenem-resistant *Enterobacteriaceae*
Clostridium spp. (notably *C. perfringens* or *C. tetani*)
Corynebacterium diphtheriae
Erysipelotyhrix rhusiopathiae
Fusobacterium
Listeria monocytogenes
MRSA (methicillin-resistant *Staphylococcus aureus*)
Mucor spp.
Mycobacterium tuberculosis complex or *M. avium*
Neisseria meningitides or *gonorrhoeae*
Nocardia spp.
Pseudomonas aeruginosa
Salmonella typhi or *paratyphi*
Shigella spp.
Stenotrophomonas maltophilia
Streptobacillus moniliformis
Streptomyces spp.
Vibrio cholerae
Yersinia pestis or *pseudotuberculosis*

Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

19.5.2. Skin allograft performance and quality issues

Viable donor skin is still considered the gold standard for the temporary covering of burns. In cryopreserved skin allografts, the viability of skin is often considered as an essential requisite and should be tested. Different methodologies are used to assess cell viability in skin grafts before and after thawing:

- quantitative, e.g. tetrazolium salt assay (MTT), neutral red test (NRT), resazurin test, oxygen or lactate consumption assay [32-33];
- and /or qualitative by histological staining, e.g. orcein, Masson, haematoxylin-eosin.

Controversy exists in the literature data regarding the importance of cell viability in graft performance and quality. It is widely considered that viable skin allografts are superior to all other dressing materials, and the majority of physicians agree that higher viability is usually associated with better wound-bed preparation and graft take [32, 54-57]. Comparison of unprocessed, cryopreserved (viable) and glycerolised skin allografts by the use of animal studies (immuno-competent Balb/c mice) revealed a better performance (evaluated by histology) of unprocessed skin and, to a lesser degree, of deep-frozen (– 80 °C) and cryopreserved (in liquid nitrogen) skin [58]. These data demonstrated that graft performance of cryopreserved skin decreased with time.

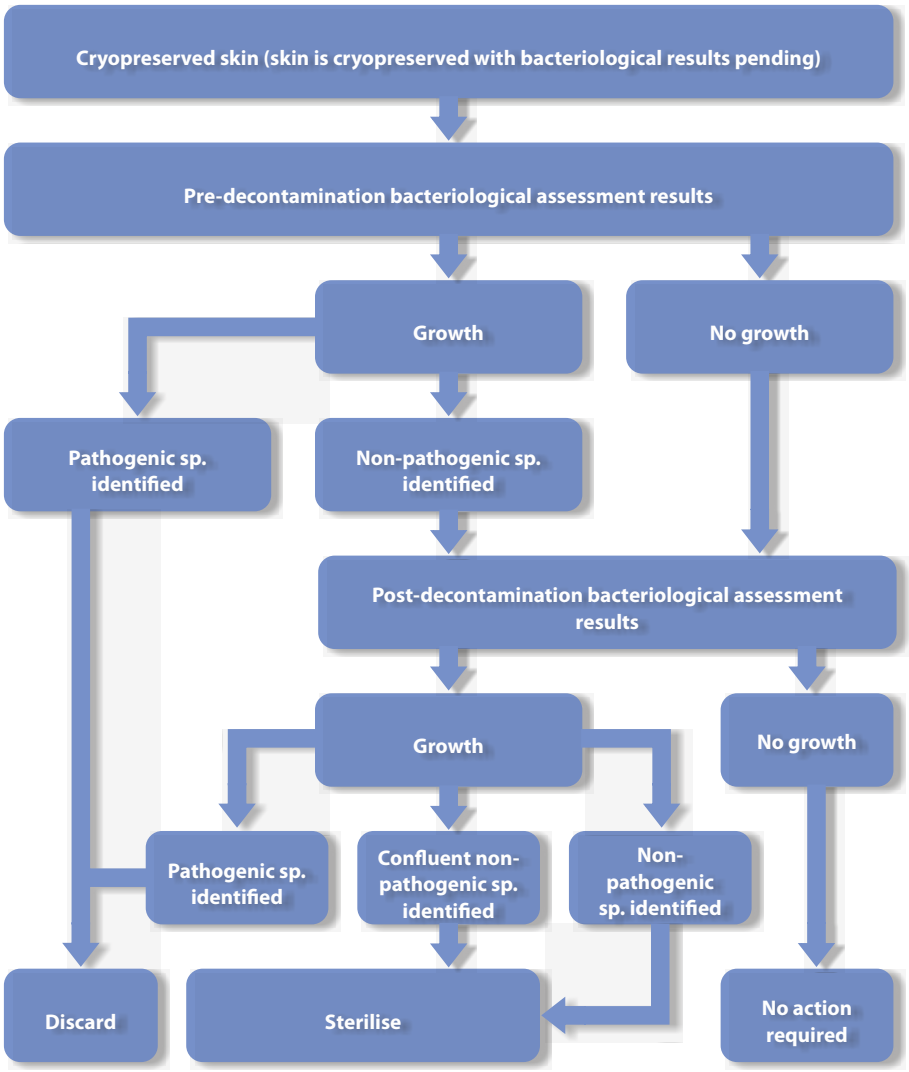
However, non-viable skin allografts can be successfully employed when viable cryopreserved skin allografts are not available or where cell viability is not required for wound treatment [4]. Literature studies [26] indicate that there is no evidence that viability of the graft influences healing outcomes. Thus, instead of viability, other aspects, such as structural integrity, clinical outcome and intrinsic anti-microbial safety of the preservation method and cost should be the primary criteria for the choice of preservation method to be used for allografts.

19.6. Packaging and labelling

The grafts are packaged in foil or polythene/polyethylene sterile containers and coded, packaged and labelled in accordance with the guidance in Chapter 14. All packages must be labelled with the name of the processing institution, a unique identifier or serial number linking the tissue to the donor, the expiry date, size and type of skin graft (e.g., cryopreserved, glycerolised).

Figure 19.2. **Algorithm for acceptance/rejection of skin after bacteriological assessment**

Note: Pathogenic spp. identified during skin assessment should be understood as those defined in Table 19.2.



19.7. Storage

Processed skin grafts are stored in various conditions depending on processing method. Glycerolised skin is usually stored in a bio-refrigerator at 2-8 °C. Lyophilised (freeze-dried) skin can be stored at room temperature. Cryopreserved skin can be stored in liquid or in the vapour phase of liquid nitrogen in a liquid nitrogen refrigerator. Viable (frozen/deep-frozen) skin allografts can also be stored in mechanical freezers, at – 45/– 60/– 80 °C or in ultralow freezers (– 130 °C), but at higher temperatures the storage time will be shorter (to maintain biological properties).

19.7.1. Expiry date

In order to ensure the safety and quality of tissues and cells, the maximum shelf-life of tissue under each type of storage condition should be specified. The expiry or retest date should come from formal validation with stability studies, which should take into account, among other factors (e.g. expiry dates of reagents), possible deterioration of the required tissue and cell properties, integrity and stability of the packaging and labelling materials, according to the requirements of Chapter 8 and Chapter 9.

19.8. Skin allograft distribution

Skin allografts are considered life-saving therapeutic materials, so TEs should have a written procedure for allocation of grafts based on clinical priority. Distribution of skin grafts for transplantation should be restricted to hospitals, TEs, physicians, dentists or other qualified medical professionals, in compliance with national regulations and the WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation (Chapter 1).

19.9. Acceptance criteria and exceptional release

The acceptance criteria should be based on validated protocols and reported in the TE's written procedures. The release criteria and specifications of skin/dermal allografts should be defined, validated, documented and approved. There should be a defined procedure for exceptional release of non-standard skin/dermal allografts under a planned non-conformity system. The decision to allow such release should be documented clearly, and traceability should be ensured.

19.10. Biovigilance

Adverse events and reactions as well as serious adverse events and reactions must be recorded, reported and investigated according to corresponding national regulations to the Health Authorities for tissues and cells.

19.10.1. Non-exhaustive list of reportable SARs

a. transmission of infective disease;
b. transmission of malignant disease;
c. allergic reaction (e.g. to antibiotics used for processing media);
d. engraftment failure/delayed engraftment (related to the tissue graft);
e. unexpected immunological reactions due to tissue;
f. bleeding (wound bed preparation);
g. aborted procedure involving unnecessary exposure to risk (e.g. wrong tissue supplied or delayed transport, discovered after patient is anaesthetised and the surgical procedure has begun [52]. See also www.notifylibrary.org.

19.10.2. Non-exhaustive list of reportable SAEs

1. Procurement:
   a. procurement without consent;
2. Processing and labelling:
   b. use of non-sterile/expired materials for tissue processing;
   c. mistaken processing media (errors in media preparation);
   d. incorrect labelling;
3. Storage:
   e. storage at inadequate temperature (e.g. in case of equipment failure; unattended alarm);
4. Transport/distribution:
   f. loss of irreplaceable autologous tissues;
   g. delayed transport of cryopreserved skin (resulting in tissue discard);
   h. incorrect tissue type, i.e. a different type of tissue is supplied than was intended or requested;
5. Testing:
   i. bacterial/viral/fungal contamination of tissues distributed for transplantation;
   j. loss of cell viability in cryopreserved viable tissue.

The Notify Library (www.notifylibrary.org) includes some well-documented cases of adverse occurrences in skin transplantation. Examples include:

- Contaminated skin graft that caused serious infection of a burn wound with *Acinetobacter* (Record Number 428).
- A case involving distribution of cryopreserved skin without review of the results of bacterial tests. Several allograft recovery cultures showed virulent pathogens ordinarily not accepted for use that prompted recall of >100 skin allografts, fortunately without any case of disease transmission (Record Number 128).
- Two cases describing incidents in which unsuitable skin grafts were released for clinical use. In one case, skin was torn upon thawing and implanting; in the second case, it was not measured appropriately, resulting in delay in patient treatment and graft loss in both cases (Record Numbers 126 and 127).

For further evaluated cases of adverse outcomes associated with skin banking, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

19.11. Developing applications

Wound healing is a major target in tissue-engineering research. In the past 40 years a large number of biological and synthetic skin/dermal substitutes have been produced. Products such as bioengineered skin equivalents and synthetic/biosynthetic materials integrated with cultured epidermal cells have been developed for this purpose, and most of them would be classified in the EU as advanced therapy medicinal products (ATMP) (Chapter 30). However, the ideal skin substitute has not yet been established and human skin allografts remain a gold standard in the therapy of major burns and skin loss treatment. Thus researchers in the field of tissue

engineering are still working on the production of an ideal artificial skin able to act as a physiological skin.

19.11.1. Epidermal cell suspensions

Epidermal cell suspensions (non-cultured autologous epidermal cellular grafting) have been used in the surgical management of vitiligo since 1992 when Gauthier and Surlève-Bazeille developed a non-cultured cellular grafting technique [59]. With this technique an epidermal cell suspension is used without cell expansion to treat larger areas (8- to 10-fold size of donor skin) on an outpatient basis with simple laboratory procedures. Epidermal cell suspensions can be useful in a variety of epidermal defects, involving both keratinocytes and melanocytes, and several approaches to delivering autologous keratinocytes/epidermal cells to restore epithelialisation have been developed [60, 61].

A recent technique based on aerosol spraying of non-cultured epidermal cells suspensions represents an efficacious and rapid way to obtain re-epithelialisation. To prepare the epidermal suspension, a cutaneous biopsy is trypsinised and epidermal cells are obtained by scraping of the dermal side of the epidermis. A suspension of autologous keratinocytes, melanocytes and fibroblasts can be delivered onto the wound bed by a spray apparatus. Therefore, epidermal cell sprays can be considered as aerosolised skin grafts that can potentially treat a variety of epidermal defects for burns and traumatic injuries, but also in scar reconstruction, donor-sites repair and in skin resurfacing techniques [60]. In consideration of the presence of melanocytes, this technique is also effective in pigmentation defects, including vitiligo and post-burn leukoderma (see §32.8).

Special commercial devices or prefabricated cellular preparation kits have also been developed to isolate and apply non-cultured epidermal cells, dermal cells or adipocytes to wounds in a one-step surgical procedure.

19.11.2. Epidermal 3D cell cultures

It was in 1975 that Rheinwald and Green [62] first described the serial cultivation of human keratinocytes in monolayer culture obtained from primary keratinocytes seeded onto lethally irradiated murine fibroblast feeder layers. Since then numerous advances have been made in the cultivation of human keratinocytes, in both two-dimensional monolayer and three-dimensional organotypic culture. Cultured epidermis was originally used to re-epithelialise severe burns, but, because of the presence of melanocytes, it was also used in vitiligo and other skin pigmentation disorders and to treat scars, ulcers and skin-graft donor sites.

Three-dimensional (3D) bioprinting, a flexible automated platform for the fabrication of complex living architectures, is a novel approach to the design and engineering of human organs and tissues [63]. A platform consists of eight independently controlled cell-dispensing channels that can precisely place cells, extracellular matrix (ECM), scaffold materials and growth factors in any user-defined 3D pattern. After the printing process, the skin tissue is cultured in media under submerged conditions to obtain a multi-layered cell and matrix structure in which human keratinocytes are grown on collagen matrices embedded with human fibroblasts.

All cell-culture methods are relevant in the field of tissue engineering and comply, when considered for clinical applications, with the ATMP regulations. Currently, the 3D bioprinter of skin is in the phase of being approved by different European regulatory authorities to guarantee that the skin that is produced is adequate for use in transplants on burn patients and those with other skin defects.

These tissues can be used to test pharmaceutical products, as well as cosmetics and consumer chemical products *in vitro* where current regulations require testing that does not use animals. See Part C for further information.

19.11.3. Skin composite grafts – nipple preservation

Practically any human tissue can be procured and banked for clinical use. Patients with loss of the nipple and areola from cancer, excision, trauma or congenital absence can undergo nipple-sparing mastectomy in specific cases after histological examination of the tissue surrounding the nipple and areola to eliminate the possibility of eventual cancer invasion. If a patient's nipple–areola complex (NAC) is available for grafting after mastectomy, it is the best material to use for reconstruction. It can be cryopreserved as a composite graft to be autografted for reconstruction of the breast after mastectomy. According to published literature and skin-bank protocols [64], a slow cooling procedure for cryopreservation is used by incubating the NAC in a cryoprotectant solution with 10 % DMSO.

The timing of transfer usually ranges from 6 months to 1 year after breast reconstruction. At the time of transfer, the cryopreserved NAC is thawed in 37 °C water and grafted on a projection made by a denuded dermal flap on the reconstructed breast.

19.12. References

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Related material

- Appendix 16. Evaluation of pigmented skin lesions

Chapter 20: **Cardiovascular tissue**

20.1. Introduction

Cardiovascular tissues can be procured from deceased donors (after brain death or after circulatory death) and living donors (e.g. heart valves from a patient undergoing a heart transplant).

The cardiovascular tissues most commonly procured are aortic valves, pulmonary valves and femoral arteries, but other cardiovascular tissues can be procured and processed, for example: ascending aorta, aortic arch, descending aorta, iliac arteries, aorto-iliac bifurcation, saphenous vein, vena cava with iliac veins, non-valvular pulmonary conduits, patches dissected from non-valvular pulmonary conduits and pericardium.

Heart valves are used mainly in paediatric cardiac surgery for treatment of congenital malformations, such as tetralogy of Fallot, valve atresia, bicuspid or monocuspid aortic valve, or transposition of great arteries. The most important reason for clinical use of heart-valve allografts in these indications is the identical morphology of the allografts and the native valve of the patient, which enables easy reconstruction of the left/right ventricular outflow tracts (RVOT/LVOT). Another advantage is that recipients, who are mainly children and neonates, do not need long-term anticoagulation because of the non-thrombogenicity of human tissue, thereby avoiding the side-effects of anticoagulation therapy in these patients. Another important indication is the Ross operation, which is often used for young female patients of child-bearing age and for athletes. This entails replacement of the diseased aortic valve with the autologous pulmonary valve (autograft) and reconstruction of the patient's RVOT with a pulmonary (or aortic) allograft.

The most common indication for the use of vascular (artery and vein) allografts is the infection of the prosthetic and/or native vascular tissue. Arteries may be used for peripheral re-vascularisation, vascular reconstruction in cases of malignant infiltration of the vascular wall (arterial, venous) [1] or reconstruction of the abdominal aorta. Iliac vessels are sometimes removed from deceased donors at the time of organ donation, and they can be used to support organ transplantation.

In light of long-term follow-up studies, it has been identified that implanted cardiovascular allografts are the subject of premature deterioration, perhaps because of immune-related deterioration [2]. Therefore, methods for the reduction of immunogenicity in cardiovascular allografts have been the subject of much research during the past two decades. This has involved the development of new procedures to decellularise cardiovascular allografts. This may also facilitate recellularisation of the graft with cells from the recipient *in vitro* before implantation or *in vivo* after implantation.

The following generic chapters (Part A) of this Guide all apply to cardiovascular tissue banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,

d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines the additional specific requirements for cardiovascular tissue.

20.2. Donor evaluation

20.2.1. Contraindications specific to cardiovascular tissue

The following exclusion criteria are specific to donation of cardiovascular tissue:

a. cardiac valvulopathy of the aortic and pulmonary valves, with moderate-to-severe stenosis or incompetence (the vessels can still be acceptable);
b. aortic dissection (detachment of the intima and adventitia);
c. direct (open) and massive traumas in the area of the body where the tissue is procured;
d. Marfan's syndrome and related diseases (e.g. Loeys–Dietz syndrome, Ehlers–Danlos syndrome);
e. bacterial or fungal endocarditis.

Other conditions to be evaluated as part of the donor-selection process are:

a. myocardial dilatation and valve alteration such as dilated roots, wall alteration of aorta or pulmonary trunk;
b. untreated pneumonia in previous days due to highly pathogenic bacteria or fungi, such as *Staphylococcus aureus*, pneumococcus or *Candida*.
c. Previous surgical interventions on the tissue to be procured.

Donor age limits vary between centres, with validations being performed to extend limits, based on the specific evaluation of the quality of the tissue. In general, the quality of cardiovascular tissue deteriorates with increasing age, and maximum age limits are a simple way to apply donor-selection criteria. However, it must be considered that other lifestyle factors, such as diet, lifestyle and history of smoking also impact on the quality of cardiovascular tissue, though these are more difficult to assess quantitatively. Where clinical demand for particular types or sizes of cardiovascular graft is not being met, tissue establishments (TEs) may choose to set higher age limits to increase the donor pool, in the knowledge that a significant proportion of the grafts donated may not be suitable for clinical application. Commonly applied age limits are shown in Table 20.1.

Table 20.1. **Commonly applied age limits for cardiovascular donors**

Arteries	male 17-45 years of age female 17-60 years of age
Aortic valves	32 weeks' gestation to 60 years of age
Pulmonary valves	32 weeks' gestation to 70 years of age

20.3. Procurement

20.3.1. Procurement team

The cardiovascular procurement teams should consist of at least two people. They should work under aseptic conditions, and be scrubbed, gowned in sterile clothing and wearing sterile gloves, face shields and protective masks.

20.3.2. *Post mortem* procurement time

It is recommended to procure cardiovascular tissue within 24 h after death, but only if the body has been cooled or refrigerated within 6 h of death. If the body was not refrigerated, then it is possible to procure tissue in the first 12 h after death. It may be possible to extend procurement times up to 48 h after death if subsequent processing has been validated to guarantee quality and microbiological safety; in these cases the blood samples for serological testing should be taken within 24 h after death (to avoid extensive haemolysis). See Chapter 5 for details of sample collection.

20.3.3. Procurement procedure

Procurement should be carried out in an environment that is as clean and controlled as possible,

ideally in the operating theatre, or in a suitable environment supported by risk assessment. For heart-valve procurement it is important to procure the ascending aorta and the pulmonary trunk with bifurcation (wherever possible) together with the heart. All efforts should be made to procure as much length of pulmonary artery distal to the pulmonary bifurcation as is practicable.

For vessel donors, the maximum possible length of the recovered vessel should be maintained, avoiding iatrogenic lesions during manipulation, and collateral branches should be cut 2-3 mm from the arterial wall to allow the surgeon to ligate or suture them during the surgical procedure to avoid unnecessary bleeding in the patient.

20.3.4. Tissue transportation to the tissue establishment

Common practice is to place procured tissues in a crystalloid transport solution (e.g. physiological saline, Ringer solution, Hanks balanced salt solution) with the possible addition of nutritional/osmotic elements (e.g. albumin) or antibiotic cocktail, and package them in at least two sterile packaging layers after procurement. The transport solution should also be buffered to ensure a physiological pH is maintained during transport.

For donors of organs, valves and vessels, it is convenient to package the heart and the vascular segments in different containers to avoid potential contamination transmission.

This package should then be placed in another container that ensures an ambient temperature of 2-8 °C during transport, and protects the recovered tissues.

20.3.5. Procurement documentation

The organisation responsible for procurement must produce a procurement report to be given to the TE. In addition to the generic requirements defined in Chapter 6, this report must contain a description and identification of the recovered material (heart, arteries, veins, valves, etc.).

20.4. Processing and storage

Procured cardiovascular tissues can be processed to facilitate longer storage periods and to reduce microbial contamination. To ensure tissue quality, it is essential that the time between cardiac arrest and cryopreservation be as short as possible. Time from procurement of the heart to dissection and disinfection should not exceed 24 h. The total ischaemia time (cardiac arrest to cryopreservation and storage) should not exceed 72 hours.

20.4.1. Cardiovascular tissue-processing methods

Processing of cardiovascular tissues includes dissection and evaluation of morphology and minimum functional requirements, incubation with antibiotics and, in some cases, anti-mycotics, cryopreservation and storage. The duration and temperature of antibiotic treatment and the composition of antibiotic cocktails should be defined by each TE, with prior evaluation of the initial tissue bioburden (i.e. before the tissue comes into contact with an antibiotic solution) and following a validation of the effectiveness of the cocktail against the most common microbes likely to contaminate the tissues. TEs should establish a clear policy stating how pre- and post-decontamination microbiology results will be used to determine whether the grafts are suitable for clinical use.

The methods used must be in accordance with current state-of-the-art and validated procedures (see Chapter 2). Different TEs apply specific preparation processes according to their own standard operating procedures (SOPs) and in accordance with relevant local authorisations.

As cardiovascular tissue is not visible for inspection once it has been cryopreserved it is recommended that the anatomical appearance, quality and other attributes noted during processing are documented to assist with the allocation of a suitable allograft for patient need. Appendix 27 provides an example of an evaluation form.

The annular diameter of valves and vessels should be measured using calibrated obturators. The length of the vessels should be recorded, as should the approximate position and size of any branching vessels. It is recommended that grafts be measured immediately prior to preservation, as measurements may alter following procurement [3].

20.4.2. Decellularisation of cardiovascular tissues

Heart valves and large vessels can be decellularised employing different methods to eliminate cellular components. Decellularisation protocols can employ physical methods (freezing, sonication), chemical methods (hyperosmotic solutions, ionic

detergents, non-ionic detergents) and enzymatic methods (trypsin, endonucleases). The most robust and effective decellularisation protocols include a combination of the three methods (see Appendix 32) [4, 5]. Quality control should guarantee maintenance of the structure and the biomechanical properties of native valves and vessels, as well as demonstrating *in vivo* function. It must be shown that residual quantities of any reagents used during the decellularisation process that are still present in the tissue do not provoke cytotoxic responses either *in vitro* or *in vivo*. Decellularisation protocols (especially enzymatic methods) should take into account that degraded collagen might have repercussions for *in vivo* re-endothelialisation of decellularised tissues.

20.4.3. Processing facilities

In selecting an appropriate air-quality specification for processing cardiovascular tissue, the criteria identified and explained in Chapter 7 should be considered alongside the factors outlined in Table 20.2.

It is vital that the processing of cardiovascular allografts takes place in a microbiologically and physically controlled environment with temperature control, ventilation and air filtration, and with validated cleaning and disinfection. Taking the factors from Table 20.2 into consideration, cardiovascular tissue should be processed in optimal environments with air quality equivalent to Grade A in EU Good Manufacturing Practice (GMP) Guidelines, with an adequate background environment. For EU countries, the background must be at least Grade D but, given the risks associated with the processing, testing and implantation of cardiovascular tissues, it is recommended that as a minimum, a Grade B or C background environment (EU GMP) be provided.

20.5. Cryopreservation and storage

Cardiovascular tissues can be cryopreserved by using a controlled-rate freezer and following a validated protocol. During the cryopreservation process, the parameters of the freezing cycle must be recorded, as well as any inconsistencies that might have occurred during the operation. After cryopreservation, the frozen tissues can be transferred to a temperature-monitored vessel (either a liquid nitrogen tank or mechanical freezer) and stored at < −140 °C. Cardiovascular tissue can be stored at < −140 °C for a storage period supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

20.6. Quality control

It is recommended that the quality-control tests on vascular grafts should consider the following minimum quality criteria to assess suitability for banking:

a. integrity of the vascular walls;
b. minimal presence of calcification, atheroma and fibrosis;
c. anatomical suitability – absence of aneurysm or stenosis.

Table 20.2. **Factors influencing the air-quality specification for processing of cardiovascular tissue**

Criterion	Cardiovascular tissue-specific
Risk of contamination of tissues or cells during processing	During processing, heart valves and vessels are exposed to the processing environment for extended periods during dissection, sizing and evaluation of their characteristics.
Use of antimicrobials during processing	Heart valves and vessels are exposed to antibiotics, and in some cases, antimycotics, with a typical decontamination period of 24 h. It is important to validate the effectiveness of the antibiotic cocktail and to list the micro-organisms that can be accepted pre-incubation as this method is not very effective compared to more robust methods that can be applied to other tissues [1].
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of a piece of myocardium or a discarded vessel for microbiological analysis does not ensure a representative sample for analysis. Storage media or solutions used to rinse the tissue should also be sampled to make this evaluation more effective.
Risk of transfer of contaminants at transplantation	Cardiovascular tissue is vascularised and can support and transmit microbiological contaminants, bacterial and viral agents. Cardiovascular tissue is used in open surgery in well-vascularised areas and frequently to replace infected tissue (endocarditis). If it is contaminated, the risk of serious infection is considerable.

Quality-control tests for heart valves should consider the following minimum quality criteria:

a. Functional competence. It should be noted that fenestrations within the margins of the lunulae are very often not a pathological finding. Provided the coaptation of the graft is ensured by adequate sizing, marginal fenestrations should not induce valve regurgitation either in the short or long term. Large fenestrations, particularly when they are in opposing cusps, should constitute a rejection criterion. Additionally, low-positioned fenestrations in the leaflets with moderate to severe leak should constitute a rejection criterion.

b. Good morphology (no fissures, no congenital defects, no/minimal calcification, or no other significant anatomical abnormality). Only small calcifications in the distal wall of the aorta or around the coronary ostia, where they are most likely not to interfere with graft functioning, can be accepted, although information on their size and location must be clearly reported to the clinical user.

c. Anatomical suitability (i.e. accurate length of conduit and diameter of annulus). Special attention should be paid to achieving an accurate measurement of the diameter of the annulus to avoid overstretching; this is particularly critical for the pulmonary valve.

d. Intact structure of the tissue matrix.

Cardiovascular allografts must be microbiologically sampled and cultured for aerobic and anaerobic bacteria, as well as fungi and yeasts, according to *European Pharmacopoeia* criteria (see Chapter 10), before antibiotic and, where relevant, antimycotic incubation.

Microbiological analyses should be carried out on:

a. the transport medium at the beginning of the processing procedure;

b. the sub-valvular (aortic and pulmonary) myocardial tissue and vessels before antibiotic incubation;

c. a final sample of each graft after antibiotic/antimycotic incubation and rinsing, and a sample of the cryoprotectant solution.

The result of the microbiological control must be negative. If a positive microbiology result is obtained, depending on the micro-organism found, a risk-assessment analysis should be done to assess the suitability of the other valve (and the rest of the tissues) obtained from the same donor. Table 20.3 lists some micro-organisms that, if detected in any culture of cardiovascular tissue (even if detected just before decontamination) require the tissue to be designated as unsuitable for clinical use. Hence, for example, detection of *Enterococcus* spp. in a pre-antibiotic sample of aortic myocardium with a negative result in all other samples (e.g. transport medium, post-decontamination aortic sample, before final packaging) should result in rejection of all valves from this donor, and a risk assessment should be done for the remainder of the tissues.

It should be noted that Table 20.3 is a suggested, non-exhaustive list, and individual TEs may have a different list of micro-organisms that result in tissue discard.

Table 20.3. **Contaminants that should result in tissue discard if detected at any stage of processing**

Aspergillus spp.
Candida spp.
Clostridium spp. (notably *C. perfringens* or *C. tetani)*
Enterococcus spp.
Flavobacterium meningiosepticum
Klebsiella rhinoscleromatis
Listeria monocytogenes
MRSA (methicillin-resistant *Staphylococcus aureus*)
Mucor spp.
Mycobacterium spp.
Neisseria gonorrhoeae
Nocardia spp.
Penicillium spp.
Pseudomonas aeruginosa or *P. pseudomallei*
Salmonella spp.
Shigella spp.
Streptococcus pyogenes (Group A)
Other yeasts and fungi

Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

20.7. Cardiovascular allograft distribution

Transportation of cardiovascular tissues can be carried out using dry-shipping containers (vapour phase nitrogen < –140 °C). This allows restorage of the tissues in the liquid or vapour phase of nitrogen without affecting the expiry date. If the tissue is to be stored at – 80 °C, for example following issue to an end user, expiry date must be reduced to a time period supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

If dry ice is used for transportation of the vascular allograft, the tissue should not be returned to liquid or vapour phase nitrogen tanks unless validated.

Transport temperatures above – 60 °C for cryopreserved cardiovascular tissues are to be strictly

avoided to ensure the stability of the product and maximum safety for the recipient. The receiving TE must ensure that all packaging and distribution processes have been carried out under controlled conditions [7].

20.8. Cardiovascular tissue thawing

Thawing, removal of the cryoprotective medium (dilution) and re-establishment of the isotonic state of the cardiovascular allograft are of critical importance in order to guarantee the integrity of the cryopreserved tissue. The record that accompanies the cryopreserved tissue must contain the detailed protocol to be used for thawing, dilution and tissue reconstitution, together with a comprehensive list of the materials required. Where cryopreserved grafts are thawed directly from vapour or liquid nitrogen, for example if they have been transported in a dry-shipper, care must be taken to ensure that rapid thawing does not cause thermal shock which can result in microcracks in the grafts. Once cardiovascular tissues have been thawed, they cannot be refrozen and should be implanted as soon as possible. A maximum period between thawing and transplantation should be defined, based on validation data or a documented rationale.

20.9. Examples of serious adverse reactions/events

The Notify Library includes some well-documented cases of adverse occurrences in the transplantation of cardiovascular tissue. Examples include:

- Donor-to-recipient transmission of hepatitis C virus (HCV) by transplantation of a saphenous vein after confirmation of transmission to a tendon recipient from the same donor. Imputability was confirmed by detection of identical HCV genotype 1a and phylogenetic nucleic acid arrangement (Record Number 564).
- Transmission of hepatitis B virus by aortic valve allograft resulting in asymptomatic seroconversion in the recipient (Record Number 424).
- Serious adverse events such as an incorrectly sized heart-valve package opened by mistake (Record Number 122) and the heart valve determined to be unusable due to excess tissue attachments (Record Number 123), both resulting in delay in treatment and graft loss [8].

For further evaluated cases of adverse outcomes associated with banking of cardiovascular tissue, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

Typical serious adverse reactions or events that may occur with cardiovascular grafts and that should be reported include:

- post-implantation infection ;
- any factors suggesting rapid degeneration/deterioration/failure of the graft, e.g. regurgitation with heart valves, or stenosis/claudication with vessels.

20.10. Developing technologies and applications

20.10.1. Veins

In addition to the long-established vascular allografts such as pulmonary valves, aortic valves and femoral arteries, over recent years there have been several publications on the use of veins, particularly saphenous veins [9, 10].

The suggested clinical indications for saphenous veins include:

- peripheral vascular disease,
- coronary artery bypass grafting (CABG),
- patients with infected fields or at risk of infection,
- arteriovenous access.

The benefits for saphenous veins are broadly similar to other vascular allografts:

- morphologically similar to the native tissue,
- resistance to infection,
- alternative to autologous veins.

The processing and storage protocols for saphenous veins are the same as those employed for other vascular allografts in that they have venous branches ligated, and they are antibiotic-treated and cryopreserved.

Other clinical indications for veins are currently being explored, as are other processing techniques such as decellularised veins (see Appendix 32).

20.11. References

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Related material

• Appendix 27. Sample forms for the evaluation of heart valves
• Appendix 32. Decellularisation

Chapter 21: **Musculoskeletal tissue**

21.1. Introduction

Human bone and soft-tissue grafts are being used increasingly in surgery as valuable materials to rebuild and replace musculoskeletal structures. Bone is the most commonly banked and transplanted tissue.

Fresh autologous bone is considered to be the 'gold standard' in bone-grafting material because it combines all the properties required in a bonegraft material: osteoinduction – i.e. bone morphogenetic proteins (BMP) and other growth factors – with osteogenesis (osteoprogenitor cells) and osteoconduction (scaffold). However, use of autografts is limited by the amount that can be procured and the risk of donor-site morbidity; so, in most cases, allografts are used.

Allografting of bone and musculoskeletal soft tissues can in most cases allow adequate and predictable restoration and functionality, including mechanical properties, analogous to the original healthy tissue at the recipient site. In addition, bone allografts offer the benefit of osteoconductive properties or even, depending on the processing applied, different degrees of osteoinduction through growth factors originally present and preserved in the graft [1, 2].

Musculoskeletal tissues can be procured from donors after brain death, donors after circulatory death and living donors (e.g. in the case of a patient undergoing hip or knee prosthesis surgery), and such tissues include bones, ligaments, tendons, cartilage and other soft tissues (e.g. fascia lata). The current indications for the transplantation of musculoskeletal tissues are, but are not limited to, tumour surgery, prosthesis replacement, filling where there is bone loss, fractures, malunion, bone fusion (spine and limbs), and ligament and meniscus replacement.

Allogeneic bone can be processed in different ways, depending on clinician needs and preferences. The processing methods include cutting or grinding into morcellised and cancellous chips, corticocancellous rings and wedges, and cortical grafts such as struts and cylinders. Bone grafts can be frozen or freeze-dried, and in some cases bone grafts are demineralised to enhance the osteoinductive properties; the result is demineralised bone matrix (DBM). Large osteochondral grafts and whole-bone segments are also provided, matched to the requirements of the recipient site [3].

This chapter defines the specific requirements for musculoskeletal tissue donation, donor evaluation, processing and preservation procedures that meet adequate quality and safety standards.

The future of musculoskeletal tissue banking is focused on the following areas:

a. developing new preservation methods to maintain the biological properties of the grafts;
b. developing new procedures such as decellularisation or specific cell seeding to improve graft incorporation in recipients;
c. improving the safety of grafts.

The following generic chapters (Part A) of this Guide all apply to musculoskeletal tissue banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,

b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines the additional specific requirements for musculoskeletal tissue.

21.2. Donor evaluation

General exclusion criteria are described in Chapter 4. Musculoskeletal tissue-specific criteria are described below.

21.2.1. Musculoskeletal tissue: specific exclusion criteria

In addition to the general exclusion criteria described in Chapter 4, screening of donors of musculoskeletal tissue should be conducted for:

a. diffuse connective-tissue disease;
b. metabolic bone diseases (severe osteoporosis, osteopetrosis, Paget disease, etc.);
c. corticoid treatment (medical director should evaluate donor suitability depending on corticoid dose and treatment duration);
d. evidence that the donor has ingested, or been otherwise exposed to toxic substances that could be transmitted in donated material in dosages that could endanger the health of recipients (e.g. cyanide or heavy metals such as mercury or gold);
e. local bacterial, viral, parasitic or mycotic infection;
f. radiation exposure at the location of the tissue to be donated (chest X-ray could be accepted);
g. evidence of trauma (e.g. open fracture) at the procurement site, or presence of joint deformities (evaluate the possibility of contraindication for osteochondral, structural bone and/or cartilage);
h. iatrogenic or degenerative tears or lesions detected during procurement of cartilage, menisci, tendons and osteoarticular grafts;
i. poor nutritional status, which may occur in, among others, donors with a history of alcoholism and can lead to reduced bone quality.

Donor age limits differ for different types of musculoskeletal tissue. These limits may be revised, based on performance of a validation study. Some countries have national guidelines or requirements but, in their absence, the following age limits, for male or female donors, are recommended:

a. for bone, the minimum age for both sexes is 6 years. No upper limit is applied unless the bone is intended to be used for structural support, in which case younger donors (age 15-55 years) are preferred;
b. for osteoarticular grafts, cartilage and menisci, the age range is 15-45 years;
c. for tendons and fascia lata, the age range is 15-65 years, although the upper limit can be extended after a biomechanical validation study.

21.3. Procurement

General principles of procurement are described in Chapter 6.

21.3.1. Procurement team in deceased donors

It is recommended that the musculoskeletal procurement team for deceased donation should be composed of at least two (but preferably three) people. The number of people involved in procurement should be determined in advance depending on the amount of donated tissues per procurement procedure. To minimise the risk of contamination during procurement it is recommended to limit the maximum number of team members [4]. Procurement team members should work under aseptic or clean conditions (clean conditions could be accepted if a validated sterilisation procedure is included in the manufacturing process) and, after hand disinfection, they should be gowned in sterile clothing and wearing sterile gloves, face shields, glasses and protective masks.

Staff must have the experience, education and training necessary to procure tissues, including significant anatomical knowledge to accurately obtain not only the regular tissues procured (femur, patellar

ligaments, etc.), but also specially requested materials (e.g. whole elbow).

It is important to define the functions of the individual members of the team for the different procurement processes (e.g. donor preparation, draping, procurement, microbiological sampling, packaging, reconstruction) and also to define the role of the team leader or person responsible for procurement.

21.3.2. Procurement procedures

The methods of tissue procurement may be similar to those used by orthopaedic surgeons in the operating room or may use wider skin incisions, applying strict aseptic techniques.

The steps for musculoskeletal procurement are:

a. Donor preparation: it includes washing, shaving and pre-operative disinfection of skin to reduce transient and resident microbial flora;

b. Donor draping: a local sterile field using disposable sterile drapes must be established before procurement to effectively reduce risk of microbial contamination;

c. Tissue procurement: all tissues must be procured using an aseptic technique. It is recommended that some rules are established to decrease the risk of cross-contamination, for instance:

i. Personnel located at one side of the donor should not change to the other side until they have finished procuring all tissues from their own side. Surgical instruments should not be shared between personnel;

ii. The packaging area should be independent and separate from procurement areas; but in an area with same environmental conditions;

iii. It is recommended that a fixed procurement sequence is established, from 'cleanest' (e.g. lower limbs) to 'dirtiest' areas (e.g. abdominal cavity);

iv. It is recommended to change gloves and surgical blade after procuring the tissues from one area (e.g. left leg).

d. Microbiological control: it is recommended to perform a microbiological control on each procured piece. Such controls can be avoided only when a validated sterilisation method is further applied during processing. Sampling methods should be consulted with a microbiological laboratory and defined in SOPs;

e. Tissue packaging: procured tissue must be inspected and identified appropriately before packaging and labelling to avoid mix-ups (see Chapter 14). Musculoskeletal tissue must be packaged in a manner that minimises contamination risk, using a validated packaging system, to assure its isolation from the external environment;

f. Donor reconstruction (see §21.3.3);

g. Procurement documentation (see §21.3.5).

The musculoskeletal tissues most frequently procured from deceased donors are:

- long bones (femur, tibia, fibula, humerus, radius, ulna, rib);
- irregular bones (iliac crest, hemipelvis, vertebrae, skull (note: CJD/vCJD risks), sternum, clavicle, scapula, mandible);
- soft tissues:
  - tendons: patellar, Achilles, anterior and posterior tibialis, peroneus longus, gracilis, semitendinosus;
  - cartilage: meniscus, acetabular labrum, costal cartilage;
  - fascia lata;
  - *dura mater* (note: CJD/vCJD risks).

Musculoskeletal tissues can also be procured from living donors:

- Allograft
  - Patients having a hip-replacement procedure can donate the femoral head that is being replaced by the prosthesis, and in some cases bone removed in knee replacement is also banked. This can be frozen or further processed and provided to other patients as a bone graft.
- Autograft
  - Cranial flaps removed during neurosurgical procedures where there is brain oedema. The tissue is stored and replaced in the same patient once brain swelling has diminished;
  - Cartilage can be used for producing autologous chondrocyte cultures for application in the same patient (see Chapter 32).

21.3.3. Reconstruction of the deceased donor's body

Once tissues have been procured from a deceased donor, the body must be reconstructed to maintain its original anatomical appearance.

For aesthetic reasons and with a view to a respectful reconstruction of the donor, a wooden or other replica (note: funeral requirements) bone approximating the size of the donated bone may be used to replace the procured bone. The subcutaneous

tissue and skin should be sutured. The use of sutures and other materials suitable for cremation should be considered.

21.3.4. Temporary storage and transportation to the tissue establishment

Once procured, if maintenance of cell viability is not crucial, musculoskeletal tissues should be kept at ≤ −15 °C until they are transported to the tissue establishment (TE). If transport occurs immediately after procurement, tissues must be refrigerated preferably not longer than 12 hours.

If tissues are obtained to be preserved unprocessed (e.g. osteochondral grafts) or during temporary storage before processing, they can be placed in a transport solution buffered at a physiological pH (e.g. Ringer's lactate solution, Hank's balanced salt solution) with the possible addition of nutritional/osmotic elements (e.g. albumin), antibiotic cocktail or culture medium, and packaged in at least two sterilised packaging layers after procurement. This package should then be placed in another container that ensures a temperature of 2-10 °C.

Temperature during temporary storage and transport as well as duration of temporary storage and transport should be validated for the related tissue to ensure protection of the procured tissues' properties.

Temporary storage must provide clearly separate and distinguishable areas for tissues and cells that remain in quarantine. To prevent mix-ups or cross-contamination, physically separate areas, storage devices or secured segregation within a storage device/unit (i.e. refrigerator, freezer) must be allocated and prominently labelled (including at least the minimum required information – see Chapter 14).

Temporary storage areas or units for tissues and cells must be monitored (and alarmed, if necessary) and checked to ensure expected environmental requirements are being met.

21.3.5. Procurement documentation

The organisation responsible for procurement must gather all relevant information associated with procurement procedures and produce a report to be given to the TE. In addition to the generic requirements defined in Chapter 6, this report must contain:

a. description and identification of the procured material (specifying all procured tissues);
b. any relevant morphological detail of procured tissues;
c. presence of lesions, including those produced during procurement;
d. non-procured standard tissues and its reason.

21.4. Processing methods

21.4.1. Processing facilities

In selecting an appropriate air-quality specification for musculoskeletal tissue processing, the criteria identified in Chapter 7 and Chapter 8 should be considered.

All stages of tissue processing should take place within a controlled environment. Although classified clean rooms are often not formally required for initial processing steps when validated sterilisation and virus-inactivation processes are applied subsequently, it is nevertheless necessary to control media quality (especially air and water) and to work with appropriately disinfected or sterilised equipment.

Table 21.1. **Factors influencing the air-quality specification for processing of musculoskeletal tissue**

Criterion	Musculoskeletal tissue-specific
Risk of contamination of tissues or cells during processing	During processing (including cutting, shaping, cleaning, grinding, etc.), musculoskeletal tissue is necessarily exposed to the processing environment for extended periods. Environmental conditions are not as critical during freeze-drying if the tissues are packaged in a validated closed system during the freeze-drying procedure.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling can be done by swabbing, immersion of a tissue biopsy in culture medium after processing or by filtering and culturing washing solutions. Different sampling methods can be combined in order to detect the possibility of contamination. For bone that is processed to small pieces or ground, representative samples can be taken for culturing. For sterilised bone, sampling is not an issue as the process is validated to achieve a certain inactivation level for micro-organisms.
Risk of transfer of contaminants at transplantation	Bone marrow, lipids and blood components placed inside grafts act as a reservoir of micro-organisms. Decontamination methods act by removing these components from musculoskeletal tissues to decrease the risk of transmission of viral and bacterial agents. Musculoskeletal tissue is used in open and well-vascularised surgeries, sometimes linked to replacement of a prosthesis, where a significant risk of infection exists.

For terminally sterilised grafts, at a minimum, the packaging step after cleaning and/or disinfection, but before sterilisation, should be conducted in a qualified clean room (see EU Good Manufacturing Practices classification). The official requirements vary between jurisdictions, but EU GMP Grade C is usually specified (WHO TRS 823, 1992).

For non-terminally sterilised grafts, the requirements for the processing environment depend upon whether the national authority mandates conformity with the EU GMP guidelines, or whether less stringent guidelines – e.g. the GTP (good tissue practice) guidelines – are applicable.

Within the European Union (EU), tissues that are exposed to the environment without a subsequent microbial inactivation process should be processed in environments with an air quality equivalent to Grade A as defined in EU Good Manufacturing Practice (GMP), with a background environment at least equivalent to Grade D (EU GMP). Many national requirements are more stringent, requiring Grade B (EU GMP) as a background, which may be more appropriate for the processing of bone and tendons that are not followed by a terminal sterilisation step. Bone that is destined for terminal sterilisation can be processed in a Grade C environment. Some factors which could influence the air quality for processing of musculoskeletal tissue are given in Table 21.1.

21.4.2. Cleansing (physical preparation/ defatting)

The methods of musculoskeletal tissue processing vary between individual TEs. Allogeneic and autologous bone allografts from living donors can be processed in the same manner as tissues from deceased donors.

The initial processing of bone and other musculoskeletal tissue generally involves mechanical steps that remove extraneous tissue. In the case of bone, residual muscle tissue and periosteum are resected, and cartilage may be debrided. Thereafter, initial pieces of musculoskeletal tissue are cut and possibly drilled and/or planed and/or shaped and subjected to additional physical or chemical downstream processing.

Residual bone marrow, lipids and blood components in and/or on tissue can have a negative effect on subsequent processing and final graft quality. Such residues may increase bioburden and/or have a negative effect on sterilisation processes and might be a cause of immunogenic reactions or delayed incorporation in graft recipients. They may also contain infectious agents derived from the donor. Such residual tissues should therefore be removed. This is generally achieved with washing processes that may combine physical and chemical components including debridement, purging (with water, saline or organic solvent solutions), ultrasound and treatment with supercritical carbon dioxide [5, 6]. High-concentrated alcohol or comparable solutions could improve the defatting process [7]. Where appropriate, delipidation processes should reliably reduce the intrinsic cellular bioburden to a level that can be eliminated/inactivated by subsequent processes [8-10].

All processes should be validated. The TE should determine what properties of the allograft are essential for safe and effective clinical application, select appropriate tests to evaluate these properties and design a validation plan accordingly. The TE may select to validate individual processes for individual graft types, or may choose a worst-case validation plan to cover all graft types. The latter should be based on the results of a risk assessment.

The types of graft that can be obtained include (but are not limited to):

- cancellous and corticocancellous chips or cubes, e.g. obtained from epiphyses of the long bones, vertebral bodies, or os ilium;
- cortical chips, e.g. obtained from diaphysis of long bone;
- bone blocks (cancellous, corticocancellous), e.g. obtained from epiphyses of the long bones or vertebral bodies;
- bone wedges, e.g. obtained from epiphyses, os ilium, calcaneus or talus;
- the whole bones;
- structural bone segments (whole or halved diaphyses, rings, struts or condyles);
- patellar or Achilles tendons with bone blocks;
- tendons without bone blocks;
- menisci, either whole (with or without bone blocks) or sections;
- fascia lata patches;
- costal cartilage segments.

Pooling of musculoskeletal tissue from multiple donors during processing is not recommended (see Chapter 8). However, in some countries pooling is permitted for some grafts (e.g. cancellous tissue). In such cases risk assessment is mandatory, taking into consideration the increased risks for the patient due to increased donor exposure and balancing benefits of the treatment.

Bone grafts should support bone healing. Appropriately processed bone grafts will provide 'osteoconduction' (i.e. they act as a scaffold and 'guide rail' for osteoclasts and osteoblasts) and therefore promote the incorporation of the graft and its remodelling

[11]. Donor bone may contain residues of functional bone growth factors. These so-called bone morphogenic proteins (BMP) are found in the organic part of the extracellular matrix and are covered by mineral, but can be exposed by bone demineralisation. This usually involves soaking bone in a hydrochloric acid solution (e.g. 0.5 or 0.6 M HCl) to significantly reduce the mineral content. The product of such a process is referred to as demineralised bone matrix (DBM).

Depending on other aspects of bone processing and the original BMP content, the exposition of BMP may promote 'osteoinduction'. This term is used when bone healing is initiated and maintained via stem-cell recruitment in environments lacking an adequate local population of osteoclasts and osteoblasts [12]. Due to its nature, demineralised bone matrix is often mixed with a carrier material to improve its handling properties and help retain the graft at the site of the transplantation/grafting/application. The safety of these carrier materials, and their effects on the essential properties of the graft, must be established.

Viable cells (e.g. chondrocytes) should be processed in a controlled and clean environment that eliminates risks of cross-contamination of tissue and culture media. This processing usually involves cell culture, and such transplants are therefore classified in the EU as advanced therapy medicinal products (ATMP) (see Chapter 30).

21.4.3. Removal of micro-organisms and virus inactivation

It is critical that the risk of transmission of microbiological agents is minimised for tissue transplants. A key factor in controlling this risk is the application of sterilisation and disinfection processes to the grafts, whether before, during or after any processing applied to the grafts. For a detailed discussion of the principles of sterilisation and disinfection, please see Chapter 8.

Sterilisation and disinfection protocols are, by their nature, aggressive processes that have the potential to damage the graft as well as to inactivate micro-organisms. It is vital therefore that, if they are to be applied, a protocol is selected that does not unacceptably impact the critical quality attributes of the graft. For example, sterilisation with gamma irradiation may damage the biomechanical properties of grafts, so should be employed with caution when applied to grafts which are expected to play a mechanical role, such as tendons or structural bone grafts. For osteochondral allografts, maintenance of donor-cell viability is crucial, so options for disinfection are limited to gentle surface decontamination.

It should also be considered that many of the routine processing techniques applied to musculoskeletal allografts, such as the use of elevated-temperature water washing (temperature range should be based on validated processes), physical removal of adherent tissues and bone marrow, and the use of washes with solvents, acids or oxidising agents can also reduce microbial bioburden.

21.5. Quality control

Quality-control tests on musculoskeletal grafts should take at least the following quality criteria into account:

a. morphology and integrity of the musculoskeletal grafts;
b. shape and size of the graft, especially for certain types of graft, for example meniscal cartilage, which require close size matching between the donor and recipient; the relevant measurements should be made using calibrated instruments when all physical processing has been completed;
c. residual moisture or available water in lyophilised or dehydrated grafts (the minimum and the maximum level to be defined according to validation studies);
d. osteo-inductive activity (*in vivo* or *in vitro*) in demineralised bone (usually demonstrated by validation rather than testing of every batch);
e. sterilisation indicators;
f. no evidence of microbiological growth;
g. number of viable cells in cell cultures (e.g. chondrocytes).

During procurement or before processing, microbiological samples should be collected to establish the initial contamination levels of tissues (bioburden) to assist in making a decision during quarantine regarding the release of procured material for further processing. The inactivation capacity of manufacturing processes (e.g. disinfection, sterilisation) should be taken into account.

Samples for microbiological testing should also be collected before or during packaging of the final product. Possible sampling techniques for microbiological testing include:

- swabs;
- destructive methods (e.g. biopsy or sacrificing a proportion of ground tissue);
- collection of the last portion of the fluid used for washing of the tissue graft for subsequent analysis, usually following filtration.

The result of the microbiological control after processing must be negative. If a positive microbiology result is obtained, the tissue should be discarded or (terminally) sterilised. Depending on the micro-organism found, a risk-assessment analysis should be done to assess the suitability of the other musculoskeletal tissues from the same donor.

Table 21.2 lists some micro-organisms that, if detected in any culture of musculoskeletal tissues (even if detected just before processing), require the tissue to be designated as equally unsuitable for clinical use or for processing. A risk assessment including the potency of any sterilisation processes employed, and the clinical relevance of the micro-organism, should be done to analyse the suitability of the rest of the musculoskeletal tissue from same donor [13].

Table 21.2. **Contaminants that should result in tissue discard if detected at any stage of processing or procurement**

Aspergillus spp.
Candida spp.
Clostridium spp. (notably *C. perfringens* or *C. tetani)*
Flavobacterium meningiosepticum
Klebsiella rhinoscleromatis
Listeria monocytogenes
MRSA (methicillin-resistant *Staphylococcus aureus*)
Mucor spp.
Mycobacterium spp.
Neisseria gonorrhoeae
Nocardia spp.
Pseudomonas aeruginosa or *P. Pseudomallei*
Salmonella spp.
Shigella spp.
Other yeasts and fungi

Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

See Chapter 10 for more detailed guidance on the principles of microbiological testing.

21.6. Labelling and packaging

Generic requirements are detailed in Chapter 14. Procured and processed musculoskeletal tissues are to be packaged in a way that minimises contamination risk. It is recommended that musculoskeletal tissues be at least double-packed in airtight packages or in sterile drapes as well as sterile containers. Each procured and processed tissue should be packed separately and labelled immediately.

21.7. Preservation/storage

After processing, grafts are stored at a TE during the quarantine period until the required test result from donor (e.g. blood cultures, serologies, autopsy and/or biopsy report) and tissues (e.g. microbiological test, biopsy report) are received (if required). The TE must confirm donor eligibility before releasing the graft.

Different preservation methods have been developed to maintain the biological properties of tissues for long periods of time, from processing to distribution for transplant.

21.7.1. Methods of preservation/storage

21.7.1.1. *Frozen and deep-frozen*

Preservation and storage of musculoskeletal tissues (including cancellous, corticocancellous and cortical bone, ligaments and tendons) by deep freezing without use of cryoprotectants is a common method. There is limited scientific evidence to justify particular temperature limits, but in general it is accepted that freezing an allograft has little impact on the mechanical properties of the tissue, and will diminish its immunogenicity. Uncontrolled freezing damages the viability of articular cartilage.

21.7.1.2. *Cryopreservation*

Cryopreservation is a process whereby tissues are preserved by cooling to temperatures of < −140 °C. This method is suitable for the preservation of some cell viability in cartilage. It is used for osteochondral bone grafts and for cartilage, although some centres also use it for other types of musculoskeletal tissue. Cryoprotectants – e.g. glycerol, dimethyl sulphoxide (DMSO) – are added to the medium to protect cells against freezing injury.

21.7.1.3. *Freeze-drying (lyophilisation)*

Lyophilisation consists in decreasing the water content of frozen tissue under vacuum through sublimation. For bone transplants, a residual moisture between 1 and 6 % is recommended. In contrast to fresh-frozen allografts, mechanical strength in freeze-dried allografts is reduced significantly, but still freeze-dried soft-tissue allograft constructs have many advantages, including limited immunogenicity, ease of graft storage, mechanical properties comparable to soft-tissue constructs, and the potential for improved biologic incorporation [14]. An alternative to freeze-drying is dehydration, where the water content should be < 15 %. Dehydration is usually performed using chemical substances.

21.7.1.4. *Fresh*

Storage of unprocessed tissues at hypothermic (2-8 °C) or near normothermic (≈ 33 °C) temperatures

allows maintenance of cell viability (i.e. osteochondral grafts) for a short period (1-3 months).

Different culture mediums and storage processes have been described. The main problem of fresh preservation is to have enough time to obtain test results before releasing the graft.

The TE should validate the storage method in order to guarantee a minimum rate (%) of cell viability.

21.7.2. Expiry dates

The designated shelf-life is dependent upon the packaging system (to guarantee the integrity and sterility of the graft) and the storage methods used (frozen, deep-frozen, freeze-dried, fresh, etc.).

Expiry dates should be established by the TE after a validation process. Each change in the packaging should be followed by a validation study of the packaging system and the expiry date.

21.7.3. Storage temperatures

As mentioned in section 21.7.1, different preservation methods require different storage temperatures (see Chapter 9), as shown in Table 21.3.

Table 21.3. **Storage temperatures for different preservation methods**

Type of graft	°C minimum	°C maximum
Frozen	– 40	– 15
Deep frozen	– 80	– 60
Cryopreserved	– 196	– 140
Freeze-dried*	+ 4	+ 30
Fresh (hypothermic)	+ 2	+ 8

* At room temperature (15-25 °C) in normal conditions of humidity.

Storage time limits will be defined by expiry dates (see §21.7.2) based on the packaging and storage system and the validity of donor-selection criteria.

21.8. Distribution and transport conditions

Transportation of musculoskeletal tissues should guarantee the preservation of graft-storage conditions from TE to end user.

Transportation systems will vary, depending on the preservation method used:

a. Frozen and deep-frozen grafts can be carried using a container with dry ice or qualified cooling systems. Once the graft has been thawed, it cannot be re-frozen;

b. Cryopreserved grafts can be carried using dry-shipping containers (vapour-phase nitrogen < –140 °C). If dry ice is used for transportation of the musculoskeletal allograft, the tissue should not be returned to liquid or vapour-phase nitrogen tanks unless validated or supported by a documented scientific rationale. Once the graft has been thawed, it cannot be re-frozen;

c. Freeze-dried grafts can be carried using a container just to protect the integrity of the package system.

d. Fresh grafts can be carried using a container that ensures the defined storage temperature.

21.9. Biovigilance

The Notify Library includes many well-documented cases of adverse occurrences in the field of musculoskeletal tissue transplantation, such as:

- Bone
  - A case of human T-cell lymphotrophic virus type-1 transmission by a deep-frozen bone allograft, resulting in asymptomatic seroconversion of the recipient, is described in Record Number 587;
  - A case of human immunodeficiency virus (HIV) transmission, through frozen femoral head used in scoliosis surgery, is documented in Record Number 19. Both donor and recipient developed acquired immunodeficiency syndrome (AIDS) 40 months after transplant;
  - Several cases of serious adverse events resulting from unsuitable bone allograft release are reported in the database. Record numbers 139, 140, 141 and 142 describe bone allografts with chondrosarcoma, lymphocytic lymphoma, Paget's disease and rheumatoid arthritis respectively; all were diagnosed during histological examination of the femoral head and resulted in discarding of allografts.
- Tendon or ligament
  - In Record Number 459, a donor-transmitted invasive group-A streptococcal infection, with the diagnosis confirmed by emm gene-sequence analysis of isolates from the blood and hemi-patellar tendon tissue of the donor and recipient;
  - A case of donor-to-recipient hepatitis C virus (HCV) transmission by patellar tendon trans-

plantation is described in Record Number 563 and confirmed by identical HCV genotype 1a and phylogenetic nucleic acid arrangement between donor and recipient;
- An HIV type-1 transmission from a seronegative organ-and-tissue donor confirmed by the recipient's seroconversion 3 weeks post-transplant (Record Number 581).

• Meniscus
- Records nos. 173 and 174 describe meniscus allografts with anatomic abnormality and fracture, respectively; both were discovered in the hospital and resulted in discarding of allografts, thereby delaying treatment.

For further evaluated cases of adverse outcomes associated with musculoskeletal tissue banking, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

21.10. Developing applications

In recent years, several innovative biological products based on decellularisation of musculoskeletal tissue (especially tendons) have been developed using biotechnological sciences, based on the experience of skin-derived and cardiovascular grafts (heart valves and vessels). More information about decellularisation processes can be found in Chapter 8 and Appendix 32.

21.11. References

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Related material
• Appendix 32. Decellularisation

Chapter 22: Haematopoietic progenitor cells from bone marrow and peripheral blood

22.1. Introduction

Haematopoietic progenitor cells (HPC) transplantation represents one of the most widely used forms of cell therapy, in part because haematopoiesis represents the best-known biological model of somatic stem cell and tissue differentiation. Following the first case reports more than sixty years ago, the procedure rapidly established itself as a life-saving treatment for adult and paediatric patients with a variety of malignant diseases. HPC transplantation also has a role when the haematopoietic tissue is functionally damaged by congenital or acquired disorders such as severe congenital immune deficiencies, metabolic diseases or bone marrow failure. More recently, the use of autologous HPC transplantation in combination with immuno-suppressive agents has been evaluated for patients with certain autoimmune diseases.

In its main field of application, i.e. as a component of the treatment of patients with poor-risk or advanced haematological malignancies, it is now well established that allogeneic HPC transplantation exerts its beneficial effects through the recognition of residual tumour cells in the recipient by donor-derived immune effectors (graft-*versus*-tumour effect, or GvT). Thus, allogeneic HPC transplantation represents a clinically useful, immune cellular therapy. Limits to the use of these therapeutic procedures are their intrinsic toxicity, dominated by (though not limited to) graft-*versus*-host disease (GvHD), an immune disorder in which donor-derived immune effectors recognise and harm the host's normal tissues such as skin, gut and liver, lungs and cornea.

The field has developed tremendously in the past half-century in developed countries, and now many emerging countries are establishing allogeneic and autologous HPC transplantation programmes. Work in the field has integrated medicinal and technical innovations, including the use of new immunosuppressive agents, the use of different sources of HPC, such as bone marrow, mobilised peripheral blood and cord blood, the procurement of cells from unrelated donors and much improved supportive care for patients. During recent years, the use of unrelated, volunteer donors has dramatically increased due to the extensive improvements in HLA-typing and matching algorithms and the growth of donor registries in the majority of European and North American countries, which are united in the World Marrow Donor Association (WMDA).

Several other biotechnological advances, including stem cell selection, lymphocyte depletion and immune effector cells activation, have entered clinical practice and made haplo-identical transplantation a valid clinical choice. However, despite these advances, procurement of HPC remains relatively unchanged. Hospitals that care for recipients often obtain autologous or allogeneic HPC from hospital-based or blood establishment-based procurement and processing facilities that are located in their immediate vicinity. Each of the procurement and processing facilities works on a typically small to medium scale. In more

than 50 % of allogeneic HPC transplantations, grafts from unrelated donors are used, which very often have to be imported from other countries or continents. Given the high rate of international exchange of donated HPC material, harmonisation of the practices in this field is of great benefit.

This chapter defines the additional specific requirements for procurement, processing, storage and transplantation of HPC derived from bone marrow – HPC, Marrow, known as HPC(M) – or from peripheral blood – HPC, Apheresis, known as HPC(A) – and the requirements for mononuclear cells (MNC) concentrates procured by apheresis – MNC, Apheresis, known as MNC(A) – either for immediate use or for further development of immunocompetent cells used after or instead of HPC transplantation.

The cells discussed in this chapter are regulated in the European Union (EU) under the Tissues and Cells Directive 2004/23/EC and its associated Commission directives. It should be noted, however, that if these cells are subjected to substantial manipulation (such as expansion or genetic modification), or are used in the recipient for an essential function that is different from the original function in the donor, in the EU they are then regulated as medicinal products. This means that their processing, storage, distribution and use in patients must respect the requirements of Regulation 1394/2007 on advanced therapy medicinal products ('the ATMP Regulation') as well as all other relevant provisions of the EU medicines rules.

The following generic chapters (Part A) of this Guide all apply to HPC transplantation and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

22.2. Recruitment of potential donors, identification and consent

Most of the patients who could benefit from HPC transplantation do not have a genotypically identical sibling donor. The chance of having a fully HLA-matched sibling donor is 25-30 % depending on the number of siblings. In some cases, an extended family search can provide an HLA phenotypically identical donor. This can happen in cases of consanguinity due to cultural or geographical reasons and can add an extra 10 % to the likelihood of finding a matched or partially matched family donor [1]. If no HLA-matched suitable donor is found in the patient's family, an alternative HPC graft can be considered. This would include search for an unrelated donor through bone marrow or umbilical cord blood registries, and extend the search to a mismatched unrelated source or an HLA-genotypically haplo-identical relative (e.g. parents, siblings). The most common donor-selection algorithm is described in the European Bone Marrow Transplantation (EBMT) handbook (see Figure 22.1) [2]. For haplo-identical transplantation using HPC(M) or HPC(A), promising protocols have been developed recently [3].

The relevance of HLA matching is dependent on a variety of factors, including but not limited to GvHD prophylaxis, transplant conditioning, graft manipulation and clinical donor characteristics such as age, sex and *Cytomegalovirus* serostatus. Biologically, mismatched HLA can be recognised by alloreactive donor T-cells, leading both to toxic GvHD and to beneficial graft-*versus*-leukaemia (GvL) as counterbalancing effects [4]. Moreover, missing self-HLA on patient cells can also lead to alloreactivity by natural killer (NK) cells, a phenomenon associated with GvL but not GvHD [5].

In unrelated stem-cell transplantation, it is generally accepted that the best donor is matched for at least 8/8 HLA-A, B, C, DRB1 alleles, with every mismatch leading to an approximate 10 % reduction in survival probability [6]. Mismatches at HLA-DQB1 and DPB1 are more controversial. For HLA-DPB1, the concept of permissive, clinically well-tolerated mismatches was pioneered on the basis of experimentally defined T-cell epitope groups (TCE). This led to the increasingly accepted notion that avoidance of non-permissive HLA-DPB1 TCE mismatches improves outcome and should be favoured when several 8/8 matched unrelated donors are available [7]. A mismatch at HLA-DQB1 seems to be unfavourable in the presence of other mismatches. In apparent contradiction to the dramatic effect of

subtle HLA mismatches in unrelated transplantation, transplantation across several mismatches or an entire HLA haplotype are possible in cord blood and haplo-identical family donor transplantation, respectively [8, 9]. This is probably due to graft composition in the former and GvHD prophylaxis in the latter, with higher proportion of regulatory and naïve T-cells in cord blood compared to adult stem-cell sources, and an important attenuating effect of agents such as post-transplant cyclophosphamide or high-dosage anti-thymocyte globulin on GvHD in the haplo-identical setting. The important question of whether and to what extent these specific characteristics will change the landscape also of unrelated stem-cell transplantation in the future will have to be answered in prospective clinical trials under way.

The first bone marrow donor registry was established in 1974 by the Anthony Nolan Bone Marrow charity in London. In 1988 the EBMT group with the Europdonor Foundation set up Bone Marrow Donors Worldwide (BMDW), based in Leiden, Netherlands. In 2017 the WMDA took over the activities of BMDW and the NetCord Foundation and now co-ordinates the collection and listing of the HLA phenotypes and other important data of volunteer HPC donors and cord blood units. The WMDA database includes almost 33 million HPC donors and almost 750 000 cord blood units (September 2018). Since ethnic minorities are under-represented in HPC donor registries, it is very important that donor centres work with these communities to explain the need to increase the ethnic diversity of the registry and to recruit new potential donors. Once the HLA-typing and personal data are entered onto the donor registry, further blood samples may be requested, leading to possible haematopoietic stem cell donation at any time in the future.

The WMDA, an organisation of representatives of HPC donor registries, cord blood banks, other organisations and individuals with an interest in HPC transplantation (www.wmda.info) plays an important role in the field of HPC transplantation from unrelated donors, providing a forum for discussion and guidelines on the more critical aspects of the procurement and clinical use of HPC. Moreover, the WMDA offers to the donor registries an accreditation programme according to internationally accepted standards.

The main role of the registries is to facilitate interactions between HPC transplant centres and donor centres. In most of the countries involved, the search processes – including HLA typing requests and donor selection – are operated using EMDIS (the European Marrow Donor Information System), an international computer network which allows fast and direct communication between registries (see Appendix 28).

Emergency rescue procedures should exist to limit consequences related to unforeseen unavailability of a donor (e.g. acute illness, accident, failed mobilisation). This could be: identifying a back-up donor, search for a cord blood unit, a haplo-identical donor or an autologous transplantation with previously cryopreserved autologous HPC.

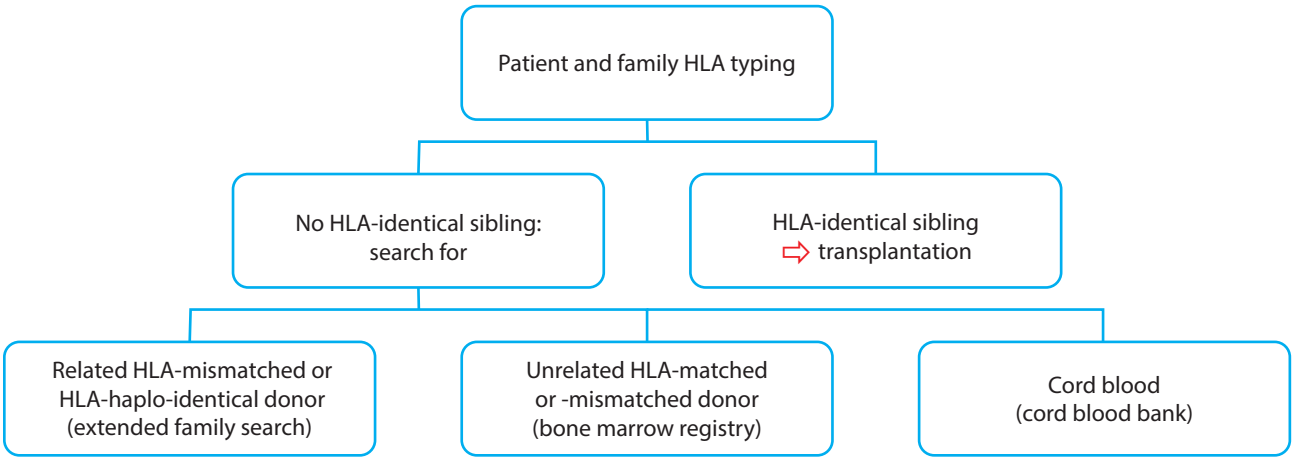
22.2.1. Donor evaluation

22.2.1.1. *Allogeneic donor*

One of the fundamental principles of volunteer stem cell donation is the right of the potential donor to proceed to donation with a minimum of extraneous influences and pressures. Protection of the donor's identity has to be guaranteed. Therefore, all the activities related to the donor's physical examination and collection of personal data must be performed in a dedicated and restricted area, as the access to all the donor information (medical and personal) must be protected and limited to authorised individuals [10]. Recommendations on assessing the medical suitability of adult stem cell donors are shown in Table 22.1.

For the evaluation of allogeneic donors, written criteria – in the form of standard operating procedures (SOPs) – should exist. Criteria must take into consideration not only the recipient's safety but also the donor's safety. Specific recommendations should be in place, especially for related donors who would not be eligible as unrelated donors due to age (e.g. young donors < 18 years, elderly donors > 60 years) or specific health issues [11-14]. The risk of donation should be evaluated and documented. To avoid a conflict of interest, the physician who evaluates the donor should be independent of the transplantation team. In any case, donor evaluation must be completed before the patient starts with the preparative regimen and, in cases involving HPC from the peripheral blood, before the donor receives the first dose of the mobilisation agent. To ensure the security of donor and recipient, a physician involved in the HPC(A) procurement procedure must be available during the procurement period, from the beginning of G-CSF (granulocyte-colony stimulating factor) injections to the post-procurement period.

Figure 22.1. **Donor selection algorithm**



Source: adapted from the EBMT Handbook [2]

Criteria for donors should include, in addition to general requirements, at least:

a. suitability for anaesthesia (for procurement of bone marrow);
b. assurance of adequate venous access;
c. additional test if appropriate (e.g. in cases of family history or elderly donor);
d. exclusion criteria (see Chapter 4);
e. policy for making decisions in cases of 'only one' donor but who does not meet eligibility criteria (e.g. only one suitable donor but with risky behaviour);
f. a donor advocate should be available to represent allogeneic related donors who are mentally incapacitated or not capable of full consent.

Donors with history of malignant disease (excluding haematological malignancies) after a minimum disease-free period of 5 years can be considered as suitable for sibling donation after careful assessment of the risk for malignant disease transmission [11].

It is up to the discretion of the transplant physician whether to accept donors with previous or existing infectious diseases (e.g. hepatitis B or others) if no other donor is available, based on careful risk evaluation. The specificity of allogeneic HPC transplantation lies in the fact that, for the vast majority of patients, the HPC graft is infused immediately after procurement. Thus, safety is reliant mostly on stringent evaluation of donors, which can be performed appropriately only if all needs are fully anticipated. In case of a risk with regard to patient or product safety, a formal acceptance of that risk should be signed by the transplant physician and the patient or their legal representative (urgent medical need).

The increasing age of recipients of allogeneic HPC transplants is related not only to the increasing age of the population but also to the introduction of less toxic conditioning regimens, allowing elderly patients and patients with comorbidities to undergo HPC transplantation. Even if the age limit to donate is well-defined for unrelated donors, and is over 18 and under 55-60 years for most international registries, these limits do not strictly apply for related donors. The decision process to collect from young (< 18 years) and elderly donors (> 60 years) must include an accurate risk assessment and appropriate informed consent of the donor stating that risk [11-13].

Decisions with regard to donor safety should be the responsibility of the independent donor physician. Some donors will present with comorbidities (discovered or not) during evaluation. If these comorbidities result in contraindication of the person for HPC donation, the physician who decided to contraindicate this person must ensure that a correct medical follow-up will be initiated in accordance with the medical condition of the rejected donor.

If the comorbidities found during evaluation allow HPC donation, the physician in charge must manage these comorbidities during the entire donation process, including specialist consulting as needed.

There should be a written plan to care for paediatric donors, donors with comorbidities and elderly donors during selection [11-14].

22.2.1.2. *Autologous donors*

For autologous donors, suitability criteria for HPC donation are less strict than for allogeneic donors. For evaluation, written criteria – i.e. SOPs – should exist. Criteria must take into consideration the patient's safety. Criteria should include threshold

values of the complete blood count before starting bone marrow procurement or leukapheresis. The risk of donation should be evaluated and documented. A donor advocate should be available to represent autologous donors who are mentally incapacitated or not capable of full consent at the time of HPC mobilisation and donation (e.g. for patients with primary CNS lymphoma).

Infectious disease markers in autologous donors should be tested as required by applicable laws and regulations (see Chapter 5). Autologous donors can donate even if results of the required tests are reactive or positive or other risk factors have been identified in patient's medical history as long as potential cross-contamination during HPC procurement, processing and storage can be prevented.

22.2.1.3. *Specific considerations for paediatric donors*

If minors are being considered as allogeneic HPC donors, in addition to the criteria shown in Chapter 3, national regulations should be followed. The use of haematopoietic growth factors and insertion of a central venous line are not recommended. Procurement methods for paediatric donors should employ appropriate adjustments for age and size to the procedure.

Children should become donors only in very specific circumstances, as family donors only and never through public registries (see Chapter 3).

22.2.2. Informed consent

Informed consent is required for an allogeneic and also for an autologous donation. In cases of autologous donation, the informed consent should include terms and conditions for the HPC storage and disposal. Volunteer HPC donors joining a donor registry express their commitment to donate, but they must nevertheless sign a formal consent before the HPC procurement. General considerations are shown in Chapter 3. Discard of allogeneic grafts from unrelated donors should comply with written agreements with donor registries.

Table 22.1. **Recommendations on assessing the medical suitability of adult stem cell donors [15]**

Assessment stage	Method	Topics to consider	Specific for this stage
Recruitment/ registration (unrelated)	History/questionnaire	Malignancy Autoimmune disease Cardiovascular disease (or a combination of risk factors therefor) Chronic disease (pulmonary/neurologic/haematologic/serious allergies) Relevant medical history (malignancy, thrombo-embolic disease etc.) Risks of infectious diseases (behaviour) Inherited/genetic disease	Look for permanent diseases or behaviour that have a clear donor risk or unacceptable recipient risk and that are relatively easy to assess
During selection stage (unrelated)	History/questionnaire Blood tests for infectious disease markers (HIV, hepatitis B, hepatitis C, HTLV, syphilis, *Cytomegalovirus*)	Update history of topics above, and also: Risks of infectious diseases – behaviour, (medical) invasive procedures, (planned) travel (Planned) medical procedures (including blood transfusion, dentist, vaccination, tattoo etc.) Serious psychosocial or psychiatric disease with impact on availability/ capacity to go through donation procedure Medication Non-prescription drug use Height and weight Blood pressure Pregnancy, pregnancy planning, breastfeeding Back problems, chronic pain	Identify contra-indications for one of the two collection methods Provide information about (possible) transmittable disease to the transplant centre Provide information to the transplant centre about any availability issues
Prior to HLA-typing (related)	History/questionnaire	As above	Identify contra-indications before concluding that the relative donor is the best match; may save time and disappointment

Assess-ment stage	Method	Topics to consider	Specific for this stage
During work-up/PE (related and unrelated)	History including full tract history; Complete physical examination; Laboratory tests: • Infectious disease markers: HIV-1,2 antibody, p24 antigen, HIV NAT, hepatitis B surface antigen and core antibody, hepatitis B NAT, hepatitis C antibody, hepatitis C NAT, validated serological testing algorithm for syphilis, on indication/per request transplant centre: HTLV I+II antibody, Chagas, Zika, Malaria, West Nile Virus, etc.; • Full blood count; ESR; if indicated: coagulation screen; blood film; haemoglobin electrophoresis; • ABO and Rh typing, screening for red blood cell and HLA-antibodies; • Biochemistry: Urea, creatinine, electrolytes, liver enzymes, LDH, ferritin; random glucose; β-HCG (for females of child-bearing age), protein electrophoresis; Chest X-ray; Electrocardiogram	As during selection stage, in addition any signs of undiagnosed disease.	Emerging infectious disease: check latest infectious disease epidemiology maps (CDC, ECDC)

22.3. Procurement

22.3.1. Haematopoietic progenitor cell graft sources

HPC(M) and HPC(A) are obtained from living donors only, either from the recipient patient (in the case of autologous transplantation) or from a fully or partly HLA-matched allogeneic related or unrelated donor. The choice of the donor is based on the best HLA matching, and other factors like age, gender, *Cytomegalovirus* status, ABO compatibility and NK alloreactivity.

For autologous purposes, nowadays HPC are obtained almost exclusively from peripheral blood stem cells HPC(A) and used to accelerate haematopoietic recovery after high doses of chemotherapy. In the allogeneic setting, the HPC graft source depends on the age and size of the donor and recipient – i.e. paediatric or adult donor, since some countries do not support G-CSF administration and apheresis in paediatric sibling donors – and the kind of disease (malignant or non-malignant), as well as the transplant protocol (myeloablative, reduced intensity, T-cell replete or deplete haplo-identical transplantation). HPC(M) are still the preferred source in allogeneic paediatric transplantation from compatible related or unrelated donors.

Indications for HLA-mismatch transplantation and selection of 'alternative' donors – in the sense of donors without a 10/10 HLA match with the recipient (considered as 'standard' donors) – are constantly implemented as they are evaluated in the context of biomedical research or registry studies. To date, prospective clinical studies comparing all sources of alternative HPC donors in different clinical settings are still ongoing. Hence, transplantation programmes should carefully follow their own local algorithms defining the 'best donor' for each patient according to the different situations, and use these algorithms to guide the donor choice.

Particular attention should be paid in HLA-mismatched donor selection (parents, brothers/sisters, adult children, or other family members). The criteria of choice should be explained in advance to the patient and potential family donors.

22.3.2. Procurement procedures

The majority of HPC are provided using two technologies: procurement of bone marrow and apheresis. The advantages and disadvantages of these technologies are shown in Table 22.2.

A risk tool (see also Chapter 6) should be used to evaluate the contamination risk factors during procurement, as shown in Table 22.3.

Table 22.2. **Advantages and disadvantages of methods of haematopoietic progenitor cells procurement**

Procurement method	Advantages	Disadvantages
Bone marrow procurement	Donor: • Single procurement; • Use of cytokines (mobilisation agents) not necessary Recipient: • Less chronic GvHD	Donor: • General or epidural anaesthesia • Invasive procedure • Considerable risk of morbidity (associated with anaesthesia, procurement method, mobilisation agents if used) • Potential tissue damage/infection at procurement site • Possible need for blood transfusion Recipient: • Slower engraftment of neutrophils and platelets • Potential graft contamination with skin contaminants • Possible contamination with tumour cells in autologous HPC procurements
Peripheral blood apheresis	Donor: • No anaesthesia Recipient: • Faster engraftment of neutrophils and platelets • Potentially less contamination of autologous product by tumour cells	Donor: • Procurement may take more than one day (i.e. several procedures may be needed) • May require placement of a central venous catheter for procurement (risk of haemorrhage, embolism, pneumothorax/haematothorax and infection) • Potential loss of platelets • Considerable risk of morbidity (associated with mobilisation agents and apheresis technique including anticoagulation) Recipient: • Increased risk of chronic GvHD

Source: EBMT [14].

Table 22.3. **Risk of contamination in the procurement of haematopoietic progenitor cells and mononuclear cells**

HPC Sources	Air quality of procurement area	Procurement system	Microbial spectrum	Risk of microbiological contamination
HPC, marrow	classified	open*	skin contaminants	low
HPC, apheresis	non-classified	closed†	skin contaminants (secondary contamination) venous catheter contaminants (patient)	very low
MNC, apheresis	non-classified	closed†	skin contaminants (secondary contamination) venous catheter contaminants (patient)	very low

* Open system: A procurement system that exposes the cells to the environment. The environment has to be controlled to minimise the risk of graft contamination (e.g. operating theatre).

† Closed system: A procurement system with equipment designed and operated such that the cells are not exposed to the environment. If closed systems are not operated accordingly (e.g. second puncture of a peripheral vein without appropriate disinfection or without clamping) the product may be exposed to the environment.

For HPC procurement and associated procedures – e.g. central venous (CV) line placement – written procedures must be established and reviewed regularly, with evidence of continued training of the staff. The International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration were developed by two organisations, JACIE (the Joint Accreditation Committee–ISCT & EBMT) and FACT (Foundation for the Accreditation of Cellular Therapy), and provide minimum guidelines for transplant, procurement and processing facilities. These standards can serve as guidance [16].

Severe adverse reactions can occur in donors with the administration of the mobilisation agents, and during and after allogeneic HPC donations. Hence, careful and documented training of clinicians and involved healthcare professionals caring for donors is needed, as is appropriate follow-up of donors. In all cases, safety of the donor is the major concern, meaning that the pre-donation work-up should be particularly meticulous [10, 11, 17-20].

22.3.2.1. *HPC from bone marrow*

Procurement of bone marrow is an aseptic process that should be undertaken in an operating

theatre by appropriately trained personnel. Special attention should be paid to the training of clinicians, to written criteria for required competence and experience in bone marrow procurement and aftercare of the donor, and to vigilance and surveillance of donors as well as of recipients. There must be provision for counselling of donors and for their routine post-donation follow-up.

Bone marrow for therapeutic use is obtained through multiple punctures, usually from the posterior iliac crests. When absolutely necessary, the anterior iliac crests can also be used. The sternum is not considered an appropriate method of bone marrow procurement. Punctures are usually undertaken under general anaesthesia. Epidural anaesthesia may be considered. A pre-anaesthesia visit of the donor is mandatory before procurement of bone marrow.

For procurement of bone marrow, written procedure(s) should be established, including at least:

a. disinfection technique;
b. preparation of media and materials used to obtain bone marrow;
c. puncture technique;
d. provision of containers for procured bone marrow;
e. monitoring of the volume of the procured bone marrow;
f. irrigation of procurement syringes;
g. bone marrow filtration;
h. quality controls (e.g. TNC counting, sterility, etc.);
i. labelling.

Bone marrow grafts contain bony spicules, fat and clots that should be filtered during procurement or processing. The bone marrow total nucleated cell number (TNC) is used to determine the adequacy of the procurement. The recipient's body weight and type of post-procurement manipulation determine the target TNC and volume of bone marrow to be procured. The procurement and anaesthesia times should not exceed 120 and 150 minutes, respectively. A maximum volume of 20 mL of bone marrow/kg donor weight should be procured. Procurement teams should not aspirate a volume more than 5 mL at each aspiration to avoid dilution of the bone marrow with blood [2]. The minimum target for autologous transplantation without graft manipulation is 2×10^8 TNC/kg recipient body weight. The target dose for most allogeneic transplantations is $2\text{-}3.5 \times 10^8$ TNC/kg recipient body weight. To increase TNC counts, mobilisation agents (e.g. rhG-CSF) can be used in autologous and allogeneic donors.

Adverse reactions related to HPC Marrow procurement are associated with anaesthesia, pain at aspiration sites, bruising and, rarely, local infection.

Blood cultures should be taken from bone marrow donors in presence of fever to investigate a possible microbial contamination of the procured HPC graft.

A 24-hour blood component donor-support protocol, including the provision of *Cytomegalovirus* antibody-negative (or equivalent), irradiated and leukocyte-depleted blood components, should be available. However, all efforts should be made to manage allogeneic donors in such a way as to minimise the need for blood transfusions. Autologous red blood cell (RBC) donation before bone marrow procurement can be considered, but potentially induces iron deficiency, because the time from final selection of donor to procurement can be short. Donation of autologous blood should be over a reasonably long period before procurement but ≥ 1-2 weeks [21]. Autologous blood must be taken in a blood-collection facility that meets applicable national/international requirements. When autologous RBC donation is considered, a protocol describing the transfusion trigger should be in place. Iron supplementation before and after bone marrow donation is recommended if possible, reducing the need for RBC transfusion.

Procurement procedures in paediatric donors should be adjusted according to donor age and size [16].

22.3.2.2. *Haematopoietic progenitor cells from peripheral blood*

In both autologous and allogeneic settings, HPC from peripheral blood should be procured in an apheresis facility by health professionals who have appropriate experience in care for patients with haematological or oncological diseases, HPC mobilisation and therapeutic apheresis. Special attention should be paid to paediatric patients and the specific circumstances pertaining to apheresis in young patients, whose weight (often < 20 kg) places them at risk of haemodynamic changes, both on commencement and during the procedure. Expertise to carry out apheresis is of particular importance for small children (< 20 kg) for autologous procurement (which is usually indicated in solid tumours or haematologic malignancies); the transplantation programme must maintain trained and experienced personnel for apheresis in paediatric units.

Before each apheresis procedure, the donor (autologous and allogeneic) should be evaluated. At least the following studies should be carried out:

a. complete blood count (including platelet count) ≤ 24 h before procurement;
b. vital signs and temperature;
c. blood cultures in presence of fever to investigate a possible microbial contamination of the procured product;
d. update of medical history.

22.3.2.2.1. Allogeneic donors

Mobilisation of HPC to peripheral blood before allogeneic procurement is ensured by recombinant human granulocyte-colony stimulating factor (rhG-CSF), which is administered to healthy adult donors in order to mobilise HPC from bone marrow into peripheral blood. The side-effects and risks associated with the procedures must be discussed with the donors. In particular, the donors must be informed about all aspects of rhG-CSF administration, including known short- and long-term effects, and given the opportunity to ask questions. Immediate and severe side-effects associated with rhG-CSF administration are rare (see Table 22.4), and raise the issue of inpatient *versus* outpatient administration. Donors who live far away from the transplant centre will require administration at home. However, because of a potential risk of allergic reaction, the first dose of rhG-CSF should be given under the supervision of trained healthcare professionals, and the donor should be followed up for at least 30-60 minutes. The transplantation programme, or the physicians in charge of mobilisation and procurement of HPC from the donor, should be informed in detail of the necessary measures to be taken in case severe adverse reactions (SARs) occur, especially for anaphylactic shock, spleen rupture, capillary leakage and acute hepatitis.

Routinely, HPC(A) procurement takes place on day 5 after 4 days of rhG-CSF administration. In cases of very low $CD34^+$ cell numbers, rescue strategies should be established (e.g. 'immediate' bone marrow procurement, administration of additional agents like HPC binding inhibitors).

Approximately 5-10 % of the donors may be asked to provide a subsequent donation of HPC or MNC concentrates to the same patient. The frequency of second donations seems to be higher for HPC(M) donors, and it may increase for the application of new therapeutic strategies. The interval between donations, for the same or for a different recipient, should be established by individual registries or transplant centres on the basis of the risks to the donor and the patient. However, these limits do not strictly apply to related donors.

22.3.2.2.2. Autologous donors

Cell mobilisation before autologous procurement is ensured by administration of various types of mobilisation regimens. RhG-CSF is the usual haematopoietic growth factor used to mobilise progenitors and can be administered alone or in combination with chemotherapy or other agents (e.g. HPC binding inhibitors such as Plerixafor, immunostimulants).

Circulating levels of $CD34^+$ cells guide commencement of apheresis. The number of cells required varies with the size of the patient and number of transplantations indicated (double grafting is indicated for some diagnoses). Procurement centres should have protocols that can determine the optimal number of cells to be procured, taking into account the patients' well-being during and after procurement, as well as their needs as future recipients.

In the selection process of the mobilisation agent, the World Marrow Donor Association document *Recommendation for recombinant human G-CSF (G-CSF) that stem cell donor registries can use – The use of biosimilar G-CSF* [22] should be consulted. The relevant mobilisation agent should be used in accordance with the latest approved Summary of Product Characteristics.

Table 22.4. **Very common adverse reactions associated with haematopoietic progenitor cells mobilisation (> 10 %)**

Agent	Adverse reaction
Rh-Granulocyte-colony stimulating factor (rhG-CSF)	bone pain musculoskeletal pain thrombocytopaenia hyperleukocytosis transitory elevation of levels of liver enzymes elevation of levels of lactate dehydrogenase headache asthenia
Haematopoietic progenitor cell binding inhibitors (Plerixafor)	diarrhoea nausea reaction at injection site

Source: EBMT [14].

22.3.2.2.3. Apheresis procurement yield

Processed blood volumes vary, depending on the procurement protocol and cell separator. Weight discrepancies between donor and recipient have to be considered as this can affect the $CD34^+$ cell yield. The target number of $CD34^+$ cells should be set before starting apheresis according to institutional protocols. The target will vary for autologous and allogeneic donations, and will depend on clinical need and regulations (as well as best available professional

practices). Target dose of $CD34^+$ cells for a single autologous transplantation ranges from a minimum of 2×10^6 $CD34^+$ cells/kg recipient weight to a more preferable 5×10^6 $CD34^+$ cells/kg recipient weight. The ability to achieve this goal is dependent on the underlying disease of the patient, therapy and mobilisation protocol.

The target for allogeneic donations is higher, because of the longer time to engraftment of neutrophils and platelets associated with allogeneic transplantation; HPC(A) doses above 4×10^6 $CD34^+$ cells/kg might be needed, especially when CD34 enrichment (a loss of $CD34^+$ cells is expected) or T-cell depletion methods are used. In addition to optimising HPC procurement, apheresis should ensure that procured cells have minimal contamination with neutrophils that could compromise subsequent processing steps or contribute to side-effects in recipients. HPC from apheresis contain small volumes of RBC (haematocrit < 5-10 %) so the risk of donation-related anaemia is very low [13]. Depending on the device used for procurement of HPC(A), the donor may experience a marked loss of platelets, in some cases resulting in post-donation values < 100 G/L. Under such circumstances a consecutive donation has to be carefully considered. Other risks related to the procurement method are given in Table 22.3.

The targeted cell dose could be reached in one or more apheresis procedures.

Some Health Authorities do not permit the use of G-CSF in paediatric donors and so bone marrow procurement might be employed.

22.3.2.3. *Mononuclear cells from peripheral blood*

To enhance immune responsiveness after HPC transplantation specific mononuclear cells are being used and/or evaluated in clinical trials. However, these immunocompetent cells are also used in patients who had not undergone an allogeneic or autologous HPC transplantation previously. As starting material, MNC concentrates are procured from an unstimulated donor. This donor can be a former HPC donor (i.e. donor lymphocytes infusion), a third party donor or the patient (autologous donor).

Donor lymphocytes infusions (DLI) can be administered to the selected patient after allogeneic HPC transplantation, either prophylactically to augment the anti-tumour immune response (following reduced-intensity conditioning protocols), in cases of mixed chimerism or of relapse of an underlying disease (mostly myeloid malignancies). The goal of this therapy is either to induce complete donor chimerism or a remission of the patient's malignancy by a process called graft-*versus*-tumour (GvT) effect.

The following cells are being used and/or evaluated in clinical trials (see also Chapter 32):

a. DLI to enhance immune surveillance against infections in patients with poor immune recovery experiencing relapsing/resistant viral infections;

b. T regulatory cells (Treg) for the prevention and control of GvHD;

Table 22.5. **Factors influencing the air-quality specification for processing haematopoietic progenitor cells**

Criterion	Haematopoietic progenitor cell-specific
Risk of contamination of tissues or cells during processing	Cryopreservation or selection of certain subpopulation of HPC is mostly done in closed systems. Processes that are closed need a less stringent specification than those that involve hours of open processing. The sterile barrier can be compromised in a moment after adding cryoprotectant, monoclonal antibodies or other solutions by sterile spikes.
Use of antimicrobials during processing	Use of antimicrobial agents during HPC processing is not applicable. Nevertheless, in some cases, even HPC contaminated by bacteria can be used (unique matching, life-saving treatment). In such cases, the recipient is protected with antimicrobial agents.
Risk that contaminants will not be detected in the final tissues or cells due to limitations of the sampling method	Obtaining adequate volumes of the sample is the main obstacle in final microbiological control, especially in CD34 selected grafts. Samples can be taken from the cells or residual components after processing, depending on the product volume. Procedures for microbiological detection should be validated for inadequate quantities of sample.
Risk of transfer of contaminants at transplantation	Nature of transplant (blood cells), minimal processing and the fact that there are no applicable decontamination procedures make these cells high-risk for transfer of contaminants at transplantation. However, application of the transplant is by infusion, during which the transplant is not exposed to the environment. Hence, the risk of contamination during transplantation is minimised. A possible risk is the thawing procedure. Thawing in a water-bath bears a risk of contamination.

c. natural killer (NK) cells as GvT effectors by alloreactivity of killer Ig-like receptors (KIRs) in donor–recipient direction;
d. viral- and fungal-specific T-cells for the treatment of several infections (e.g. *Cytomegalovirus*, Epstein–Barr virus, *Adenovirus*, *Aspergillus*);
e. vaccination with peptide-loaded dendritic cells (DC) for induction of tumour-specific T-cell responses for treatment of metastatic disease transplantations, or for treating GvHD;
f. mesenchymal stem cells to enhance engraftment in allogeneic and autologous HPC transplantations, or in treatment of GvHD;
g. autologous or allogeneic chimeric antigen-receptor (CAR) T-cells.

22.3.3. Temporary storage and transportation to the tissue establishment

Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, HPC(A) and HPC(M) should be stored in non-frozen conditions before processing and infusion or during transportation. Survival of HPC stored in a non-frozen state is dependent on the concentration of leukocytes (TNC), buffering capacity of the HPCs and anti-coagulant in the graft, product volume and storage temperature. Cell viability decreases and the risk of bacterial growth increases during storage at room temperature as well as in refrigerators (see Chapter 9, also Table 7.2). Therefore, maximum storage in the non-frozen state should be ≤ 72 h. In cases where HPCs have to be cryopreserved, this should be done as early as possible (i.e. within 48 h after procurement) to avoid cell loss and reduced viability during processing. The facility should undertake a validation study of the storage and transport conditions.

The same applies to MNC(A) concentrates procured for DLI.

22.4. Processing of haematopoietic progenitor cells

Processing of minimally manipulated HPC is intended to provide appropriate conditions for preservation and storage or to improve the risk–benefit ratio of autologous or allogeneic HPC transplantation [2, 16]. It does not affect the main biological property of the procured cells, which is to support the marrow re-populating ability (MRA) and the establishment of haematopoietic chimerism in a myelo-ablated or immuno-suppressed recipient in allogeneic transplant.

Generic requirements for processing facilities, together with standards, are described in Chapter 7 and Chapter 8.

The specificities of processing HPC are shown in Table 22.5 (see also Chapter 7, Table 7.2 on the risks of airborne contamination). While HPCs are exposed to the environment, processing should be performed in a laminar-flow cabinet of GMP Grade A with background environment to at least equivalent to GMP Grade D as required by the Directive 2006/86/EC. Considering the factors detailed in Table 22.5, it is appropriate that HPC processing takes place in a microbiologically and climate-controlled environment (control of temperature, ventilation, air filtration) with validated cleaning and disinfection. The same requirements apply for autologous or allogeneic donations.

For all processing steps, written procedures must be established and reviewed on a regular basis with evidence of continued training of the staff. The current FACT–JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration also apply to processing facilities.

22.4.1. Volume reduction

Volume reduction is either a preparatory step to further processing (including cryopreservation and storage) or a means to reduce the volume of the infused cells and, thus, prevent recipient side-effects relating to volume overload in the transplanted patient. Various centrifugation-based techniques can be used that are validated at the site. Cell loss associated with volume reduction must be evaluated and expected recoveries defined.

22.4.2. Red blood cell depletion

Red blood cell depletion is a critical step in cases where there is major ABO incompatibility between a donor and a recipient in the allogeneic setting (HLA identity does not preclude the existence of major or minor ABO incompatibility). Red blood cell depletion is almost exclusively performed if bone marrow is used as the HPC graft source, whereas HPC(A) are usually not red blood cell depleted. Various techniques for blood cell depletion are available, including buffy-coat centrifugation or apheresis cell separation. The efficiency of the technique must be monitored by measuring the residual content of red cells, which should be as low as possible. Similarly, the cell loss associated with such procedures must be

evaluated and the expected recoveries and amount of acceptable residual red blood cells must be defined.

22.4.3. Plasma removal

Plasma removal represents a critical step in cases with minor ABO incompatibility between a donor and a recipient in the allogeneic setting (HLA identity does not preclude the existence of major or minor ABO incompatibility). The necessity of plasma removal in case of minor ABO incompatibility can be judged using a titration of anti-A and anti-B antibodies in the donor blood during the period that precedes the donation. In minor ABO incompatibility, where anti-recipient-RBC antibodies are 1:256 or greater, plasma should be removed, especially from bone marrow grafts [2]. Plasma removal is usually done by centrifugation of the procured cells. The cell loss associated with such procedures must be evaluated and expected recoveries must be defined.

22.4.4. Cryopreservation, thawing and infusion

22.4.4.1. *Cryopreservation of haematopoietic progenitor cells*

The purpose of cryopreservation is to preserve HPC in such a way as to ensure their viability and potency. To minimise the volume infused, peripheral blood and bone marrow may be concentrated before cryopreservation. The volume of cell suspension per bag should be adjusted by the body weight of the patient. A maximal daily dose of dimethyl sulphoxide (DMSO) of 1 g/kg body weight, which is equivalent to an infusion of 10 mL/kg of cells in 10 % DMSO solution, should not be exceeded. Special attention should be paid if the recipient is a small child and if a patient has impaired renal or liver function.

Cryopreservation is used systematically in the autologous setting. In the allogeneic setting, cell procurement from the donor is usually synchronised with administration of a conditioning regimen to the recipient and direct infusion of the HPC product (within ≤ 48-72 h after procurement) without freezing. However, sometimes allogeneic HPC are being cryopreserved for logistical reasons, such as unavailability of the donor at the scheduled date of transplantation (procurement in advance), professional constraints, unforeseen changes in transplantation schedules or over-collection of stem cells. HPC should be cryopreserved as soon as possible. Shelf life of HPC without cryopreservation is acceptable up to 72 h. However, cell viability decreases if cells are frozen at the end of their shelf life.

The cryopreservation method for HPC(A) or HPC(M), once RBC and plasma is depleted from the latter, is the same. The method involves addition of 5-10 % DMSO to a suspension of HPC and protein-rich medium, with or without dextran or hydroxyethyl starch (HES). Immediately after DMSO addition, HPC are cooled at −1 °C to −2 °C per minute. For most therapeutic cells, the cooling rate is controlled by a controlled-rate freezer in which vapour-phase liquid nitrogen is pumped into the freezing chamber facilitating a sudden temporary drop of the temperature in order to compensate for the thermal release caused by the solidification of the suspension. Although this is the recommended method for cryopreservation of therapeutic cells, other methods may be used (e.g. freezing in a mechanical freezer) as long as they result in acceptable post-thaw viability and potency. The final phase of cooling in a controlled-rate freezer is usually quicker, with the temperature drop adjusted to 5 °C/min. When the mixture has reached approximately −100 °C to −120 °C, it is transferred to a storage container. Methods to minimise the risk of contamination or cross-contamination must be in place (e.g. secondary bag, liquid nitrogen vapour phase). Temperature fluctuations may result in a loss of viability. The validation of cryopreservation procedure must include evidence that the storage temperature is adequate to preserve the grafts.

Once frozen, HPC should be stored in vapour-phase liquid nitrogen or in liquid nitrogen at < −140 °C. Variations in cryopreservation methods include the concentration of frozen cells, the amount and source of plasma protein and the cooling rate. The method chosen must be validated.

Maximal shelf life has not been defined for cryopreserved HPC. HPC(M) or HPC(A) have been transplanted successfully even 11 years after cryopreservation. A stability programme for cryopreserved grafts should be implemented in order to evaluate viability and potency at different storage durations.

22.4.4.2. *Cryopreservation of mononuclear cells*

Processing of MNC(A) mainly involves adjustment of volume and cell number according to the clinical protocol used. The number of MNC and specifically the number of $CD3^+$ T-cells is determined by flow cytometry analysis, and further characterisation of T-cell subpopulations may be undertaken according to special requirements and needs.

For DLI, frequently a T-cell dose of 1×10^6 $CD3^+$/kg body weight of the recipient is the starting dose, and then further treatments with escalating doses may be used. The tissue establishment (TE) should validate the freezing technique in order to establish

the expected level of viable T-cells after thawing. Some protocols also include cell-selected preparation.

22.4.4.3. *Thawing and infusion*

HPC(M) and HPC(A) can be thawed at the bedside or in a processing facility. Several studies have demonstrated that the occurrence of adverse reactions during HPC infusion is related to the amount of DMSO and/or cell debris in the product, and some centres remove DMSO after thawing prior to infusion. This procedure is performed in the processing facility by manual centrifugation or by automated washing in closed systems using specific equipment. Despite the progress that has been achieved in the development of new washing methods, such as membrane filtration and new devices, automated washing is still performed only in few transplant centres because of the associated risks: cell clumping, cell loss, osmotic injury, contamination and the high cost.

Hence, washing of HPC(A) and HPC(M) must be reserved only for patients at a high risk of adverse reactions. Good practice recommends (if possible) not exposing all cells to the risk of washing procedures at once unless there is a validation that demonstrates the maintenance of morphological and functional characteristics of the cells.

HPC should be infused immediately after thawing and as fast as possible, at approximately 5-20 mL/min using standard transfusion sets, although this interval may be longer if the HPC are washed. Leukoreduction filters must not be used.

22.4.5. Cell-selected preparations

Specific Conformité Européenne (CE)-marked devices are available to select $CD34^+$ cells from bone marrow or peripheral blood on the large scales needed for clinical transplantation. The use of such medical devices requires adequate training for personnel involved in these procedures. There must be written criteria – i.e. SOPs – for cell-selection preparations and the criteria must be reviewed on a regular basis with evidence of continued training of the staff. The method chosen must be validated and ensure a sufficient purity and viability for a safe transplant and sustained engraftment.

22.4.5.1. *T-cell depletion and depletion of alloreactive immune effectors*

T-cell depletion is associated with positive (i.e. GvHD prevention) and negative (i.e. prolonged immuno-suppression) consequences that prevents its adoption in routine clinical practice, and it is rarely used in HLA-identical or HLA-matched transplantation. This is because the advantages of reducing GvHD are offset by associated increases in relapse rates and graft failures. Indications for T-cell depletion depend on the clinical protocol, such as use of haplotype-mismatch donors and transplantation for non-malignant diseases. In these situations, it is important that T-cell depletion is as extensive as possible.

Accurate determination of the residual T- and B-cell content is mandatory. The highest acceptable dose of residual T- and B-cells must be defined in advance by the medical team in charge of the recipient. CD34-positive immunoselection can also be considered a T-cell depletion method because, as in standard CD3 depletion, almost all T-cells are eliminated, including the T-cell receptor (TCR) gamma/delta-positive T-cells not involved in GvHD and exerting anti-leukaemic activity, as demonstrated by several authors. A specific depletion of TCR alpha/beta-positive cells spares the gamma/delta T-cells and is more beneficial over the depletion of all T-cell subsets. Combining this with a $CD19^+$ B-cell depletion for preventing transmission of Epstein–Barr virus (EBV) is a very encouraging strategy, especially in haplo-identical transplantation settings [24].

Other specific procedures evaluated by clinical trials include depletion of activated and alloreactive T-cells (i.e. those that can be identified by the expression of the CD25 T-cell receptor subunit). Removal of CD25 T-cells can be done using immuno-selection devices similar to those routinely used for $CD34^+$ positive cell selection or T-cell depletion. Accurate determination of the residual T- and B-cell content is critical. The highest acceptable dose of residual alloreactive T-cells must be defined in advance by the medical team in charge of the recipient and their guidance sought by the procurement team if this objective cannot be met.

22.4.5.2. *Tumour cell depletion in the autologous setting*

Autologous tumour cells procured with normal HPC may contribute to post-transplant relapse, but this has not been firmly established on the basis of clinical and biological observations. A definitive advantage for tumour-purging of autologous grafts has not been demonstrated by clinical trials. The use of $CD34^+$ cell-selection devices for this purpose is only applicable in a few clinical protocols (e.g. neuroblastoma) but, if a transplant team decides to use such a procedure, then detection of residual tumour cells should be as accurate as possible, using either immuno-histochemical techniques or flow cytometry analysis, or molecular biology techniques.

For all processing steps, written procedures must be established and reviewed on a regular basis with evidence of continued training of the staff. The current FACT–JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration also apply to processing facilities.

22.5. Quality control

22.5.1. Biological information needed to confirm donor suitability and recruitment

All clinical and biological information pertaining to donor identification, screening and recruitment must be kept, along with all information pertaining to processing and distribution. This information must remain as a permanent part of the preparation and release file.

Details on the nature of such information and the procedure to obtain it are provided in Chapters 3 and 4 of this Guide.

22.5.2. Safety controls

Detection of transmissible infections is undertaken through donor screening (using microbiological and other testing, as required by national, European and international guidance and regulations) and through microbiological testing of samples obtained at the different stages of cell procurement, processing and distribution (see also Chapter 5, 8 and 10).

Detection of donor-transmissible diseases other than occult pre-neoplastic or neoplastic diseases or other disorders is through donor screening, using medical questionnaires, physical examination and biological testing, as necessary.

The proportion of the various subpopulations of leukocytes in the procured cell preparations must be measured. High numbers of mature cells such as granulocytes or contamination with red cells may negatively affect several subsequent processing steps and may contribute to recipient side-effects at re-infusion.

Removal of red blood cells through specific processing procedures must be documented, as must the removal of T-cells or other immune effectors.

The removal of tumour cells from autologous cell preparations using specific processing procedures must also be documented where applicable.

22.5.3. Immunophenotyping and colony-forming unit assay

The number of total nucleated cells (TNC) in combination with the number of viable $CD34^+$ cells is a widely used measure for evaluating the quality of procured bone marrow. The cell dose for recipients is usually expressed in TNC and $CD34^+$/kg of recipient weight. In addition, nucleated cell counts are largely used as in-process controls to document that technical procedures have been appropriately conducted in the processing facilities (i.e. procurement of TNC following plasma removal, volume reduction, red blood cell depletion, etc.).

$CD34^+$ cell counts are used as a marker for HPC, both in the peripheral blood of individuals undergoing mobilisation regimes and in the procured cells, whether from apheresis following mobilisation or from bone marrow donation. $CD34^+$ cell counts are usually measured by flow cytometry, using monoclonal antibodies that recognise one or several epitopes on the human CD34 membrane antigen. Use of a single platform, rather than a dual platform, minimises errors in calculating cell counts. The International Society for Hemotherapy and Graft Engineering (International Society for Cellular Therapy) algorithm provides a robust and reproducible gating strategy to measure $CD34^+$ cells [25, 26].

Evaluation of $CD34^+$ cell recovery and total viability after storage and cryopreservation are an acceptable way to measure the potency of an HPC graft when the detection of colony-forming units (CFU) in clonogenic assays is not feasible. These functional tests are hampered by the delay required to produce results (usually two weeks); thus, the results are usually only available long after a non-cryopreserved cell preparation has been transplanted in an allogeneic recipient. Clonogenic assays are also hampered by poor intra- and inter-laboratory reproducibility. This particular issue could be improved by using commercially available and standardised culture media and by participation in proficiency testing and external quality-assessment schemes. The frequency of $CD34^+$ cells that form colonies differs among the sources of HPC and is higher in HPC(A) than in HPC(M). A clonogenic assay can provide additional information about the functionality of the graft; in particular, it is recommended after a long storage period. It can be used as a qualitative potency test (e.g. growth or no growth) or as a quantitative potency test. In both cases, a policy should be defined to deal with grafts where $CD34^+$ cells clone at a low frequency.

Colonies are enumerated and classified on the basis of their morphologic characteristics: CFU-GM (granulocytes and macrophages), CFU-

GEMM (granulocyte-, erythrocyte-, macrophage-, megakaryocyte) and BFU-E (burst-forming units – erythroblast).

22.5.4. Release criteria

The cell-processing facility, along with its clinical counterparts, must define which safety and quality controls serve as release criteria. It must also define which criteria must be strictly met and which ones may lead to documented waivers. Specific instructions should be established in the TE on how to deal with the recipient, donor and stem cells throughout the donation, through the processing and issue stages and all the way through to transplantation. Acceptance and release criteria may differ between autologous and allogeneic grafts. In autologous grafts, infectious disease-marker (IDM) test results may be positive (this is normally not the case in allogeneic grafts). In allogeneic grafts, which are mainly used directly after procurement, microbiological test results are pending at the time of administration and cannot serve as release criteria (in contrast to autologous grafts).

Processing and transplant facilities should agree on the cell dose (nucleated cell count, mononuclear cell count, $CD34^+$ cell count and/or clonogenic assays as appropriate for the source of HPC) required to achieve reliable and sustainable engraftment.

If cells are required for administration to a patient, a prescription for infusion is required. This prescription should list the type of cell preparation that is suitable for that patient and provide specific information on dosing. If necessary, the cells may be manipulated before infusion (e.g. washing, dilution) and this should be recorded on a worksheet and on the activity report.

22.5.5. Quality control for mononuclear cells

In addition to the safety controls (listed in 22.5.2) and definition of release criteria (22.5.4), the specific requirements include establishing the absolute number and the frequency of T-cells ($CD3^+$ and/or subpopulations) and cellular viability by flow cytometry analysis. The anti-tumour effect should be evaluated at intervals as defined in the clinical protocol.

22.6. Labelling and packaging

Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect personnel and the environment. The primary packaging must be made of a biologically compatible material. Cryopreservation requires the use of low-temperature-resistant packaging, which can also withstand contact with liquid nitrogen.

Labelling must unambiguously identify the donor, the intended recipient, the cell preparation and its nature, the additives used and the conditions under which the cells are to be stored and distributed. Following procurement, the donor identifier should be always on the 'transit' label when cells are delivered to the processing facility. The recipient must be identified (but not the donor) when cells are distributed for administration. In all cases there must be an audit trail to the donor.

International standards for labelling now exist (e.g. ISBT 128, Eurocode-IBLS) and must be used to promote consistency and traceability, aid international exchanges and facilitate vigilance and surveillance. For tissues and cells procured and distributed in the EU, the Single European Code for Tissue and Cells (SEC) must be used. See also Chapter 14.

22.7. Storage

Storage must be done in conditions that minimise the risk of contamination, cross-contamination and mix-up. A process for quarantine storage should be in place to avoid the possibility that grafts with incomplete or positive IDM test results are accidentally released without proper authorisation.

Conditions for temporary storage must be defined for each type of cell preparation and for each stage of the process, from procurement to release for administration (including pre-processing and post-thawing, etc.). There should be a stability protocol that evaluates the viability and potency of cryopreserved cellular therapy grafts, on a regular basis.

The cryogenic system used for long-term storage must be continuously monitored, and processes must be in place to detect failures in the system, such as temperature rises and changes in the level of liquid nitrogen. An emergency plan should be in place describing the actions to be taken in case a storage device fails, and a procedure should specify how to maintain the cryopreserved grafts at the defined storage temperature.

22.8. Distribution and transport conditions

Internal and external transport must be controlled. Transportation within the same institution (e.g. from the procurement facility to the processing facility, or from the processing facility to the transplant

ward) must be defined by SOPs. Periodic container validation and courier qualification should be performed. When service providers are used for transport or shipment of unprocessed or cryopreserved cell preparations, the conditions by which the service is delivered must be established and regularly audited by the cell-processing facility, which remains responsible for the delivery of cell preparations. Appropriate training of the personnel in charge of transportation should be documented. The transport containers should conform to the applicable regulations and should be secured where applicable. For cryopreserved cellular therapy grafts, a dry-shipper should be used. During shipment of HPC grafts, the temperature should be monitored, and records must be maintained by the shipping facility and shared with the receiving facility. See also Chapter 11.

22.9. Biovigilance

As an effective vigilance and surveillance (V&S) system for tissues and cells used in transplantation and assisted reproduction, the EU project SoHO V&S was developed in 2013. The *Guidelines on vigilance and surveillance of human tissues and cells* [27] were published for healthcare professionals responsible for all types of HPC (bone marrow, peripheral blood stem cells, cord blood) for human application. In EU member states, the requirements for traceability, notification of serious adverse reactions and events, and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells are detailed in Directive 2006/86/EC.

TEs and healthcare professionals should use SoHO V&S guidelines because they provide essential information for the detection, reporting/confirmation and investigation of serious adverse reactions and events (SAREs). There is general guidance on implementation of good V&S practice, as well as definitions of SAREs, in Chapter 16.

TEs should have written procedures for managing SAREs. They should also provide centres carrying out clinical applications with accurate and updated information on various SAREs in the area of HPC transplantation. Moreover, WMDA has set up a central global reporting system for its member organisations to report serious events and adverse reactions related to HPC grafts: Serious (Product) Event and Adverse Reactions or S(P)EAR. The scope of this system is to collect information on serious events and adverse reactions that occurred during procurement or processing of HPC from unrelated donors.

22.9.1. Serious adverse reactions in the recipient

22.9.1.1. *Complications related to haematopoietic stem cell infusion*

Infusion of HPC is, in general, well tolerated. Complications are consequences of immunological incompatibility, iatrogenic toxicities, microbiological contamination and manufacturing/administrative errors. Some complications are similar to the complications caused by transfusion of standard blood products, which is particularly true for allogeneic HPC preparations if they contain ABO-incompatible RBC or plasma. Other complications are specific to HPC infusion, and are related to allogeneic and autologous HPC.

Adverse reactions can be immunological and non-immunological, acute and delayed.

22.9.1.1.1. Haemolysis of red blood cells

Donor–recipient mismatching in erythrocyte antigens is not a contraindication for HPC transplantation, but haemolytic reactions due to ABO and non-ABO antibodies may occur. Reactions can be acute and delayed. The risk of haemolysis is also dependent upon the type of HPC preparation because the content of RBC and plasma is different.

Acute haemolytic reactions are severe complications of HPC infusion. They are caused by ABO incompatibility between the donor and recipient. Usually, the risk of acute haemolysis is greater if the RBCs of the donor are incompatible with the recipient's plasma (major ABO incompatibility). However, haemolysis can also occur if the donor's plasma is incompatible with the recipient's RBC (minor ABO incompatibility). In general, low titres < 1:64 are associated with mild or no reactions, whereas high titres (e.g. 1:1024) are associated with acute haemolytic reactions. The volume of incompatible RBC infused also determines reaction severity. Bone marrow contains a high amount of RBC and can cause acute haemolysis. The risk of haemolysis can be reduced by removal of antibodies from the patient's circulation or by removal of RBC from the bone-marrow preparations of the donor. In contrast, apheresis preparations usually have < 10–15 mL RBC, which is not enough for significant reactions.

Signs and symptoms of acute haemolytic reactions can be: chills, fever, dyspnoea, chest pain, back pain, headache, hypotension, oliguria, anuria, bleeding, shock and pain along veins. If an acute haemolytic reaction is suspected, cardiovascular and

renal function must be maintained and development of disseminated intravascular coagulation prevented.

Delayed haemolytic reactions may occur if the recipient is allo-immunised on the infused RBC antigens of the donor, or if the recipient receives the donor's B lymphocytes within an HPC preparation, which can produce antibodies against the recipient's RBC ('passenger lymphocytes syndrome'). If the recipient is allo-immunised on the donor's RBC antigens, infusion of RBC can stimulate an anamnestic immune response of the residual B lymphocytes of the recipient. The antibody will reach a clinically significant level within 2-14 days after HPC infusion, whereas the infused RBC will remain in circulation. The haemolysis is limited by the amount of infused RBC.

If the recipient receives the donor's B lymphocytes in a HPC preparation that can produce antibodies against the recipient's ABO or other RBC antigens, haemolysis can be more serious because of passenger lymphocyte syndrome. At greater risk are recipients who receive ABO minor-incompatible HPC. Typically, haemolysis will occur within 1-3 weeks after HPC infusion. Occasionally, life-threatening haemolysis can occur. Apheresis HPC preparations contain higher numbers of lymphocytes and carry a greater risk of delayed haemolysis.

Signs and symptoms of delayed haemolytic reactions are the same as for acute haemolytic reactions, but the severity differs.

In autologous settings and if cryopreserved HPC are used, passive haemolysis is more common.

22.9.1.1.2. Complications within the respiratory tract

HPC infusion frequently induces complications within the respiratory tract. During administration, patients often start coughing. Coughing is related primarily to application of cryopreserved autologous HPC, and is usually accompanied by nausea and vomiting. Dyspnoea, with mild decreases in the vital capacity of the lungs, is noted quite often.

Severe respiratory complications, such as acute respiratory failure, are rare. Transfusion-related acute lung injury (TRALI) can occur if granulocyte activation in the pulmonary vasculature is caused by neutrophil antibodies or bioactive mediators, which increase microcirculation permeability and allow massive leakage of fluids and proteins into the alveolar space and interstitium. Signs and symptoms of TRALI usually occur < 6 h after HPC infusion, and include acute respiratory distress, low-grade fever, hypoxaemia (oxygen saturation < 90 % on room air) and bilateral pulmonary infiltrates on frontal radiographs of the chest. If hypoxia is observed during HPC infusion, the infusion should be stopped immediately. Respiratory support should be as intensive as dictated by the clinical picture. Supplementation is necessary in almost all cases. Corticosteroids and diuretic drugs are not useful. In severe cases, transfer to an intensive care unit (ICU) may be necessary.

22.9.1.1.3. Febrile non-haemolytic reactions

During HPC infusion, patients may experience febrile non-haemolytic transfusion reactions (FNHTR). These reactions may be observed in allogeneic and autologous transplantation. FNHTR are manifested by a low-grade fever during, shortly after or ≤ 2 h after infusion of cells. FNHTR can be accompanied by chills, rigor and mild dyspnoea without evidence of haemolysis. This phenomenon may reflect the action of antibodies against leukocytes or the action of cytokines (present in infused preparations or generated by the recipient) after cell infusion.

No laboratory tests are helpful in predicting and preventing FNHTR. Any patient with fever, rigor and chills during HPC infusion should be evaluated, clinically and by laboratory tests, for haemolytic, septic or TRALI reactions. An underlying infection must also be excluded. FNHTR are short-lived complications, and anti-pyretic agents usually provide effective symptomatic relief.

22.9.1.1.4. Dimethyl sulphoxide toxicity

Dimethyl sulphoxide (DMSO) is the most widely used cryoprotectant, but it can detrimentally affect cell viability and is the cause of many side-effects observed during infusion. DMSO toxicity is the most common complication of infusion of cryopreserved HPC.

Within minutes of starting the infusion, a metabolite of DMSO is excreted through the lungs and causes a garlic-like odour that can lead to a foul taste in the mouth. Infusion of DMSO can induce a wide range of other symptoms: pruritus; sedation; headache; nausea; vomiting; abdominal cramps; diarrhoea; flushing; low-grade fever; chills; dizziness; garlic-like odour; haemoglobinaemia with red-coloured urine; elevation of levels of hepatic enzymes; elevation of levels of creatinine kinase. DMSO toxicity has been linked to cardiovascular side-effects such as bradycardia or tachycardia, hypotension and, in rare cases, myocardial infarction.

DMSO toxicity is dose-dependent. The maximum daily intravenous dose of DMSO is 1 g/kg, which is equivalent to an infusion of 10 mL/kg of cells in 10 % DMSO solution. Premedication with anti-histamines, slowing the infusion rate, increasing

the resting time between multiple infusion aliquots, dilution of thawed HPC preparations by albumin–dextran-40 solution at a ratio of 1:2 or 1:3, or removal of DMSO by washing can prevent symptoms and reduce the risk of DMSO-related toxic effects.

22.9.1.1.5. Neurological complications

Neurological symptoms during HPC infusion vary widely. Headache is common and can be related to increased intravascular volume. Occasionally, patients experience more severe side-effects such as muscle spasms and seizures. Cerebral infarcts and acute encephalopathy are rare. Simple muscle spasms often resolve spontaneously. For patients with acute mental changes, loss of consciousness or seizures, urgent intervention is necessary. HPC infusions should be stopped; rapid neurological assessment should be done as well as basic laboratory tests, including electrolytes and glucose. If seizures persist, anti-epileptic drugs are indicated. Patients should be transferred to an ICU.

Neurological complications are probably linked to a large number of non-mononuclear cells and/or caused by DMSO, but this suspicion has not been clearly demonstrated.

22.9.1.1.6. Cardiac toxicity

Cardiac toxicity is common and manifests as bradycardia and other disorders of cardiac rhythm. It is usually mild (though severe cases of arrhythmias have been reported). Severe bradycardia occurs more often in recipients of cryopreserved bone marrow, and may require aggressive supportive care.

Aetiology may be because of hypervolemia due to extensive hydration before infusion, large volume of transplant, hyperosmolality of DMSO, hypothermia, lysis of graft cells or underlying cardiac conditions.

22.9.1.1.7. Allergic reactions

Allergic reactions usually manifest as urticaria and pruritis. Most occur in patients receiving allogeneic transplants. Anaphylactic-type reactions are rare. Allergic reactions present as bronchospasm and/or laryngospasm, hypotension, severe dyspnoea, pulmonary and/or laryngeal oedema, facial burning and flushing, abdominal pain, diaphoresis, diarrhoea and dizziness.

Causes of allergic reactions are not clear. They may be related to the substances used during cell procurement, cell processing or cryopreservation, such as HES or DMSO, that can react with antibodies in the donor or recipient plasma, or with anti-immunoglobulin (Ig)A antibodies in IgA-deficient recipients. No laboratory tests can help to predict or prevent allergic reactions. Therapy is dependent upon symptoms. For mild reactions, administration of anti-histamines will be helpful or, in severe cases, corticosteroids, epinephrine and cardiorespiratory support.

22.9.1.1.8. Anticoagulation effects

Patients receiving non-cryopreserved and non-manipulated bone marrow have a greater risk of haemorrhage because of high concentrations of unfractionated heparin in bone-marrow grafts.

Risk of bleeding is increased in thrombocytopaenic recipients.

22.9.1.1.9. Hypertension/hypotension

Hypertension is more common in cryopreserved HPC or unmanipulated bone marrow HPC, because such grafts have a higher volume. Hypertension is a result of acute volume overload due to rapid infusion, prophylactic hydration and the hyperosmolality of the infused preparation.

Hypotension is also more common in cryopreserved HPC. It is linked with vasodilatation due to histamine generation. Premedication by anti-histamines decreases the incidence and severity of hypotension.

22.9.1.1.10. Acute renal failure

Acute renal failure is more common in application of a high volume of cryopreserved HPC. It is caused by a large amount of DMSO and cellular debris.

22.9.1.1.11. Bacterial contamination

Bacterial contamination of an HPC product is possible. Bone marrow, which is procured into an open system, has a higher rate of contamination than HPC collecting from peripheral blood. Contamination may occur at several steps in the process. It can be due to occult asymptomatic bacteraemia in the donor. For autologous donation, because of the particular nature of the graft and the recipient's condition due to the treatment, it is vital that HPC are procured irrespective of the possible febrile status of the patient, even if sepsis may be present. Although it is a rare occurrence, it should be kept in mind as a potential cause of bacterial contamination of grafts.

Contamination of HPC can also occur during procurement, processing, storage, thawing or sampling, due to an interruption of sterile methods.

After transfusion of contaminated HPC, symptoms of a septic reaction usually develop rapidly. High fever, tachycardia and hypotension, nausea and vomiting, and a 'shock-like' clinical picture should

arouse suspicion of bacterial septicaemia. If such symptoms occur during HPC administration, the infusion should be stopped immediately and all infusion bags and equipment examined.

Known bacterial contamination of an HPC unit is not an absolute contraindication for HPC infusion. Patients receiving culture-positive preparations require antibiotic therapy, which can be antibiogram-specific or cover a broad spectrum of bacteria, starting optimally 2 days before transplantation.

There must be criteria for administration of preparations with positive microbial culture results. A contingency plan is expected to be in place in case of urgent medical need.

22.9.1.1.12. Transmission of infectious and genetic diseases

HPC preparations should be tested for transfusion of transmissible diseases according to national requirements. However, the potential risk of transmission of infectious agents by infected donors or cross-contamination during storage cannot be removed completely. A viral infection < 6 months after transplantation must be suspected to be due to transmission of hepatitis B or C virus (HBV, HCV) or human immunodeficiency virus (HIV). For other viral infections the period will differ, depending on the incubation period.

Screening of HPC volunteer donors for genetic disease is mainly based on their medical history and on the results of laboratory tests. The risk of transmission of a genetic disease is higher with cord blood than with the other sources of HPC (bone marrow and PBSC), since some diseases might not be evident at birth or even some months later.

According to the Notify Library, very few cases of genetic diseases transmission have been described after bone-marrow transplants (cyclic neutropaenia, Gaucher's disease). Autoimmune diseases transmission has also been reported (thyroiditis, type 1 diabetes, myasthenia gravis, vitiligo, etc.) [15]. All cases of suspected post-transplantation infection or genetic disease transmission related to HPC infusion must be reported immediately to the procurement site and/or donor registry, who have to follow the requirements of the national vigilance system.

22.9.1.1.13. Engraftment failure

After HPC transplantation, recovery must occur in populations of myeloid, erythroid and immune cells. The earliest sign of haematopoietic recovery is an increase in numbers of granulocytes and platelets in peripheral blood within days and weeks after graft infusion. Engraftment of erythroid lines and immune reconstruction occurs within weeks or months. Engraftment is dependent on the dose and source of progenitor cells, method of cell preparation, function of bone-marrow stroma, intensity of the preparative regimen, donor–recipient relationship and ABO compatibility.

Measurement of granulocyte and platelet engraftments provides essential information about the success of clinical protocols as well as the quality of procurement and processing of HPC.

Primary graft failure for HPC transplantation from bone marrow or peripheral blood is defined as a lack of neutrophil engraftment 28 days after transplantation. Leukocyte recovery is designated as the first of three consecutive days in which the absolute neutrophil count is $> 500 \times 10^6$/L. Platelet engraftment is designated as the first day on which the platelet count is > 20 × 109/L in an untransfused patient. The sign of erythroid recovery is > 30 × 109/L reticulocytes or > 1 % reticulocytes in peripheral blood in an untransfused patient. T-cell engraftment is proof of mixed donor–host chimerism (5-95 % donor T-cells). Reasons for failure can be graft composition, graft source, HLA mismatch, ABO incompatibility or other reasons that can be attributed to the patient.

The transplant unit should report graft failure to the TE to enable thorough investigation of the quality and handling of grafts.

22.9.1.1.14. Graft-*versus*-host disease

GvHD is a serious and potentially lethal complication of allogeneic HPC transplantation. GvHD occurs if infused T lymphocytes engraft in the recipient and react against the recipient's tissues. Any allogeneic HPC preparations can cause GvHD. Classic acute GvHD occurs < 100 days after transplantation, whereas chronic GvHD occurs > 100 days after transplantation, and the overlap GvH syndrome may occur without limit of time. Risk factors for the development of GvHD are: donor–recipient relationship (HLA disparity, gender matching, donor parity, donor age, ABO group mismatching), stem cell graft factors (source and graft composition, cell processing) and transplantation factors (condition and post-transplant immunosuppression regimens).

Clinical manifestations of GvHD typically involve the skin, liver and gastrointestinal tract in the acute setting, but can affect (among others) the eyes, oral mucosa, vagina, lungs, joints and neurological system.

To predict the outcome of acute GvHD, scoring based on organ involvement is important. Chronic GvHD is more likely in recipients of peripheral HPC than in recipients of bone-marrow HPC. It can be

localised, affect only skin areas, and manifest as progressive systemic sclerosis, Sjögren's syndrome or primary biliary cirrhosis.

Treatment of GvHD includes high-dose corticosteroids, T-cell-suppression drugs, monoclonal antibodies targeting T-cells, extracorporeal photopheresis or mesenchymal stem cells (MSC).

22.9.2. Serious adverse reactions and events related to the graft

SAREs related to the graft – also referred to here as S(P)EAR (serious product events and adverse reactions) as defined by the WMDA – can be: inappropriate transportation, receipt of a wrong unit, receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in hospital or infusion of a unit into the wrong recipient. The transplantation centre must report these incidences immediately to the TE, to the registries if appropriate and, according to national legislation, to Health Authorities responsible for tissues and cells.

22.9.3. Serious adverse reactions in haematopoietic progenitor cell donors

Deaths in unrelated HPC donors are very rare, and few cases have been reported to WMDA. A small number of deaths have been reported also in related donors, from causes such as subarachnoid haemorrhage, sickle cell crisis, myocardial infarction and pulmonary embolism. In some of these donors, pre-existing medical conditions were identified *post mortem*, highlighting the need for stringent medical suitability criteria and assessment of all HPC donors [11, 12, 27].

Bearing in mind that HPC donations are voluntary and altruistic acts of assumedly perfectly healthy individuals, it is the ethical and professional obligation of medical professionals and also good practice to notify, document, investigate and report SARs in the living donor, and not only those influencing the quality and safety of tissues and cells. SARs are uncommon in healthy donors and rare types of SAR or emerging trends are likely not to be noticed at the national level. SARs in stem cell registry donors are followed at the international level by the WMDA. Unfortunately, no consistent follow-up exists at present for related donors. The European Bone Marrow Transplantation Group (EBMT) has established a donor follow-up, which is included in the EBMT database [27].

22.9.3.1. *Complications in haematopoietic progenitor cell (bone marrow) donors*

Donation of bone marrow is, in general, a safe and well-tolerated procedure, but some mild symptoms related to induction of general anaesthesia are common. In most cases, donors recover fully within 2 weeks.

Reactions in bone-marrow donors include constitutional symptoms such as nausea, vomiting, anorexia, insomnia and fatigue (most common).

Complications related to puncture of bone marrow, such as pain upon procurement, walking, sitting and climbing stairs, as well as minor infection, are rare. Bone and soft-tissue trauma at the harvest site may cause pain, bleeding, oedema or nerve compression. Damage to a lumbosacral nerve root or penetration into the pelvic cavity or internal iliac vessels may cause severe morbidity. Anaesthesia carries an unavoidable (albeit very small) risk of life-threatening cardiac or respiratory events, as well as the possibility of allergic or idiosyncratic reactions to anaesthetic agents. Removal of large volumes of blood may cause symptoms of hypovolemia or anaemia [15, 17, 20].

Cytopaenias (anaemia, thrombocytopaenia) and more serious reactions such as deep-vein thrombosis (DVT), thromboembolism, cerebrovascular accident and subdural bleeding have been documented. Post-donation septicaemia and anaesthesia-related complications have also been described, as well as respiratory complications such as pulmonary alveolitis and oedema.

22.9.3.2. *Complications in haematopoietic progenitor cell (apheresis) donors*

Complications are related to apheresis and administration of granulocyte-colony stimulating factor (rhG-CSF). Symptoms related to citrate infusion are the most common.

Additional complications related to apheresis include haematoma, arterial punctures, delayed bleeding, pain from injury to nerves or tendons, thrombophlebitis, local allergy, generalised allergic reactions, vasovagal reactions, haemolysis and air embolism. All severe conditions requiring hospitalisation or intervention, or resulting in death < 24 h after procurement, should be reported immediately.

Use of rhG-CSF in mobilisation is, in general, safe. Common short-term reactions related to rhG-CSF are bone pain, headache, myalgia, nausea, vomiting, diarrhoea, fatigue, fever and irritation at injection site. Most of these effects are reversible after discontinuation of rhG-CSF administration. Other rare reactions are splenic rupture, anaphylaxis, thrombosis, gout, iritis, keratitis, autoimmune

hyperthyroidism, acute lung injury, capillary leak syndrome, exacerbation of rheumatoid arthritis, insomnia and reduced numbers of thrombocytes.

Reports from long-term follow-up studies in unrelated and related apheresis HPC donors mobilised with rhG-CSF demonstrated a similar incidence of leukaemia and other malignancies to those seen in the general population. All malignant diseases in all donors treated with rhG-CSF should be reported, regardless of the time of occurrence.

22.9.3.3. *Follow-up of haematopoietic progenitor cell donors*

Chapter 16 on biovigilance also applies to HPC transplantation and must be read in conjunction with this chapter. The donor, whether related (paediatric and adult donors) or unrelated, should be followed up by the donation centre in the short, mid and long term according to the policy suggested by scientific organisations (e.g. Italian Bone Marrow Donor Registry (IBMDR) or EBMT) or as requested by national regulations (e.g. Austria, Switzerland). In particular, a short-term follow-up to document SAEs, and a long-term follow-up on a regular basis to document late effects of the donation or the mobilising agent, should be performed [15, 27].

22.9.4. Biovigilance of mononuclear cells

The same requirements as for HPC(A) and HPC(M) apply also to MNC(A) (see above).

The processing of MNC involves several steps where unexpected events that have to be documented and reported may occur (see §22.9 and Chapter 16), for instance, lower viability of frozen and thawed MNC than expected or human errors in calculating the dose of T-cells in DLI. In cases of low viability, DLI may still be used but this has to be documented and a risk analysis carried out. Possible adverse events associated with DLI are the development of acute and/or chronic GvHD and low blood counts. These complications may appear after 1 or more weeks after the administration of the $CD3^+$ cells.

As noted in section 22.9, similar complications such as DMSO toxicity, transmission of infectious diseases or GvHD may occur. In rare circumstances, serious product adverse events/reaction or complications in the donor are possible. Follow-up of the donor as stated in section 22.9 and Chapter 16 is recommended.

22.10. References

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Related material

- Appendix 28. Donor search through registries for haematopoietic progenitor cell transplantation

Chapter 23: **Umbilical cord blood progenitors**

23.1. Introduction

Umbilical cord blood (UCB) is a source of haematopoietic progenitor cells (HPC) that can reconstitute the haematopoietic and immune systems. These cells bear unique properties, including a high progenitor cell proliferation ability and immune naïvety. Usually UCB is discarded after delivery, but the blood remaining in the placenta after clamping can be collected, processed and stored safely.

Since the first transplant procedure performed in Paris by Professor Gluckman and her team in 1988 in a child with Fanconi anaemia, more than 40 000 patients have received transplants. According to the World Marrow Donor Association (WMDA), more than 750 000 units are registered at the WMDA Search & Match Service, formerly Bone Marrow Donor Worldwide [1-2]. The database is searchable for any patient in need.

The use of UCB cells offers several advantages, including no risk for the donor, prompt availability as an off-the-shelf medicinal product and clinical benefits like low incidence of graft-*versus*-host disease, even in partially matched transplants, which increases the patient's chance of finding a suitable donor. UCB banks have therefore facilitated universal access to the therapy, in particular to ethnic minorities. However, there are also some disadvantages: the number of stem cells in UCB is relatively low and often associated to slow engraftment, and it is not possible to use a donor lymphocyte infusion after transplantation. There are interesting approaches to improve outcomes, including the use of very high cellular units, double cord and intrabone transplantation, and promising protocols for progenitor cell *ex vivo* expansion. The most applied protocol in older patients uses reduced intensity conditioning and a double UCB graft approach. Recently, immune active properties like an enhanced graft-*versus*-leukemia effect have been proposed, and reconstitution of the immune system is the current research area to achieve an improvement of UCB transplant methods [3].

The regulatory framework of UCB collection and processing to make it available to patients in need has evolved considerably over the past two and a half decades. Accreditation and regulation has instilled confidence in clinicians, allowing them to select a UCB unit from across a wide range of banks in many countries.

A UCB bank is a multidisciplinary structure that is responsible for the recruitment and subsequent management of maternal donors as well as the collection, processing, testing, cryopreservation, storage, listing, reservation, release and distribution of units for administration. According to their purpose and organisation, UCB banks can be public, private or hybrid, but all must have a quality system in place to guarantee that the production of UCB units fulfils predefined specifications according to their therapeutic intention. In addition to the legal requirements of Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004, some scientific societies have developed high quality standards. Professional societies like the FACT and AABB have developed international standards [4-5]

that have a worldwide perspective to improve the quality of the products available for transplantation. The accreditation status of the UCB bank is one of the selection criteria for many transplant centres during the search for a UCB unit.

The following generic chapters (Part A) of this Guide all apply to UCB banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines the additional specific requirements for UCB banking and transplantation.

23.2. Recruitment of potential donors, identification and consent

23.2.1. Donor recruitment

The therapeutic properties of UCB-derived cells and potentially of their components require the establishment of a donation programme that enables the safe collection of residual blood contained in the placenta and cord after appropriate information of the maternal donors.

In general this donation can be made available for public or private uses. Public initiatives promote donation of UCB unit, usually to a not-for-profit organisation, with the aim to provide UCB units for transplantation or other approved clinical applications to any patient in need. Many public UCB banks offer also a service for family UCB banking in case of illness in one of the siblings or other members of the donor's family [6]. Public UCB banks are generally committed to networking in order to increase access to UCBs through worldwide registries. In a private bank, the donor keeps ownership of the product, and the organisation offering the processing and storage services is responsible for maintenance of the units for future potential medical applications. Due to the nature of these services, these banks are usually for-profit organisations.

The Council of Europe has been studying the issue of UCB donation for several years and has always been concerned about the proliferation of UCB banks dedicated to the procurement and storage of UCB for autologous or family use. This concern resulted in adoption of Recommendation Rec (2004) 8 of the Committee of Ministers to member states on autologous UCB banks, and its explanatory memorandum [7], which recommends that member states allow establishment of UCB banks only for altruistic and voluntary donations of UCB. In case of autologous (or family-use) UCB banks, those organisations must clearly inform parents about the differences between the various medical objectives of autologous and allogeneic donations and about the uncertainties relating to the medical applications of autologous UCB preservation. In any case, autologous UCB banks must meet the same quality and safety standards as for allogeneic UCB donation and banking.

The Council of Europe has produced the booklet *Umbilical cord blood banking: a guide for parents* to provide clear, accurate and balanced information about the use of UCB in medical treatment and to guide parents through their blood-storage options [8]. If a family member with a potential transplantable condition exists, related UCB units can be collected prospectively and stored for later use.

Donor recruitment (see also Chapter 3) usually starts during pregnancy, with information given by the woman's healthcare provider, but it may also occur as late as at admission into the maternity unit, as soon as parents are informed about this possibility and the mother is in good condition for signing an informed consent.

Information leaflets or brochures to inform the mothers about UCB banking are an important part of the recruitment plan. Information should include at a minimum:

a. donor exclusion criteria;
b. the potential benefit and risks of UCB donation;
c. testing to be performed;
d. potential use (transplantation or other use);
e. data protection.

Any claims made in recruitment material should be supported by scientific evidence.

It is during pregnancy that expectant mothers are encouraged to gather information and ask questions about the UCB collection procedure. Motivated personnel at collection sites are an important way to approach potential maternal donors. Training of physicians and health professionals on this step is essential to make sure that information provided to parents is accurate and that all their questions can be answered.

23.2.2. Informed consent

Informed consent has to be signed by women who agree to donate UCB of their offspring to a UCB bank. General considerations are shown in Chapter 3. Consent must cover in writing all aspects related to the donation that mothers must sign. Normally, there are questions about performing infectious disease marker (IDM) tests, contacting the maternal donor in case an IDM test is positive, using units for research, checking medical notes, etc. Asking for informed consent and providing information about it is not recommended during active labour in order to avoid distraction linked to physical and emotional stress. For private banks, informed consent is usually wider, as it is a contract between parents and bank. In this case all aspects of donation, UCB unit processing and storage, and future uses should be thoroughly explained there. Consent can be obtained in a single step prior to collection, when the donation process is initiated early in the pregnancy, or in two steps: a pre-consent followed by a full consent before the UCB unit is placed into a clinical inventory.

23.2.3. Donor evaluation

After maternal donor recruitment, trained personnel must determine the donor's eligibility. It is important to ensure that the donation is safe for future recipients. Maternal and infant donor eligibility must be determined on the basis of the results of screening and testing in accordance with national regulations. To assess donor eligibility, a donor medical history interview, which includes assessment for high-risk behaviours, must be conducted to identify risk factors for transmissible and genetic diseases (see Chapter 4 for further details). The mother will be asked to provide personal and family medical details. There must be written criteria for maternal and infant donor evaluation and management.

In general, transmissible disease, either infectious or genetic, and certain risk behaviours exclude the possibility of donation, and those factors should be taken into account when evaluating a potential donor [9]. They can be summarised in the following non-exhaustive list (see Appendix 30 for more information):

a. severe eclampsia;
b. depression, manic-depressive psychosis not regularly treated, dementia;
c. central nervous system diseases, e.g. neurodegenerative diseases, neurofibromatosis, Parkinson;
d. oncological diseases (including neoplastic haematological diseases);
e. infectious diseases;
f. inflammatory bowel disease (e.g. Crohn's disease and ulcerative colitis);
g. live immunisation (vaccination) during this pregnancy.

In addition to these considerations, there are absolute contraindications that should be taken into account when evaluating a potential donor [9]; they can be summarised in the following non-exhaustive list:

a. autoimmune diseases if the mother received treatment in the last 12 months;
b. malignancy (except basal cell carcinoma and *in situ* cancer treated and cured);
c. inflammatory bowel disease (e.g. Crohn's disease and ulcerative colitis);
d. if the mother has received donated eggs or embryos since 1980;
e. evidence of active or chronic infection;
f. live immunisation (vaccination) during this pregnancy;
g. myasthenia gravis;
h. myelodysplastic or myeloproliferative syndrome;
i. unexplained night sweats;
j. animal bite;
k. organ recipient.

Results of this evaluation must be documented in the clinical history and reviewed by trained personnel.

The medical history evaluation must be obtained while the mother is able to concentrate on the answers to the questionnaire and is not distracted by aspects of labour (see a model medical questionnaire in Appendix 29). The language used must be understood by her. It is not recommended that either family

or friends serve as interpreters or translators. Confidentiality must be preserved. If responses generate medical concerns, the collection should be rejected or cancelled. The mother's travel history to endemic areas must be obtained and documented, and eligibility determined according to national regulations. Screening for human transmissible spongiform encephalopathy, including Creutzfeldt–Jakob disease, must be documented. If history of communicable disease risk was obtained in advance of the maternal donor's presentation for delivery, the history must be updated to include information up to the time of delivery. In the case of a woman who gives birth to an infant donor not genetically hers, her communicable disease risk history must be obtained. The questionnaire must include questions to obtain at a minimum genetic history, malignant disease and inherited disorders that may be transmissible to the recipient.

In addition, IDM tests on maternal blood samples must be performed within seven days before or after collection of the UCB unit. These samples will be tested for evidence of infection of HIV1, HIV2, hepatitis B, hepatitis C, syphilis and any additional markers according to local regulations. Assays used for testing must be validated for use in volunteer blood or tissue donations. According to the EU regulation, if initial IDM marker tests do not include nucleic acid tests for HIV, HBV and HCV, then a second IDM test 180 days after donation must be performed. (See Chapter 5 for further details.)

23.3. Procurement

23.3.1. Procurement procedures

Procurement typically involves the following steps:

a. The umbilical cord is clamped as distal from the placenta as possible. No interference with labour and delivery must occur in order to protect mother and newborn safety. Nowadays, many obstetrical medical associations recommend delayed clamping. Evidence suggests that an acceptable time of 1 minute is compatible with public UCB banking [10];

b. A section of the cord is cleaned with a suitable disinfectant;

c. A needle that is attached to the collection bag is inserted into the umbilical cord vein;

d. The collection bag is filled by gravity until the cord looks 'white' and all the blood from the placenta and umbilical cord is drained into the bag;

e. The collection bag must be appropriately labelled.

There are two main techniques to collect blood from the cord vein: before the placenta is delivered (*in utero*) or after the placenta is delivered (*ex utero*). Both procurement techniques give similar results and their use will depend on the ability of the maternity unit to collect the UCB.

In any case, the individuals performing the collection must be adequately trained. In both cases the collection bag must contain an adequate volume of anti-coagulant (i.e. CPD) to prevent clotting.

After procurement, the healthcare provider in charge completes a report describing the labour phase, listing the events to be evaluated for acceptance of the unit, such as presence of fever, complications, type of delivery, etc. See Appendix 30 for further guidance on how collect this data.

Within the biovigilance process, severe adverse events and reactions need to be notified to the Health Authority in accordance with established laws and regulations (see also Chapter 16).

23.3.2. Temporary storage and transportation to the tissue establishment

Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, UCB should be temporarily stored in non-frozen conditions after collection, during transportation to the processing facility. Cell viability decreases and the risk of bacterial growth increases during storage at room temperature as well as in refrigerators. The distance from the collection and the processing facilities may be considerable and therefore, the maximum time between collection and the start of processing should be established. It is recommended that cryopreservation of unrelated UCB units should be initiated as soon as possible within 48 h and cryopreservation of related units within 72 h [4]. The UCB bank should validate the storage and transport conditions of the UCB units.

The UCB procurement bag will be identified with the following labels and paperwork:

a. unique UCB and maternal codes;

b. product name;

c. procurement site name or identifier;

d. date/time of collection;

e. name and volume/concentration of anticoagulants;

f. recommended storage temperature;

g. biohazard sign and/or other warning labels, following national regulations;

Table 23.1. **Structural components of an umbilical cord blood bank**

Facility	Characteristics
Procurement	Collection must take place in a maternity unit and be performed by trained health professionals using a validated technique without any interference in labour care. Besides the general requirements described in Chapter 6, a collection site must ensure secure storage of the UCB unit, associated samples, maternal samples and related documentation until they are transported to the UCB Processing facility
Processing	All general requirements described in Chapter 8 apply. Processing must be secure and have adequate space to perform all activities in a safe and sanitary manner. Relevant environmental conditions that could affect the safety and potency of the UCB unit need to be defined and monitored
Cryostorage	UCB units must be stored in either liquid or vapour-phase liquid nitrogen below – 140 °C. All refrigerators, freezers and cryostorage tanks used for storage of UCB units, associated reference samples, and maternal samples, must have a system to continuously monitor and regularly record the temperature. There must be an alarm system in place 24 h a day. Additional storage devices of appropriate temperature must be available in the event that a primary storage device fails
Testing	Agreements must be in place with laboratories performing cell counts, flow cytometry and potency assay. Other laboratories needed are: • accredited human leukocyte antigen (HLA) laboratory, • immuno-haematology lab, • a certified laboratory for IDM testing, • laboratories for haemoglobin screening. Testing should be undertaken in accordance with national and international regulations (see also Chapter 5).
Administrative area	UCB banks need to communicate with maternal donors, registries and transplant centres. A quality-assurance system must ensure that UCB units become available for international registry search only after processing, with medical and quality review, has been completed. Documentation related to request for UCB unit or for samples, results of testing and records and transportation and shipping between facilities must be retained in accordance with applicable national laws and regulations.

h. statement 'Related donor' where applicable;
i. donor name for related UCB units;
j. recipient family or individual name if known.

Transportation should be done using the same criteria as other progenitor cell products, especially in the requirements for containers, temperature monitoring and labelling (see Chapter 11). Shipping method must be validated and meet transportation regulations for this type of product. Upon receipt, the integrity of the UCB units and their containers will be checked, and any deviation will be recorded within the processing records.

23.4. Processing of umbilical cord blood progenitor cells

A UCB bank must have appropriate facilities and personnel for the reception, processing, testing and storage of UCB and maternal blood. All processes should be performed in compliance with national and EU regulations. Where aspects of processing, testing or storage are performed by an external party, there must be a written agreement in place between the UCB bank and the external party providing the service. Factors influencing the air-quality specification for processing HPC from umbilical cord also apply (see Table 22.5 and Chapter 7).

A UCB bank structure needs to co-ordinate different lab facilities, including a processing laboratory, a cryogenic storage area and associated testing laboratories for quality control of individual batches.

Setting up a high-level processing lab is very important since there is a clear correlation between good practice, quality certification and the outcome of UCB transplantation [11].

23.4.1. Reception at processing facility

On receipt of a UCB unit, a series of checks needs to be performed on the unit, on the associated samples and on the accompanying documentation, to verify and determine whether specific acceptance criteria are met. These include parameters such as volume, total nucleated cell (TNC) content, correct documentation and labelling, signed maternal donor consent, appropriate transport temperature, absence of large/multiple clots, transport conditions and acceptable time in transit from procurement centre to processing laboratory. Once a UCB unit meets the initial acceptance criteria it will continue on to be processed.

23.4.2. Volume/red blood cell reduction

Despite some loss of cells, volume reduction including red blood cell (RBC) depletion has practical and clinical benefits: it allows efficient storage of UCB in terms of space and cost, and, most importantly, it eliminates the following when infused after thawing:

- potentially ABO-incompatible plasma,

- free haemoglobin,
- RBC stroma.

The eliminated RBC and plasma components can be used for immediate or future testing, thereby minimising the loss of the actual UCB product for testing purposes [12].

The final product volume and cellular characteristics are dependent on the starting product as well as the processing/separation technique. Over the past decade three major methods have been used in large-scale banking which produce reproducible results that could be standardised. These include the manual method using hydroxyethyl starch for RBC sedimentation or other proprietary reagents, the semi-automated bottom-and-top method, and newer fully automated and programmable closed systems.

Whichever platform is employed, it is essential that the equipment and reagents used do not adversely affect the viability of the cells, that the process does not allow the introduction of adventitious agents or the transmission of communicable disease, and that the method be validated to allow optimal recovery of the fraction of interest.

23.4.3. Cryopreservation, thawing and infusion

23.4.3.1. *Cryopreservation*

The selection of a suitable protocol for cryopreservation of UCB is critical to optimise the recovery of functionally viable HPC [13]. Potential causes of cell damage include type and concentration of cryoprotectant, cell concentration, and cooling and warming rates, as well as level of control of storage conditions (see also Chapter 9).

Standard operational procedures (SOPs) related to cryopreservation should specify that the following information is recorded for each unit:

a. TNC concentration within a defined range;
b. the type of cryoprotectant, its final concentration, and the duration of the cell exposure prior to freezing;
c. method of freezing and end-point temperature of cooling;
d. cooling rate within a defined range;
e. freezing curve parameter within a defined range;
f. storage temperature.

UCB units must be stored in freezing bags designed and approved for the cryopreservation of human cells and placed into metal cassettes to provide protection during freezing, storage, transportation and shipping. It is important that, after filling, each freezing bag is visually examined for possible leaks and breakage of seals. As reference samples each freezing bag must have integrally attached, at minimum, two segments of adequate volume to assess identity and potency of the UCB cells prior to release.

UCB units should be cryopreserved using a controlled-rate freezer with a validated freezing program. The majority of UCB banks use cooling rates of 1-5 °C/min in order to allow the cells to slowly dehydrate as the ice phase progresses and the extracellular solute concentration increases. Cryoprotectants used for UCB are those generally established for other HPC sources. In general, a concentration of 10 % DMSO is considered optimal for UCB. When used in conjunction with DMSO, other cryoprotectants, like Dextran-40, enhance the cryoprotective effect by allowing stabilisation of the cell membrane. While alternatives have been proposed, it is generally considered that a combination of 10 % DMSO and 1 % Dextran-40 results in the best recovery rates for TNC, $CD34^+$ and colony-forming units (CFU). Prolonged exposure of cells to DMSO can result in damage to cells. It is therefore essential that the duration from addition of cryoprotectant to initiation of freezing is minimised and the maximum time allowed should be validated by the bank.

In addition to the two contiguous segments, for each banked unit it is necessary to store several samples. Netcord-FACT International Cord Blood Standards require, from each UCB unit, at least 2×10^6 TNC divided in two vials, suitable material for preparation of genomic DNA, and plasma; and, from the maternal donor, serum and/or plasma and suitable material for preparation of genomic DNA. All the samples must be stored at −70 °C or colder. Representative samples intended for viability or potency analysis must be stored under the same conditions as the UCB unit.

23.4.3.2. *Thawing and infusion*

It is important to ensure that the transplant centre receives information on how to handle and use the UCB unit. Handling includes thawing, dilution and washing of the UCB unit. Providing information about indications, contraindications and cautions is the responsibility of the UCB bank. A jointly prepared document, *Circular of information for the use of cellular therapy products*, is available online [4, 14]. Along with this circular, UCB banks should be able to provide instructions for a validated thawing method of their UCB unit. Units that have not been red cell reduced prior to cryopreservation should be washed, as recommended by FACT-JACIE, while a buffy coat

enriched UCB unit can be simply diluted [15]; see also §22.4.4.3.

23.5. Quality control

23.5.1. Biological information needed to confirm donor suitability and recruitment

All clinical and biological information pertaining to donor identification, screening and recruitment must be kept, along with all information pertaining to processing and distribution. This information must remain as a permanent part of the preparation and release file; see Chapter 2 and Chapter 15 for more details.

Details on the nature of such information and the procedure to obtain it are provided in Chapter 3 and Chapter 4 of this Guide.

23.5.2. Safety controls

In order to provide a safe UCB product for release, it is essential that UCB units are screened and tested for communicable diseases (see also Chapter 5). Maternal blood obtained within 7 days before or after the collection of the unit is used as a surrogate test for IDMs, and is strongly reflective of the infectious status of the UCB units due to the shared circulation during gestation. Testing the UCB unit for IDM provides an additional degree of safety. At a minimum, prior to release for administration, the maternal donor of each UCB unit must be tested for evidence of infection by at least the following communicable disease agents:

a. Human immunodeficiency virus, type 1;
b. Human immunodeficiency virus, type 2;
c. Hepatitis B virus;
d. Hepatitis C virus;
e. *Treponema pallidum* (syphilis);
f. any additional agents required by national regulations or locally endemic disease.

A medical and genetic history of the infant donor's family must also be obtained to prevent the transmission of malignant diseases and inherited disorders.

UCB units for unrelated use must be shown to be free of microbial contamination. Microbial testing must be performed using a system validated for the growth of aerobic and anaerobic bacteria and fungi (see §10.5.2). For related UCB units, the results of positive microbial tests must include identity and antibiogram(s) of the organism(s), and these results must be reported to the prospective clinical programme.

Prior to release for administration, each UCB unit must have undergone haemoglobinopathy screening, regardless of the family's ethnic background or history.

Mechanisms for donor counselling should be in place if there is a positive test result for any IDM (other than *Cytomegalovirus*), an abnormal haemoglobinopathy screening or any other abnormal test finding. Every effort should be made to notify the mother, and/or her physician. The UCB bank must have policies for handling specific cases.

23.5.3. Quality specifications

In order to characterise a UCB unit, identity, purity and potency assays must be performed and evaluated. Table 23.2 shows a list of reference values suggested by 6th edition of the Netcord–FACT standards to determine the quality of a UCB unit stored for clinical administration.

Table 23.2. **Specification requirements for unrelated UCB units**

Test	Specification
Total nucleated cell count	$> 5 \times 10^8$
Total nucleated cell recovery	Should be > 60 %
Fresh TNC viability	> 85 %
Viable CD34 counts	$> 1.25 \times 10^6$
Fresh CD34 viability	> 85 %
Post-thaw CD34 viability	> 70 %
Post-thaw CD45 viability	> 40 %
Post-thaw CFU	Growth
Sterility	Negative for aerobes, anaerobes and fungi
Donor screening and testing	Compliant with applicable law and regulation
Identity	HLA, ABO verified

Source: Modified from 6th edition of FACT-Netcord standards.

Meeting UCB quality specifications and having very good banking practice will ensure a successful UCB transplantation [16].

23.5.4. Release criteria

The UCB bank must receive a formal request from the transplant centre before the work-up starts. Return of unrelated UCB units is generally not permitted.

The three tests to be performed by the bank before a UCB unit can leave storage premises are: verification of donor identity; potency assessment; and safety evaluation. These tests are summed up below.

23.5.4.1. *Verification of donor identity*

UCB unit identity can be verified by performing HLA-typing using a segment physically attached to the freezing bag containing cryopreserved UCB cells. The UCB bank must have a policy in place for the cases where there are no remaining attached segments. Verifying the maternal HLA haplotype would add additional safety requirements to validate HLA typing and to ensure maternal testing and assessment corresponds to the product selected [17].

23.5.4.2. *Potency assessment*

It is a requirement to assess the functional capacity of the UCB unit prior to release to the transplant centre. CFUs are grown from functionally viable cells and the results of this assay increase confidence in UCB unit quality and ability to engraft. Therefore, it is recommended to perform CFU assay from a frozen contiguous segment prior to release for administration.

23.5.4.3. *Safety evaluation*

IDM testing of the maternal samples is understood to be a surrogate test, and strongly reflective of the infectious status of the UCB unit. Prior to release for administration the results of maternal donor screening must be available. Because of differing national regulations, testing for additional infectious agents by IDM test may be required by the transplant centres.

23.6. Labelling and packaging

Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect personnel and the environment. The primary packaging must be sterile and made of a biologically compatible material. Cryopreservation requires the use of liquid-nitrogen-resistant bags.

From procurement to distribution, labelling must unambiguously identify the UCB unit. Each label must include at least the unique identifier, the proper name of product, the intended recipient (if known), the type of manipulation, the anticoagulants and additives used and the conditions under which the cells are to be stored and distributed. The recipient must be identified (but not the donor) when cells are distributed for administration. Labelling must allow the UCB bank to ensure the link between the UCB unit and its samples and records. Because UCB bags are normally too small for a standard-size label, a partial label at distribution is acceptable and must include at least a unique numeric or alphanumeric identifier, the proper name of the product and the product code. Additional information can be included in a tie tag and/or in the accompanying documentation.

International standards for labelling cellular therapy products are now available (e.g. ISBT128, Eurocode) and their implementation is required by specific accreditation bodies. Global labelling systems promote consistency and traceability, aid international exchanges and facilitate vigilance and surveillance. For tissues and cells procured and/or distributed in the EU, the Single European Code (SEC) must be used. See also Chapter 14 and the EU coding platform [18].

23.7. Storage

In addition to the general requirements described in Chapter 9, the long-term storage required, UCB banks must have an inventory management system to ensure that each UCB unit and its associated reference samples, maternal samples and records can be located in a timely manner. This inventory management system should prevent mix-ups or contamination of the UCB units during storage, and address the duration of the storage for cryopreserved UCB units. The UCB banks need to establish and validate the duration and conditions of storage; the effects of long-term storage on the viability, potency and sterility of the UCB cells should be evaluated in a stability protocol. A procedure for quarantine to minimise the risk of microbial cross-contamination of UCB units must be in place. Release of a UCB unit from quarantine should be based upon the evaluation of the testing and screening results pertinent to that UCB unit, in accordance with applicable national laws and regulations.

Refrigerators and freezers used for the storage of UCB units and all associated reference products should not be used for any other purpose, in order to minimise the risk of cross-contamination.

UCB units are intended for long-term storage and must be stored at −140 °C or colder. Each facility should assess the potential risk of transient warming events during processing and/or storage. Examples of these events include transfer of UCB units from the controlled-rate freezer to the cryostorage tank, removal of segments for HLA verification testing

or other tests required and storage of UCB units in vapour vessels that may exhibit unstable temperatures when open. Each step should be validated to show that the viability and potency of the UCB unit have not been compromised.

23.8. Distribution and transport conditions

Internal and external transport or shipping must be controlled, and records must allow tracking and tracing of the UCB unit from UCB bank to the transplant centre. Methods of transportation and shipping must be described in operating procedures. Container validation and courier qualification should be performed periodically. Transport containers must be appropriately labelled and secured, and must conform to applicable regulations. For shipment of the cryopreserved UCB units, a dry-shipper must be used, and the temperature monitored and recorded to detect temperature excursions. A plan for alternative transportation or shipping in an emergency should be in place. Transportation records must be maintained by the shipping facility and shared with the receiving facility. Appropriate training of the personnel in charge of transportation should be documented. See also Chapter 11.

23.9. Biovigilance

Adverse events and reactions (serious and non-serious) must be recorded, reported and investigated according to corresponding national regulations for tissues and cells as described in Chapter 16.

In EU member states, the requirements for traceability, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells are detailed in Directive 2006/86/EC.

Tissue establishments must have standard operational procedures (SOPs) for managing serious adverse reactions and events (SAREs). They should also provide centres carrying out clinical applications with accurate and updated information and training on SAREs in the area of HPC transplantation.

Tissue establishments and healthcare professionals may use EU SoHO V&S guidelines as they provide essential information for the detection, reporting/confirmation and investigation of SAREs. There is general guidance on implementation of good vigilance and surveillance practice, and definitions of SAREs, in Chapter 16.

23.9.1. Serious adverse reactions and events in the recipient

23.9.1.1. *Complications related to UCB stem cell infusion*

This topic is also discussed in Chapter 22 (see 22.9.1). In the use of UCB the most frequent reactions are those related to effects derived from infusing incompatible RBC, cryoprotectant or other adventitious substances used for volume reduction. The most common infusion reactions are hypertension, nausea and vomiting, bradycardia and chest pain. Table 23.3 summarises the most common reactions.

Table 23.3. **Common reactions after UCB infusion**

Immune reactions	• haemolysis of red blood cells • febrile non-haemolytic reactions • allergic reactions
Systemic complications	• complications within the respiratory tract • neurological complications • cardiac toxicity • acute renal failure • anticoagulation effects
Biological	• engraftment failure • graft-*versus*-host disease
Transmissible	• bacterial contamination • transmission of infectious and genetic diseases

23.9.2. Serious adverse reactions and events related to the product

Despite following JACIE guidelines on UCB, there may occur what are called serious product events and adverse reactions, such as inappropriate transportation, receipt of a wrong unit, receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in hospital or infusion of a unit into the wrong recipient. In all such cases the transplantation centre must immediately report this matter to the tissue establishment and, according to national legislation, to the Health Authorities. If clinically relevant, it is recommended that a policy to inform the maternal and/or cord blood donor is adopted if there are donor consequences of (genetic) findings in donor cells in the recipient.

23.9.3. Serious adverse reactions and events in umbilical cord blood progenitor cell donors

23.9.3.1. *Follow-up of UCB progenitor cell donors*

The UCB bank must have a policy for the follow-up of both maternal and infant donors and for the management of donation-associated adverse events. The policy must define the time period within which to contact donors (see Chapter 2 for further details).

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Related material

- Appendix 29. Health assessment questionnaire for cord blood donor
- Appendix 30. Data collection for cord blood donor

Chapter 24: **Pancreatic islets**

24.1. Introduction

Type-1 diabetes mellitus (T1DM) is characterised by absolute and specific destruction of insulin-producing cells that reside within clusters of cells in the pancreas known as islets. People who do not have diabetes mellitus have ≈ 1 million islets comprising 2 % of the overall pancreas. Without lifelong insulin replacement, T1DM quickly results in coma and death. Even with optimised treatment, vascular and neurological complications often develop over time. Restoring near-normal blood glucose levels can prevent these complications. This has, however, been associated with a threefold increase in severe hypoglycaemia (low blood glucose, which can result in collapse without warning, one of the greatest fears for those living with insulin injections) [1]. Diabetes mellitus remains a leading cause of blindness, renal failure (requiring dialysis or renal transplantation) and lower limb amputation.

The ultimate goal of pancreatic islet transplantation and beta-cell replacement therapy is to restore glucose-responsive insulin secretory capacity to patients with insulin-deficient DM. This includes all people with T1DM and potentially also those with insulin-deficient type-2 diabetes mellitus (T2DM). The benefits of islet transplantation (in its current form) are reversal of life-threatening hypoglycaemia unawareness, with improved glycaemic control overall and, in over 50 % of patients, complete insulin independence for variable periods of time. It should, therefore, be available for patients who have unresolved recurrent severe hypoglycaemia despite optimised specialist management.

Hence, islet transplantation may be especially beneficial for two defined subgroups of people with T1DM: those patients with severe hypoglycaemia without warning signs and those patients with unstable diabetic control following renal transplantation [2]. Also, islet autotransplantation – as an adjunct to total pancreatectomy for benign pancreatic disease (e.g. for chronic pancreatitis) – can prevent the labiality of surgically induced severe DM.

The following generic chapters (Part A) of this Guide all apply to pancreatic islet transplantation and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,

o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

24.2. Donor evaluation

24.2.1. General criteria

Donor criteria for pancreatic islet transplantation are the same as those generally applied for pancreatic transplantation [3]. All suitable deceased donor pancreases that have not been placed for vascularised whole organ transplantation should be allocated for pancreatic islet transplantation according to a prioritised (inter)national waiting list. However, additional criteria for donation of tissues and cells (see Chapter 3 and Chapter 4) must be applied.

24.2.2. Donor characteristics

Donor characteristics – such as body surface area, body mass index and number of vasopressor types used – are predictors of successful pancreatic islet isolation. Other characteristics such as age, cold ischaemia time, and blood chemistry levels of glycated haemoglobin A1c, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), blood urea nitrogen, amylase, lipase, sodium and glucose could influence pancreatic islet isolation yield [4-6]. The tissue establishment (TE) should establish contraindications for pancreas acceptance.

24.2.3. Specific exclusion criteria for pancreatic islet transplantation

Donors suffering from diabetes mellitus type 1 or 2 are excluded from donation for this clinical use.

24.3. Procurement

The consistency of pancreatic islets manufacturing is highly dependent on the quality of the procured organ. Organ procurement should be conducted to ensure organ viability in transit, using similar procedures as for whole pancreas transplantation, but vascular access is not required. If a distant team has procured donor pancreases, the TE should have agreements with the procurement centre(s) on organ harvesting, warm and cold ischaemia time, organ preservation methods, cold preservation fluid and shipping conditions.

24.4. Processing and storage

Organs are transported to the designated isolation facility. Pancreases are processed by enzymatic and mechanical dissociation, and pancreatic islets are usually collected after density-gradient purification.

Storage of pancreatic islets in media under stringent conditions before implantation has logistical benefits: it enables additional quality-control tests, and allows time to prepare the patient for transplant or to ship pancreatic islets to a distant transplant centre [7]. The TE should guarantee that the composition of the storage medium does not alter the physiological properties of insulin-producing islets.

24.5. Quality controls/release criteria

Pancreatic islet cells exhibit a wide variety of functions that should be tested during quality control procedures. The TE should define – alongside the general tissue-and-cell release criteria – additional criteria for pancreatic islet transplantation, including:

a. quantification of the pancreatic islet cell mass (total islet number and the islet equivalent, known as IEQ), or of the number of insulin-positive cells;
b. cell viability (e.g. qualitative determination by Hoechst/propidium iodide, fluorescein diacetate/ethidium bromide or functional assessments);
c. microbiological testing;
d. bacterial endotoxin testing [8] (see §10.3.4);
e. beta-cell function (e.g. glucose-stimulated insulin secretion or insulin synthesis); but this information is not available prior to transplantation in all programmes.

Many of the currently utilised biological assays measuring islet functions and sterility are not always rapid enough for use in routine release testing because of the short period between pancreatic islet isolation and transplantation, varying from several hours to a few days. The TE should define how it will deal with incomplete test results.

Following confirmation of product identity and integrity of the pancreatic islet graft, islets will be transplanted into the portal vein as an inpatient procedure. Alternative routes of administration should demonstrate adequate safety.

24.6. Packaging and distribution

Transport temperature is usually maintained at 12-25 °C.

Pancreatic islet cells are transported in liquid media, so special notice might be necessary, depending on airline transport regulations (see Chapter 11 and Chapter 14).

24.7. Traceability

The attached documentation for the clinical transplantation centre should include, for example, details of the donor, organ transport/ischaemic time, pancreas quality, quantification of the pancreatic islet cell mass or of the number of insulin-positive cells, sterility, viability and function. Records covering the complete process from donor to recipient should be kept at the TE, and it should be possible to trace also other organ recipients from the same donor, and vice versa.

24.8. Biovigilance

Any unforeseen events influencing islet isolation and storage conditions are to be considered as adverse events that should be recorded, and reported to the competent authority. Some examples are:

- loss of pancreatic islets during isolation or temporary storage, due to failure of equipment and monitoring systems (e.g. overheating, carbon dioxide concentration, cooling),
- loss of pancreatic islets due to incorrect use of media (e.g. pH problems, sterility of media, concentration of additives, shelf-life).

Although islet transplantation is a relatively safe procedure, serious adverse reactions are not infrequent (see Table 24.1). Data collected by the Collaborative Islet Transplant Registry (CITR) show that one-third of all islet recipients have experienced at least one serious adverse reaction (SAR) in the first year after islet transplantation. Most of the reported SARs were related to the immuno-suppression therapy and the islet infusion procedure (bleeding and blood clots, intraperitoneal or liver subscapular). Approximately 91 % resolved with no residual effects [9, 10]. The incidence of SARs has declined significantly in recent years. Life-threatening events occurred in 24 % of recipients in 1999-2003 and only 4 % in 2011-2014 [10, 11]. For further guidance on biovigilance, please refer to Chapter 16.

Table 24.1. **Non-exhaustive list of possible reportable SARs in islet transplant recipients [10, 11]**

Infusion procedure-related	
	• haemorrhage • portal thrombosis • transient transaminitis
Immunosuppression-related	
Haematological	• anaemia • leukopaenia • neutropaenia
Metabolic	• dyslipidaemia
Gastro-intestinal	• oral ulcers (sirolimus) • diarrhoea (mycophenolic acid) • CMV colitis
Respiratory tract	• upper respiratory infections • interstitial pneumonitis (sirolimus)
Neurological	• neurotoxicity (tacrolimus)
Genito-urinary	• urinary infections • ovarian cysts • dysmenorrhoea • nephropathy • proteinuria
Cutaneous	• infections • cancer

24.9. Developing applications for patients

In the last several years, some innovative applications have been investigated and developed, based on somatic cell and gene therapy:

- human embryonic stem cells differentiated into pancreatic beta-cell precursors [12];
- encapsulation of insulin-producing cells;
- hepatic insulin gene therapy (pre-clinical).

24.10. References

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Chapter 25: **Hepatocytes**

25.1. Introduction

Hepatocyte transplantation is an alternative treatment to liver transplantation for patients with metabolic liver diseases or acute liver failure, or as a temporary support for patients with liver failure while waiting for an organ transplant [1, 2]. Patients with metabolic liver diseases are characterised by deficiency of one particular enzyme or protein, giving rise to hepatic and/or extrahepatic disease while all other liver functions are unimpaired. Thus, replacement of the whole liver by liver transplantation may not be required, and selective replacement of a fraction of the liver cell mass should be therapeutic. There is evidence that replacement of 5 %-10 % of the liver with healthy donor hepatocytes can correct a wide range of inherited metabolic liver diseases [3, 4]. In patients with acute and chronic liver failure, hepatocyte transplantation could provide temporary liver support until the native liver has recovered or a whole liver is available for transplantation.

Hepatocyte transplantation has potential advantages over whole-organ transplantation: the procedure is a less invasive approach, resulting in lower morbidity and mortality; it can be repeated several times; and it is reversible. Functional hepatocytes can be isolated from unused segments of donor livers that had been retrieved for whole-organ transplantation; and, in contrast to whole organs, cells can be cryopreserved and stored until needed.

This chapter defines the additional specific requirements for liver tissue and hepatocyte isolation for transplantation only. The following generic chapters (Part A) of this Guide all apply to hepatocyte isolation and transplantation and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

25.2. Donor evaluation

Liver tissue for hepatocyte isolation can be procured from donors after brain death (DBD) and from donors after circulatory death (DCD).

Liver tissue can also be procured from healthy living donors. In theory a healthy living donor could donate a part of their liver. However, this procedure

has been rarely performed so far because of the risk of morbidity and mortality for the living donor when balanced against the results obtained in clinical hepatocyte transplantation [5]. The liver from a living donor can also be the explanted liver in a so-called domino procedure where a patient is undergoing a liver transplantation [6], provided that the indication for the transplant (for example maple syrup urine disease) [7] is not considered to be a contraindication for the hepatocyte recipient. However, explanted livers of patients with familial amyloidotic polyneuropathy (FAP) are usually used for transplantation in another recipient rather than for the preparation of hepatocytes.

At present, hepatocyte transplantation is limited by the scarcity of donor livers rejected for whole-organ transplantation, that is, by the lack of adequate sources for viable human hepatocytes. Steatotic donor livers, which are becoming more common with the increasing incidence of obesity in European populations, are currently not considered to be a viable source of cells for hepatocyte transplantation, as steatotic hepatocytes display impaired metabolic function and lower engraftment [8]. The average hepatocyte yield after perfusion varies from 3×10^6 to 2×10^7 hepatocytes per gram of tissue, with variable viability yields reported (20-85 %) [8]; several billion cells are generally infused into one patient. Primary human hepatocytes do not proliferate *in vitro* and therefore cannot be expanded. Cryopreservation may have harmful effects on the viability and metabolic function of the cells [9].

All these limiting factors have prompted researchers and clinical teams to investigate the use of hepatocytes produced by the differentiation of pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) [10], which can both be indefinitely amplified and have the potential to become a permanent source of quality-controlled hepatocytes. Clinical-grade hepatocytes derived from these cells are now being produced by small companies and should be tested for clinical use in the coming years.

Donor criteria for hepatocyte donation are the same as those generally applied for organ donation for liver transplantation. Donors positive for HIV, HBV or HCV, as well as for malignant tumours, are excluded. Conditions to be evaluated as part of the donor-selection process are:

a. liver-originated disease of the donor that could be transferred to the recipient and cause disease, e.g. hyperoxalosis, familiar amyloidotic polyneuropathy;

b. alterations to the liver vessels that could complicate perfusion and isolation of hepatocytes (though this is uncommon);

c. donor liver characteristics that might affect hepatocyte quality, such as the size of liver tissue, the degree of steatosis and the length of both warm and cold ischaemia and hypoxia [11, 12].

Neonatal livers are not generally considered for organ transplantation in view of the increased incidence of thrombosis and due to size limitations. Neonatal livers may, however, be a valuable source of hepatocytes and their function is comparable to (may even be superior to) hepatocytes derived from adult donors. Post-thaw viability of cryopreserved neonatal hepatocytes is significantly higher when compared to adult hepatocytes [13, 14].

25.3. Procurement

Liver tissue is usually procured from deceased donors by the surgical liver-retrieval team of the transplant unit. Staff performing the procurement must be adequately trained in liver retrieval. Liver tissue should be flushed either *in situ* or *ex vivo* with an appropriate organ-perfusion solution through the hepatic artery and/or portal vein.

The procured liver is then placed in an appropriate organ-storage solution and triple-packaged in sterile packaging. This package should then be placed in another container that ensures a temperature of 2-8 °C and protects the recovered tissues during transport. Organ-perfusion machines are currently being evaluated for storage and transportation of liver tissue for organ transplantation and could potentially lead to a change in practice.

25.4. Processing and storage

Organs are transported to the designated isolation facility for processing. Depending on the size of the organ, the liver may be divided and perfused in parts. It has been reported that liver tissue preserves liver function better than isolated hepatocytes, so for repeated infusions of fresh hepatocytes (i.e. not cryopreserved) it may be better to isolate hepatocytes from different segments at different times to assure good perfusion and to minimise the time of isolated cells in suspension [15]. Vessels are cannulated to ensure perfusion of the liver tissue.

The liver tissue is perfused in a 2- or 3-step procedure at 37-38 °C. First, buffer containing ethylene glycol tetra-acetic acid (EGTA) is pumped through

the tissue to remove divalent ions, thereby disrupting cell–cell connections, then the EGTA is washed away by perfusion with buffer only. Finally, the tissue is perfused with collagenase/protease to digest extracellular matrix [8]. In some protocols, the second (wash-out) step may be omitted. Addition of the antioxidant N-acetylcysteine to the perfusion solution when isolating hepatocytes from fatty liver has demonstrated significant improvement in cell viability and metabolic function, and may be added for isolation of hepatocytes for clinical use. Isolated hepatocytes are purified by low-speed centrifugation. Cells that meet the release criteria after quality assessment (see §25.5) can be transplanted immediately after isolation, or cryopreserved and stored. Cells for transplantation are suspended in transplant medium (Plasmalyte or Eagle's minimum essential medium) containing 300 mM glucose heparin and human serum albumin (4 % final concentration) at a concentration of approximately 1-2 × 10^7 cells/mL [16].

25.5. Quality controls/release criteria

Hepatocytes exhibit a wide variety of functions that can be individually tested. Indeed, quality testing could be made appropriate to the recipient's disease, e.g. measurement of urea synthesis for recipients with urea cycle defects, or phase II conjugation activity for patients with Crigler–Najjar syndrome [17]. However, when fresh hepatocytes are used, there is limited time for functional assessment before infusion. The most important quality-control tests are viability (should be > 50 %), as assessed by trypan blue exclusion, and number of cells. It should be remembered, however, that the trypan blue exclusion test detects only cell-membrane damage; it cannot detect apoptotic cells nor determine metabolic or physiological function. Functional tests should, however, be performed on aliquots of hepatocytes from the same batch used for transplantation, either in parallel or afterwards, for the evaluation of hepatocyte function for each batch/donor.

Cryopreserved hepatocytes have the advantage that more extensive quality and genetic testing can be performed, which is not possible when using fresh hepatocytes due to time constraints. However, current cryopreservation protocols induce severe hepatocyte damage, which decreases both viability and function [9]. Testing should therefore be repeated after thawing of aliquots.

The following tests could also be considered as quality-control tests:

a. plating efficiency on coated plates (collagen, laminin, fibronectin or EHS matrigel), ability to attach to each other (spheroid formation);
b. enzyme activities (cytochrome P450 activities, conjugation of bile acids, metabolism of molecular probes such as EROD, PROD, CDFDA);
c. synthesis (albumin, A1AT, bile acids, lipoproteins);
d. urea cycle activity, metabolism of ammonia into urea;
e. markers of apoptosis.

Since none of these endpoints have specifically been demonstrated to correlate with engraftment or *in vivo* proliferation of hepatocytes, no specific assays can be mandated at this time. If such assays are conducted, the data from any individual assay should not be considered sufficient cause to exclude the use of the cells for a transplant. These assays will provide additional information on hepatic function that can be used in conjunction with additional data, including trypan blue exclusion, to help in the decision whether to use or not use cells for a transplant or to evaluate outcome.

Limited testing (viability tested on trypan blue and sterility on Gram staining) is used when fresh cells are transplanted; however, subsequent analysis allows for retrospective data on sterility and function of the cells.

25.6. Packaging and distribution

Hepatocytes can either be transported under hypothermic conditions (2-8 °C) or cryopreserved. Hepatocytes transported under hypothermic conditions should be stored in an appropriate preservation solution. Transportation time under hypothermic conditions should be kept as short as possible, because hepatocytes decrease in viability and function over time [15].

25.7. Administration of hepatocytes

Although few cases of intraperitoneal and intra-splenic administration have been described, the most common route of infusion is into the portal vein. Intra-splenic infusion has been used in cases of liver cirrhosis, where intra-portal infusion is not possible or too risky. Intraperitoneal infusions are used when temporary liver support is needed for bridging. Intra-portal infusion is the main cell delivery route for clinical HT with the portal venous system accessed

by percutaneous transhepatic puncture or inferior mesenteric vein catheterisation [18].

There are two routes of infusion in the portal vein, either by percutaneous transhepatic catheterisation of a portal vein branch, or through catheterisation of an ileal vein by a mini-surgical approach. The transhepatic approach is now technically well mastered by radiologists but entails a risk of intraperitoneal bleeding and of portal thrombosis. Although both risks are low in patients with normal liver function (most hereditary metabolic liver diseases), they may be much higher in patients with liver failure and coagulation disorders (acute liver failure and acute-on-chronic liver failure). A complete study of coagulation disorders before administration and potential corrections of the coagulation disorders by intravenous infusion of fresh frozen plasma and platelets might be necessary. Clinical (intensive care unit) and radiological (ultrasonography) surveillance are required after the administration in order to detect intrahepatic haematoma, peritoneal bleeding or portal vessels thrombosis.

The ileal vein approach requires a McBurney incision. Catheterisation of a small ileal vein may be difficult in patients with unstable haemodynamic status.

Another risk of portal infusion of hepatocytes is obstruction of sinusoids by clumps of cells, resulting in an increase of the portal pressure [19]. A continuous monitoring of the portal pressure during the infusion should performed. Infusion rate must be slow: 1-2 mL/min [16]. It is followed by immunosuppressive treatment.

25.8. Traceability

Records covering the complete procedure – from donor selection to recipient transplantation – should be kept at the tissue establishment (see Chapter 2 and Chapter 14). If the donor also donated other organs, special care should be taken to ensure traceability from the organ donor to all other organ and tissue recipients, and vice versa.

25.9. Biovigilance

For all relatively new clinical applications of human cells, documentation of all adverse events and reactions is of particular importance because we can learn from them. For example, the above-mentioned lack of *in vitro* endpoints that correlate with engraftment or proliferation of hepatocytes *in vivo* will only be clarified after collecting sufficient data as well as monitoring adverse events during procurement and processing of hepatocytes (see also Chapter 16 for management of adverse reactions).

25.10. References

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Chapter 26: **Adipose tissue**

26.1. Introduction

Autologous fat transplantation in aesthetic and reconstructive plastic surgery has revolutionised surgical treatment for soft-tissue defect correction or volume augmentation in recent years. In 1893, Neuber reported the first autologous fat grafting [1]. With the invention of liposuction in 1977 and the proposed technique of reinjecting aspirated fat in the late 1980s [2], lipofilling procedure has become one of the most popular procedures performed by plastic and aesthetic surgeons [3]. Nowadays, the most common method of adipose tissue procurement and transplantation is Coleman's technique from 1994 [4].

Unlike with synthetic materials, there is no risk of rejection and the implementation costs are reasonable. Autologous fat transplantation can be used in both aesthetic and reconstructive plastic surgery for soft-tissue augmentation. In addition, it does not induce an immune response in the recipient and, as a filler material, is abundantly available.

Primarily, procurement, banking and transplantation of autologous adipose tissue should non-restrictively be supported in cases of reconstructive indications (e.g. Romberg's disease, depressed scars, eyelid depression, pitting acne, post-traumatic defects, subcutaneous adipose atrophy of senility, breast reconstruction, improvement of function and appearance of irradiated tissues, correction of asymmetry in Poland's syndrome, soft-tissue defect correction), though results after autologous fat transplantation are better in cases of aesthetic indications (e.g. wrinkles, volume augmentation), probably due to better recipient site condition [2].

Autologous fat transplantation represents a simple solution to restoring the profile of the breast during reconstruction procedure. In fact, in breast cancer surgery, lipofilling is usually used for the correction of defects and asymmetry following tumour excision [5]. Adipose tissue is preferred over other types for the correction of volume and contour defects because fat is autologous, abundant and easily procured [6].

One of the most common indications for this therapy is reconstruction after tissue removal in patients with breast cancer. Other indications for this procedure can also include lipodystrophy due to acquired immune deficiency syndrome (AIDS), so positive results in infectious disease testing can be accepted, as the tissue will be for autologous use. For more than 100 years, autologous fat transplantation has been used to correct subcutaneous lipoatrophy, resulting from hemifacial atrophy, acne, trauma, lipodystrophy and sclerodermia, cutaneous lupus erythematosus and defects resulting from accident, infection or surgery. Adipose tissue has been used in post-mastectomy pain syndrome; in fact, breast-conserving surgery has become a well-established alternative to mastectomy in the treatment of breast cancer, providing a less invasive treatment [7]. Fat transplantation is efficient also for breast augmentation in patients suffering from micromastia, postexplantation deformity, tuberous breast deformity and Poland syndrome [8]. Adipose tissue has been used

also for the correction of cicatricial ectropion [9] and for superior sulcus deformity [10].

Most of the clinical data obtained from adipose tissue transplantation are from patients receiving lipofilling directly after procurement in a one-step surgical procedure.

So far, the main obstacle to achieving favourable outcomes is its unpredictable long-term results due to the high rate of resorption in the grafted site, which means overcorrection of the treated zone or additional grafting including repeated procurement, leading to increasing cost and surgical risks as well as discomfort for the patient. There are several approaches to improving fat graft survival, including changes to procurement and processing techniques.

The initial isolated adipose tissue is composed of adipocytes and stromal vascular fraction (SVF) cells, which include adipose stem cells, preadipocytes, fibroblasts, vascular endothelial cells, and a variety of immune cells [11]. It has become apparent through extensive research in the past decade that SVF cells and adipose stem cells might improve fat graft survival, largely through their angiogenic properties [12, 13].

In order to avoid multiple procurements, protocols are developed to store adipose tissue in tissue establishments, sufficient for several treatments. In 2001 Shoshani *et al.* reported the successful frozen storage of adipose tissue for repeated fat injection in a domestic freezer [14]. One year earlier another group was less enthusiastic and advised against storage [15].

Autologous adipose tissue from liposuction is being used increasingly in plastic surgery for reconstructive procedures. Some of the implanted tissue is resorbed, so surgeons treating large defects frequently apply a staged approach; its absorption rate has been reported to be 30-70 % [16, 17]. This approach can be facilitated by storing all or part of the tissue collected from the initial liposuction and implanting it during subsequent interventions. 'Fat banking' eliminates the need for repeated liposuction and, thereby, reduces cost and the risk of morbidity. However, the overall quality of the cryopreserved adipose tissue is still less ideal than the fresh one.

The following generic chapters (Part A) of this Guide all apply to adipose tissue banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

26.2. Donor evaluation

The criteria for donor selection to be applied are the criteria for autologous donation. The patient must be provided with sufficient information on the process (including the planned storage period and tests performed) and must sign an informed consent form.

Additionally, it should be ascertained that donors do not have any major systemic diseases or lipid disorders, and that they are not underweight. If the adipose tissue is to be stored and not only used in the same surgical procedure, infectious disease testing must be performed for all autologous adipose tissue patients, as described in Chapter 5. Patients known to have HIV or hepatitis B/C can be accepted for autologous use. In this case, the tissues and cells must be labelled accordingly (e.g. CAUTION: BIOLOGICAL HAZARD) and stored separately or under special conditions. (For further details, see §9.2.8 and §9.2.9).

26.3. Procurement

Usually, adipose aspirates are only used for immediate autologous fat grafting; therefore, adipose aspirates obtained from liposuction are usually discarded because currently there is not a widespread and well-established cryopreservation protocol to store the aspirates.

Risk assessment on the conditions of procurement, processing facilities and storage should be conducted, and appropriate mitigating actions should be taken to prevent cross-contamination. Particular attention should be paid to procurement conditions, because they support the initial quality and low bioburden of the adipose tissue.

Before surgery, the various adipose areas of the body are examined to identify natural fat deposits. The most common donor site is abdominal fat because it is one of the largest fat deposits. The second most common sites are the greater trochanteric region and the inside of the thighs and knee [5, 6].

Various procurement and preparation techniques have been introduced to obtain better and more reliable survival of adipose tissue. The fat tissue is usually procured with a specific cannula with negative pressure from abdomen, thighs and hip with Coleman technique [4, 16], but several techniques for procurement are currently being employed. Adipose aspirates are collected in a specific container (for example, a Luer-lock syringe) and should be transferred immediately (at a transport temperature of 4 °C) to the processing unit.

26.4. Processing

There are several published protocols for processing adipose tissue, but there is no evidence to prefer one technique above another.

Tissue processing includes washing (e.g. 0.9 % NaCl [2]), centrifugation (e.g. 300-3400 rpm for 3 min [2]) or decanting, eventually antibiotic decontamination, controlled-rate freezing (≈ 1 °C/min) with cryoprotective agents and then storage < −140 °C (in vapour phase of liquid nitrogen) to preserve maximum viability.

It should be taken into account that adipose tissue is very sensitive to external treatment (centrifuge, processing methods and temperature). In particular, Moscatello *et al.* described the requirements for cryoprotectants and controlled freezing/storage, and listed components which can affect the viability of transplanted adipose tissue [18]:

- procurement → procurement method, source location, donor age;
- processing → wash solutions, centrifugation, disaggregation;
- storing → media, cryoprotectants, storage temperature;
- recipient bed → infusion solutions, growth factors;
- implantation → method, location, flexibility.

There must be written protocols for all procedures related to liposuction and tissue transfer to tissue-processing facilities. Appropriate measures should be taken to minimise the risk of microbiological contamination, including possibly the addition of an 'antibiotic cocktail' to the lipoaspirate.

26.5. Quality control

To cryopreserve adipose tissue, quality control is an essential issue. Adipose tissues must be processed under sterile conditions and in an aseptic manner. All biological tests should be performed as described in Chapter 5 and Chapter 10, if the tissue is processed (regardless of the location where this is done).

Quality control must include microbiological testing of each batch. Histology, cell-viability and functionality controls are highly recommended. Microbiological analyses of procured tissue, rinsing solutions and tissue after possible decontamination must be carried out according to the *European Pharmacopoeia* (*Ph. Eur.*). In cases of positive results after decontamination, the adipose tissue should be discarded.

26.6. Preservation/storage

Literature clearly recommends the use of cryoprotectants when long-term storage of adipose tissue is desired [19-25].

Cryopreservation is one way to indirectly overcome the problem of absorption of the autologous fat graft results in repeated procurement procedures (with increasing cost and risks for the patient), as the patient only needs to undergo one procurement procedure. Unfortunately, an optimal technique for long-term preservation of adipose tissues is not available, and outcomes following implantation are mixed. Further studies need to be done in order to develop a better cryopreservation method for long-term preservation. The selection of cryoprotective agent is one of the key issues for obtaining optimal viability of adipocytes.

The most common cryoprotective agents used for adipose tissues are dimethyl sulphoxide (DMSO), trehalose [26, 27] and glycerol. A higher recovery of adipose tissue after cryopreservation can be achieved if cooling is performed slowly and thawing is done by fast warming [28, 29].

Additionally, Hwang *et al.* [30] described the storage of adipose tissue at – 20 °C.

When autologous adipose tissue is procured and stored, positive test results will not necessarily lead to discarding the tissue. For such tissues, isolated storage possibilities should be considered in order to exclude risk of cross-contamination or mix-ups.

A state-of-the-art research or validation study on the shelf life of preserved adipose tissue should be performed in order to determine the expiry date

according to the used processing and preservation protocol.

Processed adipose tissue should be kept below – 80 °C, but preferably in the vapour phase of liquid nitrogen (< – 140 °C). The thawing protocol must be gentle, when removing the cryoprotective agent as well the amount of free lipids and debris associated with loss of adipocytes.

26.7. Biovigilance

Any adverse reaction or event occurring during procurement, processing, thawing or reinjection of tissue must be notified, as described in Chapter 16.

Serious adverse reactions for adipose tissue transplantation include:

- graft failure (e.g. volume loss, calcification);
- malignancy possibly attributable to the transplanted tissue (mainly due to cancer stem cells in the autologous transplant);
- fat embolism.

Serious adverse events include:

- wrong tissue supplied for the intended surgical procedure;
- tissue supplied was damaged or transported at wrong temperature;
- tissue supplied beyond its expiry date.

No entries have so far been found in the Notify Library (www.notifylibrary.org) for the banking of adipose tissue.

26.8. Developing applications

Adipose tissue may also be a source of SVF or stem cells and can be cryopreserved before the cells are isolated [31, 32]. A search for clinical trials using SVF in www.clinicaltrials.gov reveals that 15 studies are currently actively recruiting for patients. Adipose-derived stem cells isolated from SVF and expanded *in vitro* are under investigation for a whole range of diseases [33, 34] (see §32.10).

26.9. References

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Chapter 27: **Medically assisted reproduction**

27.1. Introduction

This chapter refers to the medical procedures used to achieve pregnancy and live birth involving the identification, procurement (collection), processing and/or storage as well as distribution of at least one of the following reproductive tissues and cells: oocytes, ovarian tissue, sperm, testicular tissue, embryos. These procedures may be carried out using freshly collected and/or cryopreserved gamete(s), zygotes or embryos originated from the couple being treated ('partner donation') and also from gamete donors ('non-partner' or 'third partner' donation). For the procurement, processing and/or storage of ovarian and testicular tissue, we refer to Chapter 28: Fertility preservation. These contexts are, in general, addressed separately due to the different risks involved. Ovarian stimulation or any other clinical procedure that does not involve gamete procurement is not addressed in this chapter.

Medically Assisted Reproduction (MAR) is also referred to as Assisted Reproductive Technology (ART). However, MAR is a broader term, which includes ART, but also includes ovarian stimulation and intra-uterine insemination of sperm, whereas the term ART refers only to procedures where *in vitro* handling of gametes or embryos is included.

MAR is carried out in centres specialised in treating patients with fertility problems. These centres are usually a combination of a tissue establishment (TE) and an organisation responsible for human application, bringing together a clinical team and a laboratory team in a multidisciplinary unit.

Procurement, processing and/or storage and the distribution of reproductive cells may also be performed in separate gamete cryobanks not connected to a fertility clinic. The recommendations in this chapter concern all institutions where reproductive cells are handled.

MAR comprises various procedures, such as:

- Processing of sperm for the purpose of intra-uterine insemination. Sperm provided by the partner, or originating from a non-partner donor, is processed and transferred to the uterus directly prior to the estimated time of ovulation [1];
- *In vitro* fertilisation (IVF), either conventional, whereby collected and prepared sperm and oocytes are co-incubated (so-called routine or standard IVF), or intracytoplasmic sperm injection (ICSI), whereby a single spermatozoon is injected into a mature oocyte. IVF involves procurement (collection) and processing of gametes, fertilisation, culture and transfer of embryos into the uterus. Oocytes and/or sperm might be provided by a partner or by non-partner donor(s);
- Cryopreservation and storage of gametes, embryos and/or gonadal tissue;
- Pre-implantation genetic testing (PGT) that uses genetic identification methods to diagnose or screen oocytes or embryos *in vitro* to exclude known inherited disease or chromosomal rearrangements incompatible with the birth of a healthy child. These methods include pre-implantation genetic diagnosis (PGD, now

named PGT-M for monogenic/single gene defects, and PGT-SR for chromosomal structural rearrangements) and pre-implantation genetic screening (PGS, now named PGT-A for aneuploidy testing).

Procedures such as cryopreservation of gametes or gonadal tissue can also be used in patients with certain diseases (e.g. cancer, some chronic diseases) for whom treatment may be potentially harmful to their fertility. In those cases, long storage of their cryopreserved reproductive tissues and cells may be proposed to children, adolescents and male or female adults. This approach, called 'fertility preservation', is addressed in Chapter 28 and is also an option for fertility preservation for non-medical reasons. MAR treatments can also be proposed to couples at risk of transmitting a serious transmissible disease – e.g. human immunodeficiency virus (HIV) or hepatitis B and C viruses (HBV and HCV) – to the partner and/or the child. These practices are applied only after risk assessment of vertical and horizontal disease transmission and taking into account the patients' health condition. In some countries, MAR can be undertaken in single women or homosexual female couples. In a few countries in Europe, under stringent conditions, surrogate motherhood is allowed for women without a uterus or with a non-functional uterus, or for male homosexual couples. Through insemination or embryo transfer, the surrogate mother carries and gives birth to a child for the intended parents.

MAR is performed in most countries in Europe. Each year, the European Society of Human Reproduction and Embryology (ESHRE) publishes a report of activity in European countries, based on voluntary declarations. The latest published ESHRE data (from 2014) include data from 1 279 clinics in 39 countries and report 776 556 treatment cycles, including 508 433 IVF/ICSI, 192 027 of frozen embryo replacement (FER), 56 516 of egg donation (ED), 292 of *in vitro* maturation (IVM), 15 894 of PGT and 3 404 of frozen oocyte replacements. European data on intra-uterine insemination using husband/partner's semen (IUI-H) and donor semen (IUI-D) were reported from 1 364 institutions in 26 countries. A total of 120 789 IUI-H and 49 163 IUI-D cycles were included [2].

This chapter aims to provide guidelines that can help to conceive healthy singletons carried to term, which is the ultimate goal of MAR. The medical activities involved may in some countries be considered ethically sensitive. The procedures described here are intended to achieve efficient results in terms of delivery rates and also address the safety of patients, donors and children born. For partner donation, priority is given to using the reproductive cells from the partner even if the sample exhibits poor quality (e.g. ICSI for patients with decreased sperm quality) and/or at least one partner is infected by HIV or poses a high risk of transmission of a genetic disease.

In addition, the following generic chapters (Part A) of this Guide all apply to MAR and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent, but only for non-partner donors,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling, but only for non-partner donors,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

This chapter defines additional specific requirements for MAR. Procedures may vary from country to country as determined by national legislation.

27.2. Quality management, risk management and validation

The implementation of a quality management system is mandatory and will contribute to compliance as well as to the success of a given MAR programme. This section should be read in conjunction with Chapter 2; however, certain MAR-specific matters concerning quality management are addressed below.

27.2.1. Risk-assessment analysis for laboratory activities

Risk management will help in assessing and prioritising the possible existing hazards in order to monitor and control them, so that the proba-

bility of an adverse event occurring will be kept to a minimum. The most commonly applied methods of risk assessment are: FMEA (Failure mode and effects analysis), FMECA (Failure mode, effects and criticality analysis) and Hazard analysis and critical control points (HACCP). (The process of risk assessment is described in §2.17.)

27.2.2. Validation

For MAR procedures, currently no test system with the necessary biological sensitivity exists, apart from an assessment of the actual clinical performance. New equipment should be qualified and methods should be validated before entering clinical use. Such validation should include installation, operational (technical) qualification and performance qualification, by comparison with laboratory key performance indicators (KPI) for similar types of equipment or methods already existing in the laboratory. The Special Interest Group in Embryology of ESHRE, in collaboration with Alpha Scientists in Reproductive Medicine, have established minimum performance (competence) levels and aspirational (benchmark) values for the IVF laboratory. Based on the information presented, each laboratory should select its own set of KPIs founded on laboratory organisation and processes [3].

Likewise, it is desirable to ensure that the necessary research and development has been conducted before bringing new techniques into clinical practice, preferably by means of well-designed randomised control trials with a follow-up of all children born from the procedure. The steps needed to validate a new technology before its introduction into clinical practice have been described (see Figure 27.1) [4, 5]. In some countries, a formal authorisation by Health Authorities is required before introducing a new technology into clinical practice.

27.2.3. Materials, consumables and reagents

All consumables and media should be quality-controlled, fit for their purpose, of embryo-culture grade and, when available for the intended use and providing at least equal results as qualified alternatives, be CE-marked.

Specific culture media that fulfil the requirements of gametes and embryos are needed during all processing, fertilisation, culture, cryopreservation and other processing activities in MAR.

Patient or donor serum or follicular fluid should not be used as a protein supplement. Commercial suppliers of human serum albumin or media containing a serum-derived protein source should provide evidence of screening according to European and/or national regulations.

27.3. Recruitment of potential donors, identification and consent

27.3.1. Donor recruitment – non-partner donation

As with any tissues and cells, the donation of reproductive material should follow the principles of voluntary and unpaid donation, as described in Chapter 3 (see §3.2.1.2, which specifically relates to MAR). However, expenses related to the donation can be reimbursed, and loss of earnings may be compensated for.

National regulations will need to pay special attention to the existence of advertising and false or misleading promotion. In addition, the activities of TEs related to donation should have a non-profit character, which means that only the actual costs of the additional services (those required to allow the donation to be performed) should be charged.

27.3.2. Donor consent – partner and non-partner donors

As mentioned above, MAR treatments can be undertaken with partner gametes or non-partner-donated gametes (i.e. 'partner donation' or 'non-partner' donation). Chapter 3 describes consent-giving procedures for donation by living donors, and this also applies to gamete and embryo donors in the case of non-partner donation. Also for partner-donated gametes, fully informed written consent is mandatory, and this section describes additional aspects of these specific consent forms that should be addressed. In MAR, consent forms may be separated for the female and male patients, although for certain treatments – and especially in partner donation – they could be combined in one document. It is important to emphasise that both partners need to sign these combined documents on partner donation. Examples of separate consent forms for treatment and storage are given in Appendix 11 and Appendix 12 for the female patient and in Appendix 13 for the male patient.

The couple (or individual) to be submitted to MAR treatment should receive written and oral information (during medical consultation with the

physician or paramedical personnel, through information sessions, leaflets, website etc.) concerning the following:

a. national legislation about MAR and its implications for those who have access to assisted reproduction;
b. in cases of non-partner donation and embryo donation, the implications of current national legislation for the possible anonymity of the donor and for the possible right of the offspring to know their origins;
c. possibility of withdrawal of consent to treatment;
d. chances of success based on their medical history, the degree of invasiveness and the possible risks of the treatment (including multiple pregnancies); and, in treatments involving hormone administration, special reference must be made to ovarian hyperstimulation syndrome (OHSS) and the risks linked to oocyte retrieval (e.g. bleeding, infection or perforation of bladder or bowel);
e. testing for genetic and infectious diseases, and evaluation carried out in gamete donors in non-partner donation;
f. full description of the treatment at each stage of its implementation;
g. possibility of cryopreservation and storage of gametes and supernumerary embryos, and the options for future use according to national legislation;
h. total cost of the procedure, and existing reimbursement policies, if applicable;
i. possibility of the physician not proceeding with the entire treatment (or some of its parts) for medical or deontological reasons;
j. possible ethical issues regarding MAR;
k. possible psychological effects resulting from treatment using MAR;
l. possible risks to the offspring resulting from MAR treatment, particularly in multiple pregnancies, and the limited follow-up data on the long-term health of those born from the treatment.

In addition, psychological counselling should be offered to the couple/patient.

MAR treatment normally comprises a series of individual treatments, so consent forms should be signed for each treatment or else be valid for consecutive treatments until the treatment is successful, until a predefined date or until relevant circumstances change.

If the treatment is undertaken with cryopreserved gametes or embryos, consent for thawing should be given for each treatment. This policy would prevent a treatment from being initiated by either of the partners without the knowledge of the other.

In the consent form, the couple should state whether embryos or gametes may be cryopreserved or not. Depending on the possible options, the couple should receive information on the different success rates and on the implications of national legislation regarding the fate of cryopreserved gametes or embryos. The destiny of cryopreserved gametes or embryos could be: keep for own reproduction, donate to another couple, donate for scientific research or destroy. The consent form could also specify the duration of storage.

There should be specific consent whenever additional methods beyond IVF and cryopreservation are used. A very specific case in MAR is the possibility, in some countries, of consent for the use of the remaining gametes or embryos after one of the partners has died ('posthumous donation'). This needs to be clearly specified in the consent form.

A woman who enters IVF treatment could decide not to use all of her oocytes for her own treatment, but to donate some of them to other couples/individuals for reproductive purposes. This procedure is called 'oocyte sharing' and implies that this woman should be considered both a patient and a non-partner donor. Screening should therefore be conducted as described in Chapter 4 and Chapter 5, and specifically for ART in §27.5.1 and §27.5.2 below.

27.4. Donor evaluation

27.4.1. Evaluation of partner donors

27.4.1.1. *Interview*

Couples who experience problems in conceiving should be evaluated together because infertility constitutes a mutual medical problem for the couple. Counselling before, during and after treatment is widely practised and is recommended because fertility problems, investigation and treatment can be causes of psychological stress.

27.4.1.2. *Taking of medical history and physical examination*

Full medical history – including surgical, sexual, contraceptive, genetic, family and pregnancy history, as well as travel history for the assessment of certain viral diseases – should be taken from both

partners. Both partners should also undergo a physical examination.

27.4.1.3. *Screening of the female*

Screening of the female should include:

a. assessment of ovulation, with a complete menstrual history; ovulatory dysfunction can be due to hypothalamic, pituitary or ovarian dysfunction;

b. assessment of ovarian reserve, including biochemical tests and ultrasound imaging of the ovaries;

c. assessment of tubal patency;

d. assessment of uterine abnormalities, such as submucous fibroids, polyps, adhesions or other Müllerian malformations (septae, bicornuate uterus);

e. testing for immunity to rubella should be carried out before treatment; vaccination should be offered to seronegative women before they commence any MAR treatment.

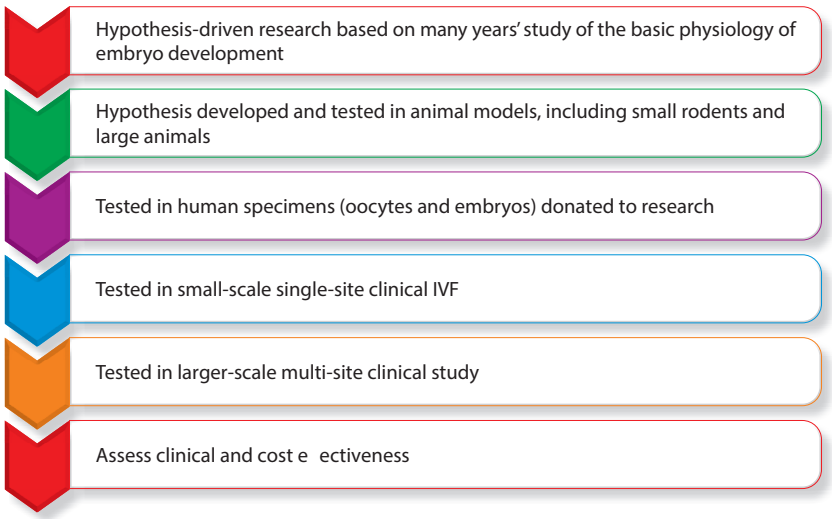
27.4.1.4. *Screening of the male*

a. At least one diagnostic semen analysis should be carried out before starting treatment. If the seminal parameters are altered, a second semen evaluation can be repeated after 2/3 months; procedures and reference values are described in the *WHO laboratory manual for the examination and processing of human semen* [6];

b. men with azoospermia or severe oligozoospermia should be screened for genetic abnormalities (e.g. Klinefelter syndrome or Y-chromosome deletions) and, if a chromosomal abnormality is detected, appropriate genetic counselling should be offered; in the presence of obstructive azoospermia, cystic fibrosis or renal-tract abnormalities should

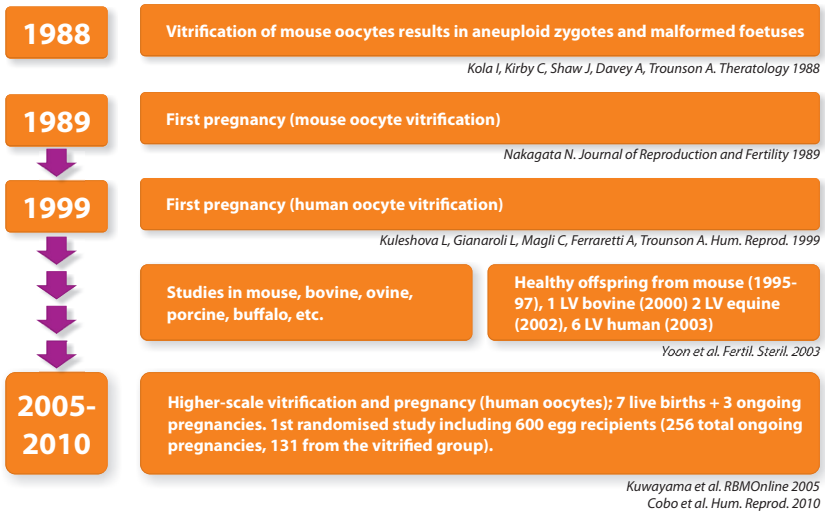
Figure 27.1. **Validation of new technologies in medically assisted reproduction**

1. Theoretical steps to be followed when introducing a new technology into clinical use in medically assisted reproduction



Adapted from "When and how should new technology be introduced into the IVF laboratory?" *Hum Reprod* 2012.

2. Example of a validation process in medically assisted reproduction



Source: Adapted from Harper J, Magli MC, Lundin K et al. When and how should new technology be introduced into the IVF laboratory?" *Hum Reprod* 2012;27(2):303-13.

be screened for; besides genetic testing, there should be hormonal testing and a scrotal ultrasound performed in order to establish a diagnosis of testicular failure.

27.4.1.5. *Inclusion/exclusion criteria for treatment*

A full medical evaluation will help to determine if a couple is suitable for MAR treatment. The risk–benefit analysis should be estimated on an individual basis.

The number of repeat cycles should be based on the individually estimated probability of a live birth.

27.4.2. Evaluation of non-partner donors

The purpose of evaluation in non-partner donors is to ensure that donors whose gametes may cause a health risk in the recipient or to the offspring (e.g. infectious disease, genetic disease) can be excluded. In addition, ensuring that the donation process does not cause harm to the health of the donor is equally important.

To donate his/her sperm/oocytes, the potential donor must be submitted to:

a. consultation and counselling with a healthcare professional;
b. completion of a health/medical/donor family history questionnaire;
c. psychological assessment;
d. medical examination: gynaecological examination and ultrasound for female donors, and genital examination in males;
e. laboratory testing (including screening for infectious diseases);
f. blood group typing;
g. genetic testing as indicated by family history and prevalence of carrier status in specific populations; karyotype testing is strongly recommended, and other extensive genetic screening for common recessive genetic mutations – like carrier testing for cystic fibrosis and spinal muscular atrophy (SMA) – is now available and should be considered in order to reduce the risk of transmitting genetic disease to the child;
h. semen analyses for sperm donors; freeze–thaw test may also be recommended, to assess the quality of the sperm after freezing and thawing;
i. assessment of ovulation and ovarian reserve (including endocrine work-up) in oocyte donors;
j. informed consent before any procedure.

27.4.2.1. *Exclusion criteria for oocyte donors*

a. age < 18 years or > 36 years;
b. positive results in tests for dominant genetic disease and/or infectious disease;
c. any risk factor to her own health;
d. unsuitability for donation based on interview.

27.4.2.2. *Exclusion criteria for sperm donors*

a. age < 18 years and > 45 years;
b. positive results in tests for dominant genetic disease and/or infectious disease;
c. poor sperm quality;
d. unsuitability for donation based on interview.

In cases in which embryos are donated, the partners of donors from whom the gametes originated must both be considered non-partner donors and must comply with the general criteria for non-partner donation in this section and in Chapter 4 and Chapter 5.

27.4.2.3. *Psychological examination (non-partner donation)*

In MAR, psychological evaluation of non-partner donors is highly recommended and should focus on a psychological anamnesis (including but not limited to: looking at motivation, looking at pattern of personal stability, discussing the psychological ramifications of being a gamete donor, giving psychological guidance in the preparations for becoming a gamete donor), often in combination with a personality and/or psychological diagnostic test.

27.4.2.4. *Welfare of non-partner gamete donors*

To secure the welfare of gamete donors is very important. Although the minimum age limit is 18 years, it could be good clinical practice not to include very young gamete donors, but to recruit an older donor group who have proved their fertility. It is important to counsel male and female non-partner gamete donors that the donors' DNA will be transmitted to any future children. Therefore, donating gametes may have a potential impact on the donor and his/her partner and family, including their own future children and their offspring. Although the donor can be unknown to the recipient, in some countries it is possible that the identity of the donor might be disclosed to the child. It is therefore also important, where applicable, to address the possibility of contact between future donor children and their gamete donors and to make sure that existing regulations about future contact are clearly described in the consent for donation.

Regarding female donors, the risk of OHSS, bleeding, infection and ovarian torsion should be minimised, as it should be in all women submitted

to MAR treatment. The number of times an oocyte donor may donate may be determined by several factors, such as the number of children and/or families achieved with this donor's gametes, the medical and psychological risks to the donor and the relevant legislation in the country of donation. Oocyte donors should preferably be accepted after having achieved a successful pregnancy of their own. Also for male donors, the number of donations should be determined by the number of children and/or families achieved with this donor's gametes, the psychological risks to the donor and the relevant legislation in the country of donation. Implementation of national registries for gamete/embryo donors as well as for recipients should be encouraged.

27.5. Donor testing

The purpose of testing gamete donors is to prevent transmission of severe infectious and genetic diseases from the donor to the recipient and their offspring, and to protect the staff while handling the patients and their gametes.

Testing of gamete donors is discussed here separately for each type of donation:

a. partner donation
b. non-partner donation.

Less strict biological testing is justifiable in the donation of reproductive cells between partners who have an intimate physical relationship (i.e. for partner donation).

27.5.1. Testing in partner donation

The following tests must be carried out:

a. anti-HIV-1 and anti-HIV-2;
b. HBsAg (HBV surface antigen) and anti-HBc (HBV core antigen);
c. anti-HCV.

Beyond these tests, TEs should, based on analyses of risk or depending on stricter national legislation or recommendations, also carry out additional tests:

a. syphilis (a treponemal-specific test or a non-specific treponemal test can be used);
b. testing for human T-lymphotropic virus (HTLV)-1 antibody for donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or where the donor's parents originate from those areas;
c. additional testing may be required in certain circumstances, depending on the donor's history of travel/exposure as well as the characteristics of the tissue or cells donated, e.g. RhD (D antigen), diagnostic tests for malaria, Zika virus, *Cytomegalovirus*, *Chlamydia* and *Trypanosoma cruzi* (infectious agent for Chagas disease).

Blood samples for serology testing must be obtained before the first donation. In European Union (EU) member states, this must be done ≤ 3 months before the first donation. For further partner donations, additional blood samples must be obtained according to national legislation, but ≤ 24 months from the previous sampling.

Positive serology test results do not exclude donation between partners. Nonetheless, robust procedures should be in place to prevent the risk of contamination, to partner or to personnel, and of cross-contamination. If results for tests of HIV-1 and -2, HBV or HCV are positive, or if the donor is known to be a source of infection risk, a system of separate handling and storage must be put in place. In the case of positive serological tests, partner donation should be performed after consultation with a specialist on viral infections.

If the TE can demonstrate that the risk of cross-contamination and exposure to personnel has been addressed through validated processes, biological testing may not be required in the case of sperm processed for IUI and not intended for storage.

27.5.2. Testing in non-partner donation

The following biological tests must be carried out for each donation:

a. anti-HIV-1 and anti-HIV-2;
b. HBsAg and anti-HBc;
c. anti-HCV;
d. syphilis (a treponemal-specific test or a non-specific treponemal test can be used);
e. in male donors: *Chlamydia trachomatis*. In the EU, this must be done from a urine sample by a nucleic acid test (NAT) but recent scientific data suggest ejaculate testing may be more sensitive.

In some cases, further tests may be required:

f. if required by stricter national legislation, e.g. in some countries, testing for HTLV-1/2 is mandatory;

g. testing for HTLV-1 antibodies must be done in donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or where the donor's parents originate from those areas;

h. additional testing may be required in certain circumstances, depending on the donor's history of travel/exposure and the characteristics of the tissue or cells donated, e.g. RhD – D antigen, diagnostic tests for malaria, antibodies to *Cytomegalovirus*, antibody to *Trypanosoma cruzi*, Zika virus infection. Latest epidemiological updates can be found at the European Centre for Disease Prevention and Control (http://ecdc.europa.eu/en/Pages/home.aspx).

All serum samples must be obtained at the time of donation. Sperm donations must be quarantined for ≥ 180 days after the last collection, after which repeat testing is required. If, at each donation, serology testing is combined with NAT for HIV, HBV and HCV, quarantine is not necessary unless further tests are required as mentioned in points f, g, h above. It is recommended that the same testing approach be used for oocyte donors, allowing for the safe use of cryopreserved or fresh oocytes if NAT is done at the time of donation. Oocyte donation could be considered as starting at the first day of stimulation, with the sample for testing to be taken at that time.

27.6. Procurement

27.6.1. Sperm

27.6.1.1. *Collection by masturbation*

Semen is usually obtained through manual stimulation or penile vibratory stimulation, or in rare cases through intercourse using a specially designed condom free of spermicidal substances. Patients should be given clear instructions regarding the collection of the sperm sample (hygiene, sexual abstinence, timing, etc.).

After thorough cleaning of the hands and genital area, semen is collected into a sterile collection container. The circumstances under which a semen sample is collected and delivered to the laboratory can influence the results of semen analyses. Since the time that spermatozoa are kept in the ejaculate can affect their survival, motility and fertilising ability, the start of diagnostic/therapeutic treatments must be standardised. If the sample can be collected in a special room adjacent to the laboratory, the risk of delays during transportation and cooling of the sample is minimised. This situation calls for appropriate design and equipping of the laboratory and semen-collection room. In general, patients are asked to collect a semen sample after 2-7 days of abstinence from ejaculation. Both too long and too short period of abstinence may influence the quality of the sample.

Semen samples should be collected into sterile, plastic containers (preferably tested for sperm toxicity). The use of spermicidal condoms, creams or lubricants must be avoided. The container should be clearly labelled, and correct identification should be confirmed by the patient. After collection, the sample should be delivered to the laboratory as soon as possible, avoiding extreme temperatures (< 20 °C and > 37 °C). Analysis should start within one hour of collection.

For traceability of samples to be used for treatment, records should be kept of the type of container used, the time of collection and the time interval between collection and analysis/preparation. The use of medication, fever during the previous months and completeness of the ejaculate collection should be documented.

27.6.1.2. *Surgically retrieved sperm*

In patients diagnosed with non-obstructive or obstructive azoospermia, sperm can be retrieved by surgical means from the testis or epididymis in an operating room. The method used is dependent upon the nature of the cause. This method may also be used for patients who cannot produce an ejaculate by masturbation.

27.6.1.2.1. Collection of sperm from the epididymis

Percutaneous epididymal sperm aspiration (PESA) is a method for sperm collection if the vasa deferentia are blocked. It involves the use of a sterile needle to aspirate sperm from the epididymis without a surgical incision.

27.6.1.2.2. Collection of sperm from the testis

An alternative to sperm collection from the epididymis is collection of sperm from the testis. This can be performed by testicular sperm extraction (TESE), and possibly by tissue removal (testis biopsy), and could be accompanied by a histopathology study for diagnosis. TESE can also be undertaken *via* a percutaneous approach – testicular sperm aspiration (TESA) – using a sterile fine needle or a biopsy needle. This is a less invasive procedure but usually results in less material than when TESE is undertaken.

27.6.1.3. *Retrograde ejaculation*

In cases of retrograde ejaculation, the sperm ends up in the urinary bladder after ejaculation. Although a procedure rarely used today, sperm can in these cases be collected from the urine after voiding, where the urine pH has been increased by intake of bicarbonates. Should this method yield very low-quality sperm, epididymal or testicular biopsies could be a better option for these patients.

27.6.1.4. *Collection by electro-ejaculation*

In some patients (e.g. in case of injury to the spinal cord, pelvic surgery, multiple sclerosis, diabetes mellitus with nerve involvement, unexplained anejaculation), ejaculation by masturbation is not possible. In these cases, ejaculation can be stimulated using a rectal probe with electrodes. This low-voltage stimulation is usually sufficient to produce a semen ejaculate. However, the quality of the ejaculate is often not as good as that obtained by masturbation. Also in this case, epididymal or testicular biopsies could represent a better option.

27.6.2. Oocytes

Before oocyte collection from the ovaries, also known as oocyte retrieval, the patient will be given hormonal treatment to stimulate the growth and maturation of the follicles in the ovaries (so-called controlled ovarian hyperstimulation or COH). During treatment, the patient is monitored closely to follow the response to the hormonal treatment and to assess the risk of OHSS.

Oocytes are collected through transvaginal ultrasound-guided ovarian puncture and aspiration of follicular fluid. The procedure can be carried out under local anaesthesia (paracervical block), sedation or general anaesthesia.

27.7. Processing

Safety and quality issues covered in Chapter 8 also apply to the processing of human gametes and embryos. However, there are some specific issues that must be taken into consideration.

The following section is based largely on *Revised guidelines for good practice in IVF laboratories* by ESHRE [7]. These guidelines were drawn up by the Special Interest Group (SIG) in Embryology, and constitute the minimum requirements for any laboratory performing MAR procedures.

27.7.1. Premises for processing of gametes and embryos

27.7.1.1. *Laboratory design*

The laboratory handling gametes and embryos must have adequate space and should be as close as possible to the operating room in which clinical procedures are carried out. Laboratory construction must ensure aseptic and optimal handling of gametes and embryos during all phases of treatment. To ensure this, high-efficiency filtration of particulates and volatile organic compounds in the air supplied to the laboratory and rooms in which clinical procedures are carried out should be considered [8]. In addition, a number of protective measurements should be implemented to minimise the risk of contamination (see Table 27.1).

According to the EU Tissues and Cells Directive, tissues and cell processing must be performed in a Good Manufacturing Practice (GMP) Grade A environment with a background of at least GMP Grade D. However, if it is detrimental or not feasible to carry out a specific procedure in a Grade A environment, or if a validated microbial inactivation process is applied, a less stringent environment may be acceptable. If so, an environment must be specified and it must be demonstrated and documented that the chosen environment achieves the quality and safety required.

27.7.1.2. *Laboratory equipment*

All equipment must be validated as fit for its purpose, and its performance must be verified by calibrated instruments; it should preferably be CE-marked.

The laboratory must contain and identify all essential/critical equipment required for IVF, in numbers appropriate to the workload. Incubators in which gametes and embryos are cultured should be organised to facilitate their identification. The number of incubators is critical and should be based on the number of cycles and embryo-culture duration. Gametes and embryos should be conveniently distributed across incubators to minimise door openings and to maintain stable culture conditions.

Devices for the maintenance of a constant temperature during manipulation of gametes and embryos that are out of incubators must be in place (i.e. warm stages, heating blocks). Regular checks of critical parameters such as temperature, pH related to CO_2 and O_2 levels must be carried out.

Table 27.1. **Criteria to be considered in determining the risk of culture contamination in assisted reproductive technologies processing facilities (EuroGTP guidance)**

Criterion	Explanation
Risk of contamination of tissues or cells during processing	Although very rare, accidental contamination from the environment during processing might occur. Working under oil in the laminar flow hood minimises this risk.
Use of antimicrobials during processing	Use of antimicrobials during processing reduces the risk of contamination from the patient and/or the environment. Culture media for processing of oocytes, sperm and embryos usually contain antibiotics (e.g. penicillin, streptomycin, gentamycin).
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Destruction testing is not possible in partner donation or in non-partner oocyte donation. Therefore, in MAR, preliminary microbiological testing of donors before procurement and the methodology of processing are more important. Eventually additional testing of conditioned culture media can be of use to assess microbial contamination.
Risk of transfer of contaminants to patient	Working under oil in the laminar flow hood minimises this risk. Cells in only a minimum amount of culture media are transferred into the uterus, so the risk of contamination is very low. Furthermore, other measures such as cleaning of the cervix on transfer will help to reduce the risk of infection.

A sufficient number of cryostorage units should be available and be continuously monitored and equipped with alarm systems, detecting any out-of-range temperature and/or levels of liquid nitrogen (LN_2).

27.7.2. Handling of gametes and embryos

As stated in Chapter 2, approved SOPs for all activities influencing the quality or safety of tissues and cells, including SOPs for handling of gametes and embryos, should be developed and maintained.

Handling of biological material should be performed in laminar-flow hoods (Grade A environment) equipped with heating stages and pre-warmed heating blocks, using aseptic techniques at all times. Certain processes, such as ICSI and embryo biopsy, can be done outside the laminar hood since they need to be undertaken under an inverted microscope. Class-II hoods should be used for documented contaminated samples (e.g. HIV, HCV) since they provide protection to the operator.

Measures must be taken to ensure that oocytes and embryos are always maintained at the appropriate temperature, pH and osmolality during culture and handling. Exposure to volatile or toxic substances, or harmful radiation, should be minimised.

Pipetting devices must be used for one type of procedure only and must never be used for more than one patient. If possible, unit-dose sterile disposable pipettes are preferred. Each sample must be handled individually and its processing should be completed before moving to the next sample in order to prevent cross-contamination or mix-up of samples (see §27.13 on Biovigilance).

27.7.2.1. *Oocyte processing*

Oocyte retrieval is a particularly sensitive procedure, and special attention should be given to temperature and pH, as well as efficient and quick handling. An identity check before oocyte retrieval is mandatory. The time between oocyte retrieval and culture of washed oocytes should be minimal. Prolonged oocyte exposure to follicular fluid is not recommended. Appropriate equipment must be in place to maintain oocytes close to 37 °C. Flushing medium, collection tubes and dishes for identifying oocytes should be pre-warmed. Follicular aspirates should be checked for the presence of oocytes using a stereomicroscope and heated stage, usually at 8-60 × magnification. Exposure of oocytes to high-energy light should be minimised. Timing of retrieval, number of collected oocytes and identity of the operator should be documented.

27.7.2.2. *Sperm processing*

A test sperm preparation before starting a treatment cycle may be advisable in order to propose the most suitable insemination technique; a frozen back-up sample should be requested if difficulty in sperm collection on the day of oocyte retrieval is anticipated. Before starting sperm processing, an identity check is always mandatory. In the case of ejaculated sperm, the sample preparation aims to:

- eliminate seminal plasma, debris and contaminants;
- concentrate progressively motile sperm;
- select against morphologically abnormal sperm.

On the day of oocyte retrieval, an appropriate sperm-preparation method should be chosen,

according to the characteristics and origin of the individual samples. The swim-up technique and discontinuous density-gradient centrifugation are most frequently used and widely accepted.

In case of azoospermia on the day of oocyte retrieval, a second semen sample should be requested before considering alternative sperm-retrieval procedures or oocyte cryopreservation.

For surgically retrieved sperm, several techniques are available to maximise sperm recovery and to select viable sperm among immotile testicular sperm cells [9]. In case of epididymal recovery, the aspirate is generally processed by swim-up or discontinuous density-gradient centrifugation, depending on the sperm cell number available. For testicular sperm, mechanical procedures to harvest the sperm from the tissue may be combined with enzymatic treatment in order to increase the sperm recovery rates.

27.7.2.2.1. Specific treatments

Although less often used, phosphodiesterase inhibitors (pentoxifylline, theophylline) or the hypo-osmotic swelling test are sometimes used in absence of motile sperm.

Enzymatic digestion of testicular tissue by collagenase may be applied if no sperm are observed.

27.7.3. Insemination of oocytes

Oocytes can be inseminated by conventional IVF or by ICSI. The insemination/injection time should be decided on the basis of the number of hours elapsed from ovulation trigger and/or oocyte retrieval, also keeping in mind that fertilisation will need to be checked 16 to 18 hours later.

27.7.3.1. *Conventional in vitro fertilisation (IVF)*

The number of progressively motile sperm used for insemination must be sufficient to optimise the chance of normal fertilisation. Typically, a progressively motile sperm concentration in the fertilisation dish between 0.1 and 0.5×10^6/mL is used.

The final sperm suspension should be in a medium compatible with oocyte culture.

Co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a shorter period may be sufficient.

27.7.3.2. *Intracytoplasmic sperm injection (ICSI) procedure*

27.7.3.2.1. Preparation of oocytes for intracytoplasmic sperm injection

When removing cumulus cells from oocytes, hyaluronidase concentration and exposure should be kept to a minimum. In order to prevent oocyte damage, pipettes with appropriate lumen size should be used and vigorous pipetting avoided. After denudation, oocytes should be thoroughly washed to remove traces of hyaluronidase. The maturation stage of the oocytes should be recorded.

27.7.3.2.2. The injection procedure

See Figure 27.2. It is recommended to keep records of the injection time (start and end of the procedure) and the performing operator. The duration of sperm identification and immobilisation followed by injection should be minimised. The number of oocytes transferred to the injection dish should relate to the operator's skills and the sperm quality. Appropriate temperature and pH should be maintained during injection. Viscous substances such as polyvinylpyrrolidone (PVP) can be used to facilitate sperm manipulation. In case of only immotile sperm cells, a non-invasive vitality test can be used to select viable sperm for injection. After injection, oocytes should be washed prior to culture.

Figure 27.2. **Intracytoplasmic sperm injection**

Source: Image provided by María José De los Santos Molina (Spain).

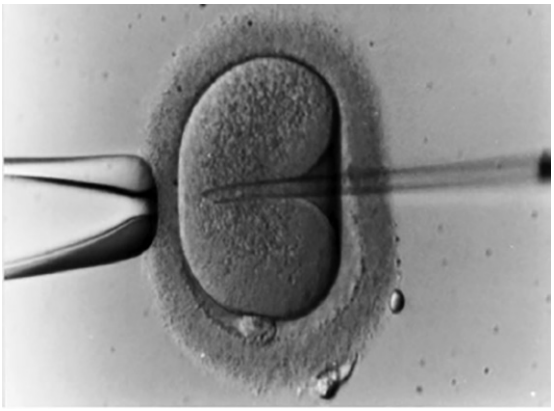
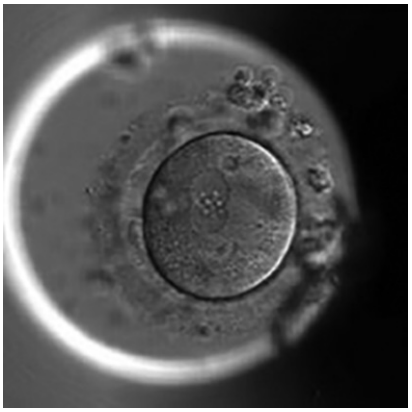
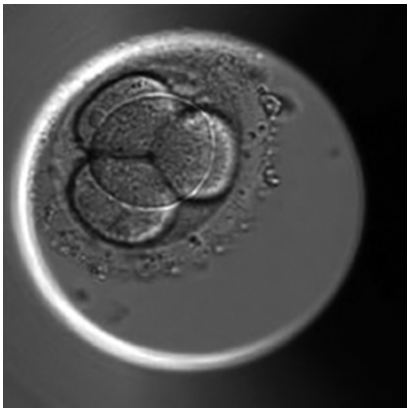


Figure 27.3. **Zygote with 2 pronuclei and 2 polar bodies**



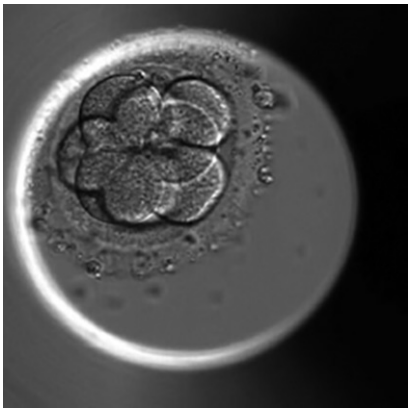
Source: Image provided by María José De los Santos Molina (Spain).

Figure 27.4. **Embryo at the 4-cell stage**



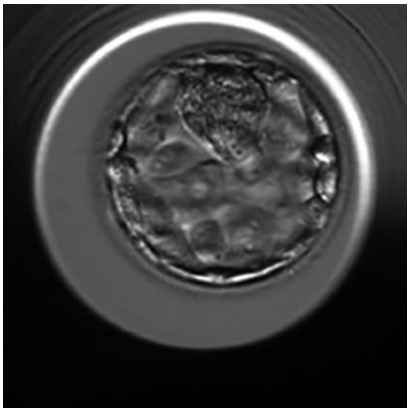
Source: Image provided by María José De los Santos Molina (Spain).

Figure 27.5. **Embryo at the 8-cell stage**



Source: Image provided by María José De los Santos Molina (Spain).

Figure 27.6. **Embryo at the blastocyst stage**



Source: Image provided by María José De los Santos Molina (Spain).

27.7.4. Assessment of fertilisation

All inseminated or injected oocytes should be examined for the presence of pronuclei (PN) and polar bodies at 16 to 18 hours post-insemination. A normally fertilised oocyte (zygote) contains 2 PN and 2 polar bodies (Figure 27.3). For conventional IVF, cumulus cells must be removed and 2 PN oocytes transferred into new dishes containing pre-equilibrated culture medium.

Fertilisation assessment should be performed under high magnification (at least 200 ×), using an inverted microscope equipped with Hoffman or equivalent optics, in order to verify number and morphology of pronuclei.

27.7.5. Embryo culture and transfer

In order to optimise embryo development, fluctuations of culture conditions should be minimised. Precautions must be taken to maintain adequate conditions of pH, temperature and osmolarity, to protect embryo homeostasis during culture and handling.

Embryo scoring should be performed at high magnification (at least 200 ×, preferably 400 ×) using an inverted microscope with Hoffman or equivalent optics. Evaluation of cleavage-stage embryos should include cell number, size and symmetry, percentage of fragmentation, granulation, vacuoles and nuclear status (e.g. multinucleation). Blastocyst scoring should include expansion grade, blastocoel cavity size and morphology of the inner cell mass and trophectoderm. Assessment should be performed at standardised times post-insemination. Embryo development can also be assessed using time-lapse imaging, allowing a dynamic evaluation of the timing of consecutive events during embryo culture. These systems also allow more stable culture conditions that may be of benefit. For an overview, see [10].

Embryo-quality assessment records should include the operator(s), date and time of assessment, and embryo morphological characteristics, which should be noted with the developmental stage (see Figure 27.4 and following).

Embryo selection for transfer is primarily based on developmental stage and morphological aspects. Other selection parameters, such as time-lapse kinetics, may be considered.

Single embryo transfer is recommended to avoid multiple pregnancies. The decision on the number of embryos to transfer should be based on

embryo quality and stage of development, female age, ovarian response and rank of treatment. It is recommended never to transfer more than two embryos.

Supernumerary embryos may be cryopreserved, donated to research or discarded, according to their quality, patient wishes and national legislation.

If the laboratory is at some distance from the embryo transfer room, arrangements should be made to maintain temperature and pH while transporting embryos.

A double identity check of the patient, the patient file and the culture dish(es) is mandatory immediately prior to the transfer.

27.7.6. Pre-implantation genetic testing

Oocytes and pre-implantation embryos can be biopsied and the obtained genetic material tested for certain monogenic disorders or chromosomal abnormalities. The biopsy procedure may be carried out by:

a. removal of polar bodies;
b. blastomere biopsy at day 3 (Figure 27.7);
c. trophectoderm biopsy at the blastocyst stage (Figure 27.8).

Cell(s) destined for genetic analysis are removed in the IVF laboratory using glass microtools on a micro-manipulation set. The embryology laboratory has the responsibility of providing unique identification between biopsied polar bodies, blastomeres or trophectoderm cells and the corresponding oocyte, embryo or blastocyst, respectively. All cells and embryos for genetic investigation must be handled individually, avoiding DNA contamination from other cells, from the samples or from the operator. They must be identified and labelled carefully, and tracked during the entire procedure. During these steps, double identity checks are necessary. The biopsy sample should be subjected to diagnostic procedures in an accredited laboratory for medical genetics. Traceability for embryo identification must also be guaranteed during the analysis in the reference genetic laboratory.

The purpose of PGT-M (for monogenic/single gene defects) and PGT-SR (for chromosomal structural rearrangements) is to identify embryos generated *in vitro* that carry certain hereditary genetic diseases or chromosomal abnormalities and exclude those embryos from transfer. Due to a minimised risk of transferring affected embryos, these tests are an alternative to induced abortion.

Genetic counselling must be available to all couples known to carry a (severe) hereditary disease. The recipient must be informed that due to mosaicism of the tested embryos and the limitations of the test, genetic testing on embryos does not substitute for prenatal analysis, such as amniocentesis.

PGT-A (pre-implantation aneuploidy screening) is used to analyse whether a cell biopsy from an embryo has the correct number of chromosomes, and such screening is used particularly for women of advanced reproductive age and for women who have had recurrent miscarriages or implantation failures. It is considered as a complement to standard morphological selection of embryos for transfer. Recent studies indicate that, for women of advanced reproductive age, embryo selection using PGT-A can decrease the number of embryo transfers necessary to obtain a pregnancy, thus reducing the time to pregnancy. However, the cumulative results are similar as when no PGT-A is used; see e.g. [11]. Bearing in mind the scarce data from prospective clinical trials and meta-analyses, PGT-A should be offered with caution, and full information on its present value should be provided to the patients.

Another possible future use for PGT could be to reduce the transgenerational risk of transmitting mitochondrial DNA disorder. Other utilisations such as the selection of histocompatible siblings can be also applied, case by case.

In some countries, PGT may not be allowed or allowed only in specific circumstances according to national legislation.

27.7.7. *In vitro* maturation

IVM refers to the maturation in culture of immature oocytes in specialised media after recovery from follicles that may or may not have been exposed to exogenous gonadotropins before retrieval [12]. During IVM, such oocytes progress from prophase I – i.e. from germinal vesicle (GV) stage – to reach metaphase II (MII). However, reaching the morphological criterion for MII (release of the first polar body) does not necessarily mean that the oocyte is competent for normal development.

Bearing in mind the lack of sufficient data from prospective clinical trials and meta-analyses, IVM should be considered an experimental procedure and not be used outside a system of ethical approval, and full information on its present value should be provided to patients.

27.7.8. Processing of samples from seropositive donors in partner donations

In couples with one or both partners being seropositive, MAR may still be applied for procreation, considering the risks of horizontal or vertical trans-

mission of the infection, after appropriate counselling and with the informed consent of patients.

For couples with seropositive males, the process includes density-gradient separation of the semen sample and optional swim-up.

Processing of samples from seropositive partner donors should be handled according to specific SOPs to protect personnel and avoid cross-contamination.

Hepatitis B-seronegative individuals with seropositive partners should be offered vaccination before ART [13].

Good quality and safety laboratory practices in assisted reproductive technologies for serodiscordant couples must be in place and should include personal protection of patients and staff, protocols for risk reduction of cross-contamination and proper decontamination of the work area [14].

27.8. Cryopreservation

27.8.1. Methods for cryopreservation of human gametes and embryos

Sperm, embryos and, more recently, oocytes are being cryopreserved for future use in MAR treatments (supernumerary embryos or oocytes, fertility preservation, non-partner donor gametes for banking). At present, the two most used methods for cryopreserving gametes and embryos are slow freezing and vitrification.

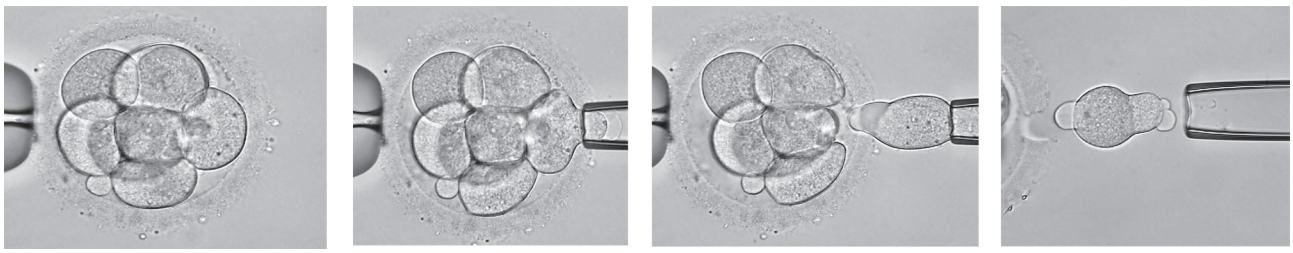
Slow freezing is a method using programmed step-wise decreases in the temperature of the solution in which the specimen is cryopreserved. This usually involves specific computerised equipment and programmes for cooling different types of tissues and cells in solutions with cryoprotectant substance(s).

Vitrification is an ultra-rapid cooling method that relies on very fast temperature drops (4 000-6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals. This is a fast method that does not require special cooling equipment (although special consumables are required), and is performed with the addition of specific cryoprotectants in higher concentrations (compared to slow freezing) for shorter exposure times.

There are significant differences in the sensitivity of different types of male and female gametes and different-stage embryos concerning the cooling process and cryoprotectant agents used [15, 16, 17, 18].

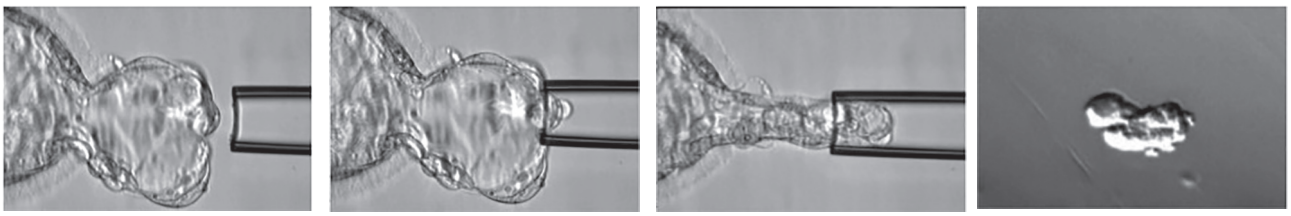
Concerns about the safety and quality of cryopreserved human gametes and embryos are raised regarding cell damage (disassembly of meiotic spindles, membrane rupture), toxic effects of cryoprotectants (anti-freeze substances), osmotic damage and incomplete dehydration, all having an effect on the cell viability [19].

Figure 27.7. **Embryo biopsy of an 8-cell stage embryo**



Source: Image provided by María José De los Santos Molina (Spain).

Figure 27.8. **Embryo biopsy of blastocyst**



Source: Image provided by Cristinas Magli (Italy).

27.8.1.1. *Cryopreservation of sperm*

For sperm, slow freezing is still the method of choice, but rapid cooling is a possible alternative [14, 15]. Freezing/thawing of human sperm is a well-proven technology. Sperm samples are usually cryopreserved in glycerol-based cryoprotectant solutions in cryovials or straws, frozen in a programmed cell-freezing device or incubated in liquid nitrogen vapour and then plunged in the liquid phase.

Seminal plasma, immotile and damaged sperm can be removed (by sperm processing) before freezing to select a population of sperm with a better chance of survival. It is recommended to process testicular biopsy samples before freezing.

27.8.1.2. *Cryopreservation of oocytes*

In recent years, successful cryopreservation of MII human oocytes has been reported worldwide, with rapidly increasing success rates due to optimisation of protocols. These data strongly suggest that vitrification may be the method of choice in oocyte cryopreservation, since improved rates of survival, implantation and pregnancy have been obtained using this method compared to slow freezing [16, 17, 18].

27.8.1.3. *Cryopreservation of embryos*

Zygotes, early-cleavage embryos, morulae and blastocysts have been cryopreserved successfully and used later for 'frozen embryo transfer'. Slow freezing or vitrification can be used, with vitrification/warming in dimethyl sulphoxide-based cryoprotectants resulting in better survival rates; see e.g. [18]. Exposure time to the cryoprotectant before vitrification is crucial and must be strictly respected.

27.9. Storage

Regarding cryostorage premises, the main aspects to be considered are location, ventilation and construction materials (e.g. flooring must be sufficiently durable for liquid nitrogen spill).

From a practical point of view the storage room with the liquid nitrogen tanks should be located close to the laboratory, so that the cryopreserved gametes or embryos can be easily, rapidly and successfully transferred to the storage room and into the liquid nitrogen tanks.

It is recommended to have a system in place whereby a forced ventilation system can be automatically activated when low oxygen tension is detected. For electricity-dependent equipment, alarms etc., the storage facility should be part of the clinic's general emergency plan whereby, in case of loss of electrical power, a generator or uninterrupted power supply (UPS) system must be in place.

The type of construction materials should be similar to the ones used in the procurements and processing facilities, with smooth surfaces and easy to clean. A special consideration in the choice of construction materials is that the floor should be resistant to large changes in temperature caused by liquid nitrogen spills.

27.9.1. Storage limits

There is no scientific evidence that gametes, embryos and gonadal tissue, if kept under appropriate storage conditions, deteriorate after a certain time of storage; hence, they can be stored for long periods of time. Use of frozen sperm through assisted reproductive techniques has led to the birth of healthy offspring more than 20 years after initial storage [20], and successful storage over a long period for oocytes and embryos has also been published [21, 22]. However, at defined time points, contact with patients should be made to determine the destiny of their cryopreserved material. In some EU countries, national laws determine a maximum legal storage period. Patients must declare in writing the destiny of their reproductive material when this maximum storage period has ended (see also §27.3.2 on donor consent).

A periodic inventory of the cryobank is recommended, including cross-referencing contents with storage records.

27.9.2. Storage temperature

Optimal storage temperature is based on the type of tissue, cryoprotectant and freezing method used. However, a temperature < -136 °C for gonadal tissue, embryos and gametes is appropriate, and > -130 °C is detrimental to the survival and quality of the material frozen. Even though storage in liquid nitrogen or liquid nitrogen-vapours is common practice, it is important to ensure that the minimum temperature is also maintained when handling the stored samples (< -140 °C). Special attention should be given to temperature changes when handling vitrified material stored in liquid nitrogen vapour.

27.9.3. Storage devices

Several devices can be used to store reproductive material. Sperm can be stored in straws or vials, whereas gonadal tissue is stored mostly in vials. Embryos and oocytes are stored in straws, whereby

one straw can hold one or more embryos or oocytes. It is, however, advisable to store only one embryo per straw to encourage single embryo transfer and to keep traceability between the quality of the oocyte or the development of the embryo frozen.

In the case of using straws for storage (and especially for storage of oocytes), open or closed systems can be used. Using open storage systems means that, at some point in the processing of reproductive tissues and cells, there is direct contact of the cells with liquid nitrogen. In a closed system, there is no direct contact between cells and liquid nitrogen.

27.9.4. Cross-contamination during storage

Introduction of contamination in the storage vessel is due to human manipulations during processing. Viral and microbial agents may survive during long periods of time in liquid nitrogen. However, no reports have shown cross-contamination between these environmentally induced pathogens and the preserved reproductive material. Also, storage of reproductive material originating from patients carrying infectious diseases in liquid nitrogen has not led to cross-contamination of other frozen reproductive material residing in the same vessel [23, 24]. Even though evidence is lacking, it should be considered good laboratory practice to store reproductive material of patients with positive serology and negative serology separately. Vapour-phase storage containers have been proposed as an alternative to liquid nitrogen containers. Periodic thawing and cleaning of storage vessels is recommended for extending the lifetime of the vessel as well as periodic decontamination of viral and microbial agents.

27.9.5. Storage safety

Storage in liquid nitrogen or vapour nitrogen vessels is definitely the most common infrastructure used to store gametes, embryos and gonadal tissue. Cryopreservation and thawing of material is a daily process in a fertility clinic. Therefore, it is of the utmost importance that personnel working in the cryogenic room have received appropriate training on how to handle liquid nitrogen, and that they are aware of the potential hazards. Personnel must be equipped with specific protective garments (gloves, boots and goggles) and use special forceps for manipulation of straws.

27.10. Distribution, import/export

Transport of tissues and cells within the EU is usually referred to as distribution (see Chapter 11). During transport of gametes and embryos, measures need to be taken to ensure the quality, safety and traceability of reproductive tissues and cells. Before transport, some specific actions need to be taken using the appropriate documents:

- a signed transport agreement between expediting and receiving institutions;
- presence of valid documentation (patients and sample identification, import/export permission when applicable, in accordance with legislation, biological test etc.);
- a protocol for adequate sample handling during transport, storage and thawing;
- a protocol of acceptance, checking for possible damage to container, for samples and patient identification and for presence of valid documentation;
- signed consent for sample transportation by patients and/or by institutions.

It is also necessary to consider and strictly control the conditions during the actual transport because cryopreserved material is highly sensitive to any fluctuations in temperature. See also Chapter 14 and section 27.9.

For export to and import from countries outside the EU, different requirements need to be met; for details see Chapter 11.

27.11. Packaging and labelling in assisted reproductive technologies

As addressed in Chapter 14, the coding, packaging and labelling of tissues and cells have an important role during banking procedures. Packaging applies only to cryopreserved gametes and embryos in storage and transport. Frozen gametes and embryos are packaged and stored in straws/cryovials as described in section 27.9.3.

Labelling is intended to identify gametes and embryos unambiguously. Labelling and identification systems may vary between centres and countries. As mentioned in section 27.7.2, procedures must be in place that ensure correct identification of patients at all stages of handling, using at least two points of identification (e.g. treatment number, name, colour code and/or date of birth) and should include at

least the names of partners (when relevant) and date of processing. For frozen samples, colour coding of cryovials and straws should also be used.

At cryopreservation, documentation on biological material should include labelling of devices, cryopreservation method, date and time of cryopreservation, operator, embryo quality and stage of development, number of oocytes or embryos per device, number of devices stored per patient, location of stored samples (tank, canister). Cryo-devices must be clearly and permanently labelled with reference to patient details, treatment number and/or a unique identification.

At thawing, documentation on biological material should include thawing method, date and time of thawing, identity of operator and post-thawing sample quality.

27.12. Traceability

Identification of patients and traceability of their reproductive cells are crucial aspects in MAR treatments. Each IVF laboratory must have an effective and accurate system to uniquely identify, trace and locate reproductive cells during each procedural step. A proper identification system should ensure that the main characteristics of patients (or donors) and their tissues and cells, together with relevant data regarding products and materials coming into contact with them, are available at all times.

Proper training in traceability procedures for all laboratory staff is highly recommended.

Before commencing any procedure, the laboratory must be provided with each patient's unique identification, which has to clearly and easily refer to the patient's documentation. Each treatment cycle must be assigned a unique identification.

Corresponding consent forms, clinical data and details of serological exams undertaken by patients/donors prior to admission to the treatment should be available to the laboratory staff.

Rules concerning the correct identification and processing of reproductive cells must be established in the laboratory by a system of codes and checks that considers all the following:

a. Direct verification of patient identity and correspondence with their assigned unique identification is required at every critical step. Patients should be asked to give their own identifying information (at least full name and date of birth) before procurement or assisted insemination/embryo transfer.

b. Labelling of dishes/tubes containing gametes and embryos must be permanent and on the container itself, not only on the removable lid.

c. All devices containing biological material must be clearly and permanently labelled with the unique patient and cycle identification.

d. Biological material from different patients must not be processed in the same working area at the same time.

e. Incubators and cryostorage systems should be organised to ensure easy access and identification of the biological materials therein.

f. During critical steps, traceability must be verified through correct identification of the reproductive cells and tissues. This can, for example, be executed by use of the four-eye-principle of witnessing (i.e. a double check of the identification by a second person) and/or by use of an electronic identification system.

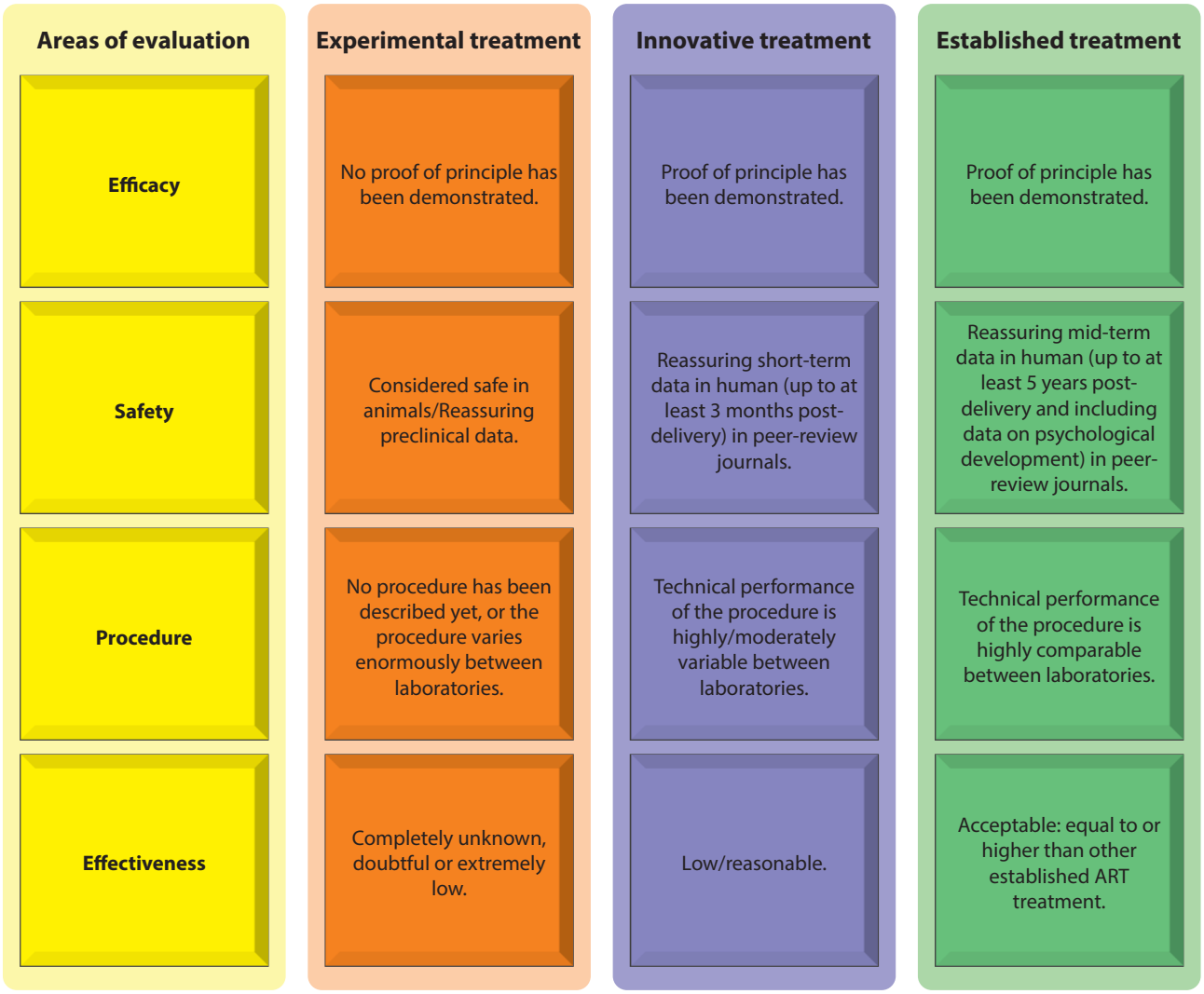
g. Products and materials used with biological materials must be traceable. The date and time of each manipulation and the identity of all operators and witnesses must be documented throughout the treatment. These records should be kept for a specified period of time according to European and/or national legislation.

h. Gametes and embryos from non-partner donation require specific coding for those countries that are regulated according to European Commission directives, specifically Directive 2015/565 amending Directive 2006/86/EC (see also §14.2.3).

i. Transport of reproductive cells and tissue requires identification of distributing, importing and exporting institutions, as well as identification of the biological material and its conformity for clinical use. At both institutions, the accompanying documentation and sample identification on the storage device must be checked to ensure that they correspond with patient records.

j. TEs that store and distribute non-partner gametes should label containers with an appropriate unique donation identification. In the EU, the coding requirements for non-partner donation apply (see §14.2.3.1 and following).

Figure 27.9. **Experimental, innovative and established treatments**



Source: adapted from Provoost V *et al.* Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014 Mar;29(3):413-17 [5].

27.13. Biovigilance in medically assisted reproduction procedures

Deviations from SOPs in TEs or other adverse events that may influence the quality and safety of tissues and cells should result in SARE reporting to the Health Authority. Adverse reactions can also be related to ovarian stimulation and surgical procurement of gametes, in partner and non-partner donors.

Examples of SAREs reported for MAR are given below. In addition, the Notify Library (www.notifylibrary.org) includes many well-documented cases of adverse occurrences in MAR treatments. The database is publicly accessible and can be searched by substance, adverse occurrence and record number.

All patients involved should be informed as soon as possible and should be offered counselling and support.

27.13.1. Serious adverse reactions and events

27.13.1.1. *Serious adverse events*

Serious adverse events may be, for example, mix-ups or loss of gametes, embryos or tissues, and may occur at any stage of clinical or laboratory processes (collection, insemination, embryo transfer, cryopreservation). Reasons for mix-ups or loss of cells or tissues can be multiple processing steps, mislabelling, contamination, human factor involvement, misidentification, absence/failure of witnessing and/or poor-quality systems. The consequences may include reduced or no chance of pregnancy, (genetic) disease

transmission, psychological impact and ethical/legal issues. Causal factors should always be investigated.

27.13.1.2. *Examples of SAEs reported in MAR*

27.13.1.2.1. Mix-ups/loss of traceability

a. mix-up of sperm samples during preparation/treatment;
b. sperm sample contaminated by another sample (e.g. with a used pipette);
c. oocytes fertilised with spermatozoa from the wrong person;
d. insemination of a woman with sperm cells from wrong donor;
e. wrong embryos thawed;
f. labelling error of tubes/dishes containing the oocytes/sperm/embryos.

27.13.1.2.2. Accidental loss of gametes and embryos

a. loss of gametes or embryos resulting in total loss of chance of pregnancy in one cycle (e.g. technical failure of incubator, cryomachine or cryotank, accident with culture dishes, accidental thawing);
b. embryos destined for culture or freezing were instead destroyed (error in transmission of information);
c. gametes or embryos lost due to microbiological contamination.

27.13.1.2.3. Adverse events after treatment with donated gametes

a. genetic condition discovered in a sperm/oocyte donor years after the gamete donation (for further information see section 27.13.2).

27.13.1.3. *Serious adverse reactions*

All serious reactions related to ovarian stimulation and procurement of the tissues and cells should be reported to the Health Authorities under the category of SARs in donors. Hospitalisation due to ovarian stimulation (OHSS) should be considered as an adverse reaction (non-serious adverse reaction if it is for observation only).

Although the birth of a child with a genetic disease inherited from the donor is an affected offspring it should be reported under the category of SARs for recipients (see e.g. 27.13.1.3.2.d)

27.13.1.3.1. Examples of SARs reported in MAR for donors

a. severe OHSS leading to hospitalisation;
b. bleeding after oocyte retrieval;
c. ovarian torsion.

27.13.1.3.2. Examples of SARs reported in MAR for recipients

a. salpingitis after intra-uterine insemination;
b. bacterial infection of the recipient due to infected sperm;
c. ovarian torsion after ovarian stimulation;
d. mix-up of samples in the genetics or IVF laboratory (PGT-M treatment), causing the birth of a baby carrying a genetic disease.

27.13.2. Transmission of genetic diseases by medically assisted reproduction with non-partner donations

Donors may unknowingly carry genetic defects causing a (severe) disease. Thereby gamete banks, for example, when distributing sperm or oocytes from a non-partner donor to multiple recipients, could potentially be spreading a (severe) genetic disease. TEs should keep this in mind, especially when informing non-partner donors and recipients of non-partner donations. National registries to facilitate traceability of non-partner donors and offspring are strongly recommended.

Non-partner donors should be strongly advised to inform the procurement centre/TE if they are diagnosed with a genetic abnormality. It is recommended to contact the recipient in the case of a diagnosis that may seriously affect a child's health.

Recipients of non-partner donations should be advised to inform the clinic where they received fertility treatment and also any physician treating a child with a genetic disease that the child was conceived through a non-partner donation, so that appropriate investigations about the origin of the genetic defect can be put in place. Measures should be put in place to prevent the use of gametes from the same donor until an appropriate investigation and risk assessment has taken place. Subsequent measures may include launching international rapid alerts if gametes from the same donor have been distributed or exported to other countries.

These examples emphasise that forward and backward traceability is of the utmost importance in MAR treatments.

27.13.3. Cross-border management of serious adverse reactions and events

Individuals travel abroad to access fertility treatment for various reasons (legal restrictions, long waiting times, treatment costs, lack of expertise, quality of treatment). If patients travel home after treatment, there is a risk that an SARE might occur

that might not get reported to the professionals who carried out the treatment or to the Health Authorities. As a consequence, no investigation of potential causes is done and no preventive measures are taken. It is strongly recommended that medical teams involved in both countries communicate with each other to ensure adequate treatment and follow-up. Healthcare professionals should report any SARE to their national Health Authorities, even for cross-border treatments.

27.14. Additional considerations

Fair, clear and appropriate information must be provided to donors and recipients at all stages of MAR treatments. The chances of success (including the live-birth rate) should be discussed appropriately. Clinicians, embryologists, technicians, nursing staff and all involved professionals need to communicate at all times to ensure optimal teamwork for the benefit of patients.

OHSS risk, appropriate selection of laboratory methods, the risk of multiple pregnancy and its complications, and the need for follow-up of children must all be addressed. In this sense the use of a unique European database for donor–recipient allocation would be critical in achieving prompt, rapid and reliable SARE report management.

All establishments are strongly encouraged to document internal data and results, and to benchmark with international standards [2, 3]. It is also important to keep track of developments that may increase safety and quality.

In order to ensure global consistency and harmonisation when communicating regarding MAR, a consensus and evidence-driven set of terms and definitions has been generated [1].

27.15. Developing applications

MAR is a rapidly evolving field. Development and implementation of new technology may affect not only donors and recipients, but also future generations. It is therefore important that these procedures are proven to be safe and efficient.

A methodology for the introduction of new techniques and treatments into clinical practice has been proposed by Provoost *et al.* [5], involving three levels: from experimental, then innovative, to established. A scoring tool is used at each level to determine whether a threshold has been reached of sufficient efficacy, safety, procedure and effectiveness. (see Figure 27.9). For more information on the introduction of new methodology see Chapter 29.

27.15.1. Non-invasive pre-implantation embryo genetic testing

It is known that embryo morphology performs relatively poorly in determining embryo quality and predicting implantation. Many embryos that do not implant or end in miscarriage are indeed carriers of chromosomal abnormalities due to inherited meiotic and mitotic errors.

The discovery of cell free DNA in human embryo-culture media supported the research on new non-invasive biomarkers of embryo viability that could eventually replace the current invasive pre-implantation embryo genetic testing (PGT) screening methods [25]. This new technology would be able to determine the euploidy status or even single gene mutations of human embryos by measuring cell-free DNA in the spent culture media. Validation of the methods still needs to be undertaken. Recent studies have shown good prediction power with high sensitivity and specificity values in a specific situation of beta thalassaemia [26].

27.15.2. Whole genome screening

Recent research developments in the field of genomics have made possible the comprehensive testing of the human genome by combining the methods of next-generation sequencing with advanced bioinformatics. In this way, a complete picture of each individual genome, including single nucleotide- (SNV) and copy number- (CNV) variations, leads to expanded DNA screening. The application of this approach permits PGT and non-invasive prenatal testing, with expanded carrier screening, but also the disclosure of gamete donor anonymity. The comprehensive information derived from whole genome screening has benefits as well as limitations and risks, and its introduction into clinical practice requires prudence and genetic counselling [27].

27.15.3. Gene editing

Gene editing includes a group of technologies that allow modifying the genome by inserting, deleting, replacing or modifying genetic material at specific locations in the DNA sequence. In cases of genes carrying a mutation, the enzyme cuts the mutation and replaces it with the correct DNA sequence, making it of great interest in the prevention and treatment of human diseases. This approach can be used for somatic gene editing for the treatment not only of a genetic disorder, but also of cancer and infectious diseases. Ethical concerns arise when gene editing is used to alter genes in gametes or in embryo,

introducing changes that will be passed to future generations.

In 2015, a study from China was published, reporting genome editing by CRISPR-Cas9 on non-viable human embryos [28]. The following year, the United Kingdom issued the world's first endorsement of a national regulatory authority for research on human embryos using genome editing. Additional studies have demonstrated how human germline gene modification is rapidly progressing from the experimental field to clinical research applications [29].

The results obtained so far raise high expectations regarding possible therapeutic applications in humans, but much remains to be considered before clinical applications, including the reproducibility of the technique and possible long-term consequences.

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Related material

- Appendix 11. Example of consent form: female (NHS, UK)
- Appendix 12. Example of consent form: female (CNPMA, Portugal)
- Appendix 13. Example of consent form: male (NHS, UK)

Chapter 28: **Fertility preservation**

28.1. Introduction

Despite some differences in technical approaches and expected results, today fertility preservation (FP) can be applied for both medical and non-medical (also known as social) reasons.

FP involves actions taken in order to avoid, delay, diminish or circumvent the exhaustion of the germ-cell pool of the individual. In most current circumstances, either in anticipation of cytotoxic therapy treating a severe disease or for a number of possible reasons for postponing parenthood, this involves cryopreservation of gametes, gonadal tissue or embryos.

FP techniques are usually proposed to prepubertal girls and boys and to males and females of reproductive age at risk of losing their reproductive potential due to either malignant or non-malignant diseases. Gamete cryopreservation is also an option for individuals for non-medical reasons, such as to postpone parenting, previous to vasectomy or other reasons.

This chapter describes the indications for male and female FP and the techniques actually available for the cryopreservation of reproductive cells and germinal tissue. The collaboration between paediatricians, oncologists and reproductive specialists is essential to ensure proper evaluation and counselling for each patient. Patient assessment and approach will depend on disease, age and treatment, and information about possible options and future use of cryopreserved gametes or germinal tissue should be discussed with patients, or parents (in the case of minors). It is important to realise that FP and restoration may include techniques that are in an experimental state, and their availability may be restricted according to national legislation.

This chapter must be read in conjunction with Chapter 27: Medically assisted reproduction and the following chapters (Part A) of this Guide:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent, but only for non-partner donors,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling, but only for non-partner donors,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

28.1.1. Female fertility preservation

Female FP should be considered whenever fertility loss is predicted as a consequence of a cytotoxic treatment for a specific disease (e.g. in cancer patients) or due to the disease itself (malignant or non-malignant, e.g. severe endometriosis). This part includes indication for FP under medical conditions (oncological and non-oncological) as well as for non-medical reasons.

28.1.1.1. *Medical reasons*

All prepubertal girls and women at reproductive age newly diagnosed with specific medical conditions (e.g. certain cancers or rheumatoid arthritis) whose treatment may cause premature ovarian insufficiency (POI) should be referred to a fertility expert to be counselled about the risk of infertility and informed about fertility preservation. FP should ideally be offered before treatment is started, but should not delay treatment. The risks of undergoing FP, including possible delay of the cancer treatment, should be weighed against the benefits of having reproductive cells and/or tissues stored for future use. Sometimes the patient's health may be too impaired by the disease to justify FP.

28.1.1.1.1. Oncological reasons for fertility preservation

Chemotherapy and radiation therapy may cause depletion of the pool of primordial follicles in the ovaries of girls or premenopausal women and thus render them infertile. Once the ovaries are exhausted of follicles, the patient will experience POI and infertility. In the case of pre-pubertal girls, loss of the entire stock of primordial follicles will mean that the girl will not enter puberty spontaneously and that she will not be able to become pregnant with her own oocytes later on in life. This is of course a very serious side-effect of an otherwise efficient cancer treatment, and is by many considered to reduce the quality of life significantly. As more and more girls and women at reproductive age survive a malignant disease today, these unwanted side-effects will affect an increasing number of adults in the population.

It is well known that chemotherapeutic drugs belonging to the group of alkylating agents cause the most damage to the ovaries. Alkylating agents, such as cyclophosphamide or busulfan, are used to treat various cancer forms, including breast cancer, lymphoma and sarcoma, and are also used in the preconditioning protocol before haematopoietic progenitor cells (HPC) transplantation. As alkylating agents cause damage to both dividing and resting cells, they are very toxic to the oocytes and granulosa cells of the primordial follicles, as this is the most immature and 'dormant' type of follicle [1].

Radiation therapy, whether given to the abdomen or the spine, will also affect the functionality of the ovaries. Radiation therapy is very toxic to the oocytes, and doses as low as 2 Gy will destroy half of the pool of follicles. Whenever possible, the ovaries are shielded or moved away from the field of radiation, but scatter dose is inevitable [2].

28.1.1.1.2. Non-oncological reasons for fertility preservation

Non-malignant diagnoses – such as kidney disease, autoimmune conditions or haematological diseases like aplastic anaemia or thalassaemia – can sometimes be life-threatening and require treatment with alkylating agents or even HPC. Women affected by any severe disease requiring these treatments may also need FP.

When surgery to the ovaries is planned, as in the case of severe endometriosis or benign ovarian cysts, or borderline cysts, healthy ovarian tissue containing primordial follicles will inevitably be excised in connection with the operative procedure. These procedures may pose some threat to the reproductive potential of the patient, and in these cases FP should also be offered [3].

Certain genetic conditions – such as Turner mosaicism, galactosaemia, Fragile X mutation carrier status or blepharophimosis, ptosis or epicanthus inversus syndrome – will cause premature exhaustion of the pool of primordial follicles in the ovaries, and girls and women at reproductive age suffering from any of these conditions can also be potential candidates for FP.

Several options exist to preserve fertility in post-pubertal girls and women. Oocytes, ovarian tissue and embryos can be cryopreserved depending on the characteristics of each individual case and considering the most efficient alternative for every patient. Examples of consent forms for female FP are given in Appendix 11 and Appendix 12.

Also cross-hormone treatment for transgender persons is potentially harmful to their fertility. Therefore, transgender female-to-male patients may undergo oocyte collection and storage before cross-hormone treatment and sex-reassignment surgery.

28.1.1.2. *Non-medical reasons for fertility preservation*

Non-medical FP can be also considered in young women who want to postpone maternity (age-related fertility preservation) [4].

In all cases the women should be aware of additional issues such as the expected survival rate of oocytes or the minimal amount of oocytes required to optimise the likelihood of successful live birth; in this sense, the creation of *ad hoc* prediction models is an interesting approach that may guide patients and clinicians [5].

Specifically, for age-related FP cases, the women should be adequately informed about the medical problems connected to any late pregnancies.

In the case of female-to-male transgender treatment, patients should be informed of the possible use of their gametes in the future. This approach may be different in different countries according to national legislation.

28.1.2. Male fertility preservation

28.1.2.1. *Medical reasons*

FP is indicated in all boys and men facing gonadotoxic treatment or surgical procedures affecting semen production and deposition. All patients at risk of fertility loss should be informed about FP options.

28.1.2.1.1. Oncological reasons for fertility preservation

Chemotherapeutic agents and radiation treatments can adversely affect the male gonadal epithelium. Thus therapies used to cure cancer (but also used for several non-malignant conditions) may render the patient temporarily or permanently infertile. The amount of damage depends on the regimen, the cumulative dosage of treatments used and the individual capacity of recovery. Dividing spermatogonia are highly sensitive to cytotoxic treatments and radiation. Low doses of these treatments deplete the pool of differentiating spermatogonia, while spermatogonial stem cells (SSC) may initially survive, and spermatocytes and spermatids can continue their maturation into sperm. Testicular involution occurs when no new precursors are provided from the stem-cell pool and the differentiating germ cells mature into spermatids and are released from the seminiferous epithelium [6, 7].

Significant damage is reported after treatment with alkylating agents, and different thresholds are given in the literature (e.g. for cyclophosphamide and cisplatin-based drugs). Both alkylating and platinum-containing agents cause direct DNA and RNA damage, and so can affect even non-dividing, reserve stem cells. The gonadal epithelium is highly susceptible to radiation-induced damage. Differentiating spermatogonia are sensitive to scattered doses of radiation as low as 0.1 Gy, leading to short-term cessation of spermatogenesis. Cumulative doses above 3 Gy affect SSC and cause long-term azoospermia, whereas doses in excess of 6 Gy deplete the SSC pool and can lead to permanent infertility. Fractionation of radiotherapy increases the germ-cell toxicity. Overall, post-treatment infertility problems are reported in up to 60 % of cancer patients [8].

28.1.2.1.2. Non-oncological reasons for fertility preservation

Certain non-malignant pathologies that require potentially gonadotoxic treatments could require FP. Transgender male-to-female patients may wish to store semen for FP. Transgender persons planning to start cross-hormone treatment and undergo sex-reassignment surgery can benefit from FP. Analogous considerations previously explained for female-to-male trans patients also apply for male-to-female transgender patients.

28.1.2.1.3. Non-medical reasons for fertility preservation

These indications include groups such as men in military services, who are at risk of potential harm to their fertility.

28.2. Consent in fertility preservation

After referral of the patient, informed consent for FP should be obtained by a clinician. However, since pre-pubertal children can also benefit from FP techniques, informed consent should in this situation be signed by the parents or legal guardians of the child. It is important that, in the case of FP for pre-pubertal children, care should be taken to explain the future use of banked gonadal tissue.

Individual countries may have their own legislation regarding FP, and therefore consent forms can differ. Examples of generic consent forms for cryopreservation of sperm appear in Appendix 13 and Appendix 31. The forms can be used as a template that can be adjusted according to national legislation or common practice of the MAR centre. Information with regard to the process, legal time of cryostorage and potential risks can be reported in the consent form or in a related information document.

28.3. Patient evaluation

Patient evaluation of post-pubertal women or men undergoing FP is similar to patient evaluation for patients undergoing MAR techniques (Chapter 27). The future use of the stored gonadal tissue or gametes is eventually their use in MAR techniques with the aim of obtaining embryos in a partner donation treatment.

Patient evaluation for pre-pubertal boys and girls needs special care in cases where gonadal tissue is removed and banked. Close collaboration between paediatric, surgical, oncologic and fertility specialists is essential concerning FP in pre-pubertal children. When the patients are first seen in the oncology department, the influence of cancer treatment to the patient's future should be discussed and fertility and preservation options explained. The referral to a fertility clinic should be possible in very timely fashion because of the short time available for certain patients to undergo FP. In the fertility clinic, detailed information on the possibility and the process of FP will be offered on an individual patient basis. Additionally, the future use of the preserved reproductive material must be addressed. In order to accommodate the patients, certain aspects of the FP process can be considered: To minimise trauma to the patient, the surgical recovery of gonadal tissue should be combined with other interventions requiring anaesthesia, such as bone marrow sampling or implantation of venous ports. Close interdisciplinary co-operation between paediatric oncologists and gynaecologists, urologists, paediatric surgeons, psychologist or other medical specialist is required.

In the case of pre-pubertal boys, measurement of testicular volume is helpful in predicting the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has been induced – are comparable to those of adult patients, irrespective of the underlying disease [9-11]. In the case of pre-pubertal girls, the assessment of the ovarian reserve by anti-Müllerian hormone (AMH) may be investigated [12]. No further gynaecological investigations should be performed, since they can be perceived as intrusive and an emotional and psychological burden for these patients.

28.4. Procurement

28.4.1. Female

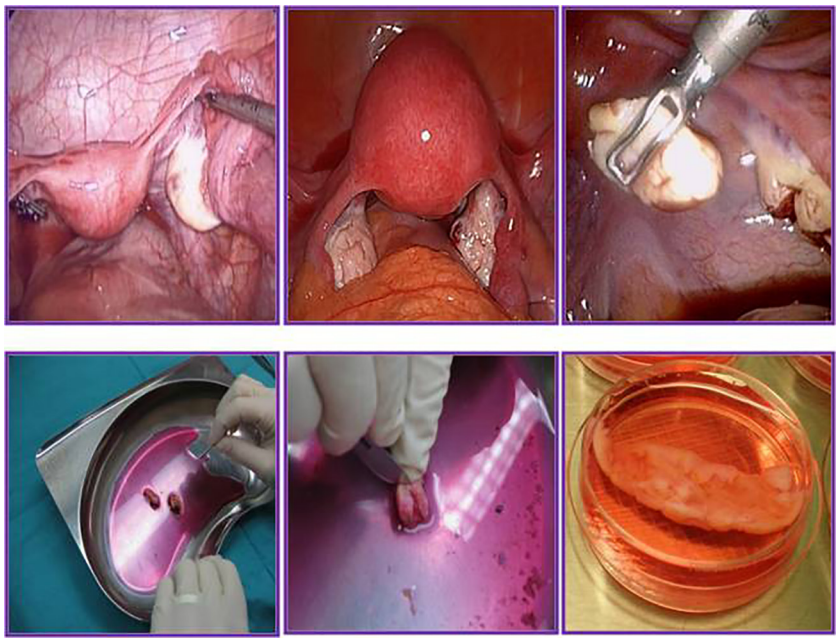
28.4.1.1. *Ovarian tissue*

Procurement of ovarian tissue can be performed at any time of the menstrual cycle and can be done at short notice.

By the procurement of ovarian tissue, thousands of follicles can be preserved. The follicles lie within the cortical tissue of an ovary, with the vast majority of follicles in the outermost 1-2 mm of the ovary. An entire ovary, a semi-ovary or ovarian cortical biopsies are removed by an operative procedure under general anaesthesia and prepared for cryopreservation [13]. See Figure 28.1 and Figure 28.2.

Figure 28.1. **Ovarian tissue procurement by laparoscopy: stage 1**

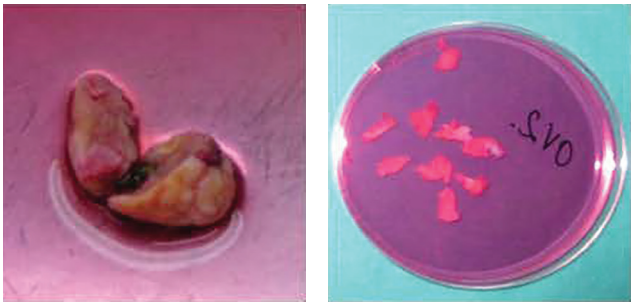
Images in the upper panel show the surgery steps taken to obtain the tissue. The lower panel shows processing steps: the medulla of the ovary is removed.



Source: Images provided by Sonia Herraiz, Valencia, Spain.

Figure 28.2. **Ovarian tissue procurement by laparoscopy: stage 2**

After the medulla removal the remaining ovarian cortex is then cut into small pieces of 10 × 5-10 mm



Source: Images provided by Sonia Herraiz, Valencia, Spain.

Ovarian tissue procurement is offered to pre-pubertal girls, and to post-pubertal girls not ready to undergo ovarian stimulation, endovaginal ultrasound monitoring and oocyte retrieval in order to procure and bank oocytes. Adult women who do not have the time to undergo stimulation for procurement of oocytes, either because cancer treatment is imminent or because the cancer is hormone-dependent, are also candidates for ovarian tissue cryopreservation.

Although, technically, there is the possibility of storing ovarian tissue at the time of sex-reassignment surgery and thus during cross-hormone therapy, this approach is highly experimental [14]. For all patients, including transgender men, ovarian procurement should be performed before the start of gonadotoxic treatment.

28.4.1.2. *Oocytes*

In order to collect oocytes, controlled ovarian stimulation is needed. This stimulation is similar to the stimulation for IVF (Chapter 27). The number of oocytes that can be collected depends on the age of the patient and her ovarian reserve. Special considerations are necessary to avoid high oestrogen production during ovarian stimulation in patients with oestrogen-dependent diseases. In cases of breast cancer, stimulation protocols have been developed to reduce the risk of an unwanted high level of oestradiol. Ideally, stimulation should start on the third day of the menstrual cycle, but can be started at any time in the menstrual cycle, including in the luteal phase, with apparently good results. Any pre-menopausal patient with a sufficient ovarian reserve can be considered for oocyte collection for FP. Post-pubertal girls may sometimes be able to undergo ovarian stimulation and tolerate endovaginal ultrasound monitoring and oocyte retrieval.

Oocytes will be collected by aspiration via the transvaginal route following the same steps previously described in Chapter 27.

28.4.2. Male

28.4.2.1. *Testicular tissue*

Testicular tissue is mostly procured in pre-pubertal boys when there is no possibility to produce a sperm sample. Collection of testicular tissue can be performed at any time. In general, unilateral procurement takes place, with a maximum of half of the testis.

The procedure used for testicular biopsy in pre-pubertal boys is quite simple and similar to the technique described in adults. Basically, it should be performed at the cranial pole of the gonad, to avoid damage to the main testicular artery. After making a transverse or midline scrotal skin incision of 2-3 cm, the *tunica vaginalis* is opened and the lateral surface of the testis is exposed. The *tunica albuginea* is incised (0.5 cm in length) and the testes are squeezed to make the testicular tissue protruding. A biopsy of 2-3 mm^3 is then cut with scissors. The *tunica albuginea* and the skin are closed over. Besides being useful in fertility preservation, testicular biopsy in pre-pubertal boys is a minor procedure that can provide valuable information for predicting the risk of malignancy and fertility, as described in Faure *et al.* 2016 [15] (see also Figure 28.3).

The amount of tissue procured for FP will have an effect on future testosterone production, and hormone replacement therapy could possibly be needed. However, it has been shown that the development of the testis in boys after biopsy of gonadal tissue for FP did not have an effect on the testicular growth [16].

A balance between the amount of tissue retrieved and the amount conserved is important to achieve adequate levels of testosterone. Since the testis volume in very young pre-pubertal children can be limited, one third of the testis is generally procured in this patient population. Immunohistochemical staining is necessary to assess the presence of SSC in the procured and stored tissue [17].

28.4.2.2. *Sperm*

Sperm samples are mostly obtained through masturbation. Sperm samples can be collected in adult men, postpubertal boys and in peri-pubertal boys if the patient is ready to obtain a sample through masturbation [10, 18-20]. In cases of failure to produce a semen sample by masturbation, assisted ejaculation techniques such as penile vibratory stimulation or

electro-ejaculation under general anaesthesia could be considered as a second-line treatment option.

Special care should be taken to clearly explain to young post-pubertal boys how to produce a sample by ejaculation, since not all patients are already sexually active.

28.5. Processing

MAR tissue establishments such as MAR centres and banks can process and store gonadal tissue, gametes and embryos for FP. The techniques for processing and storage are described in Chapter 27. For further microbiological testing, refer to Chapter 10. Processing and storage of gonadal tissue require a tissue establishment with the facilities, licence and expertise to perform the procedure, and to process and store the tissue. These are described in more detail below.

Based on risk analysis, and offered testing for infectious disease (HIV, hepatitis) separate processing and storage of infectious material will be performed.

28.5.1. Female

28.5.1.1. *Ovarian tissue*

Ovarian tissue should be transported on ice in a transport medium (e.g. Leibovitz L-15), supplemented with serum albumin. Processing of the ovarian tissue starts with the ovarian biopsy or with bisecting the ovary, in the case of a whole ovariectomy. The medulla, the inner part of the ovarian tissue, is removed by careful scraping with a scalpel to prepare the cortical tissue to the required thickness of, on average, 1-2 mm. The cortex is subsequently cut into smaller fragments (5 × 5 mm). These fragments are then treated with a cryoprotectant (dimethyl, DMSO), to protect the cells from cryodamage, and generally subjected to controlled slow freezing in a programmable controlled-rate freezer [21]. Vitrification of the ovarian tissue is another optional methodology.

During ovarian tissue processing, the medulla should be further minced into small pieces in a petri dish with medium and examined under a stereomicroscope for the presence of cumulus oocyte complexes (COC). These COC can be collected and subjected to *in vitro* maturation in order to obtain metaphase II oocytes that can be collected and stored. This collection, with *in vitro* maturation and storing of oocytes obtained during the processing of ovarian tissue, is considered a highly innovative FP technique, since so far only one live birth has been described in Europe [22]. However, it opens the possibility of maximisation of FP in the case of ovarian tissue procurement and storage.

Transport of the procured tissue from different centres to a centralised tissue establishment is a realistic and efficient system to be considered [21].

28.5.1.2. *Oocytes*

Oocyte cryopreservation is the preferred option for FP in post-pubertal patients who can be submitted to controlled ovarian stimulation. Vitrification is the technique of choice, due to the excellent results obtained in IVF patients in terms of survival, embryo development and implantation [23, 24]. The methodology is described in Chapter 27.

28.5.1.3. *Embryos*

Although oocyte cryopreservation is generally practised today, embryo cryopreservation can also be considered for FP in the case of couples. However, cryopreserved embryos will not be available for future use if the couple separate.

28.5.2. Male

28.5.2.1. *Ejaculate*

Sperm cryopreservation is performed for male FP in post-pubertal males. Semen characteristics may vary with both patient age and type of disease, with testicular cancer patients having the worst semen quality. For adolescents, in more than 80 % of cases semen can be cryopreserved. However, up to 20 % of adolescent or adult patients may either fail to produce a semen sample or may present with azoospermia. Measurement of testicular volume is helpful in predicting the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has been induced – are comparable to those of adult patients, irrespective of the underlying disease.

The methodology for sperm cryopreservation is described in Chapter 27.

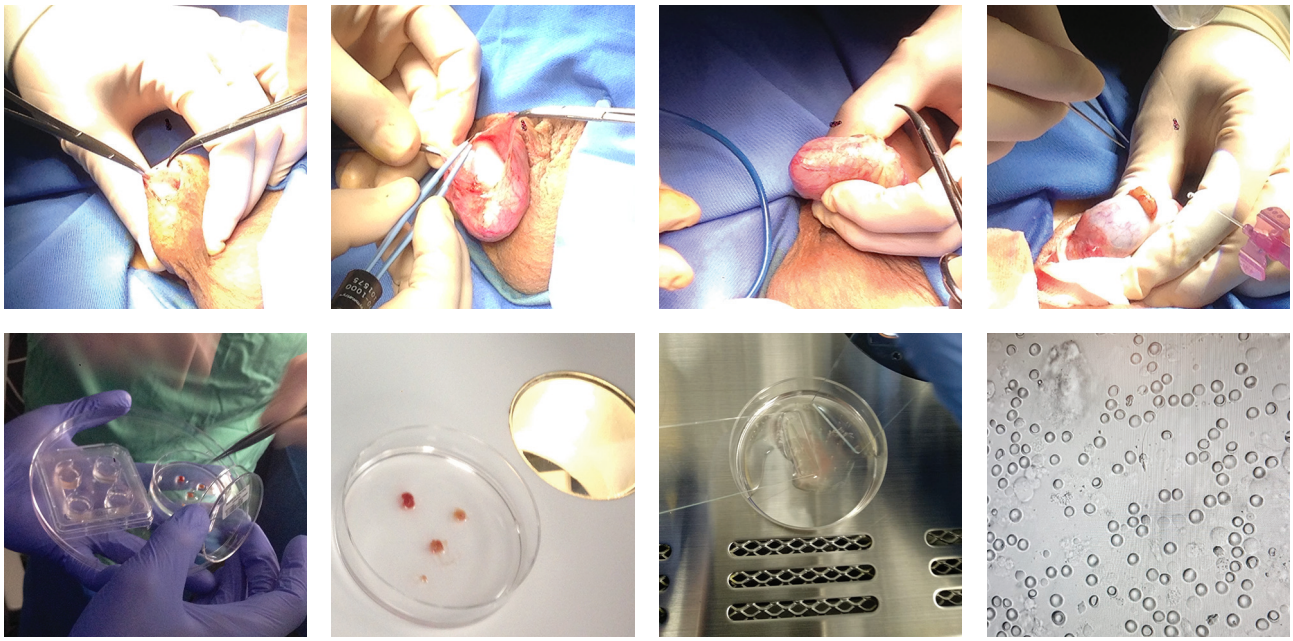
28.5.2.2. *Testicular tissue/epididymus*

For pre-pubertal boys and also for azoospermic patients, surgical sperm procured by testicular sperm-extraction procedures should be offered. Sperm can be retrieved by microsurgical aspiration of the epididymal fluid or by testicular biopsy/testicular sperm aspiration (TESA) or testicular sperm extraction (TESE).

Testicular tissue should be transported on ice in a transport medium (e.g. Hepes-buffered DMEM/F12), supplemented with serum albumin (in general, 10 % HSA).

Figure 28.3. **Conventional testicular biopsy and tissue processing for sperm recovery**

Images in the upper panel show a surgical procedure for testicular tissue extraction. Lower panel shows processing steps: the small pieces of tissue are cut into smaller pieces. Isolated testicular spermatozoa and/or the tissue can be cryopreserved and thawed for future use.



Source: Images provided by Dina Pabón (Spain).

Processing of the testicular tissue consists of cutting the tissue into small fragments, submerging the pieces in medium supplemented with a cryoprotectant to protect the cells from cryodamage and then subjecting them to controlled slow freezing. However, no standardised protocol for cryopreservation of immature testicular tissue is available. Most groups are using DMSO-based cryoprotectants (0.7-1.4 M DMSO) with or without addition of sucrose. Slow-freezing protocols are mostly applied. Vitrification may also be effective when using higher doses of cryoprotectants. Vials/straws are thereafter submerged into liquid or vapour-phase nitrogen. Since the reproductive potential of cryopreserved immature testicular tissue has still to be demonstrated in humans, the technique remains experimental.

The legislation and recommendations for FP in males differ between countries. There are no strict limitations on semen quality or sperm numbers for FP strategies and there are no international guidelines for the duration of storage of spermatozoa, whether ejaculated or procured directly from the testis.

28.6. Storage

The permitted storage period of cryopreserved sperm, oocytes and embryos and reproductive tissues varies according to national legislation.

Long-term storage of ejaculated or testicular spermatozoa, ovarian cortex, oocytes or embryos does not negatively affect the quality of the frozen material, but constant storage conditions with a temperature of ≤ −140 °C are mandatory [25-29].

28.7. Clinical application

28.7.1. Female fertility restoration

When a patient wants to use her preserved tissue or oocytes/embryos for MAR treatment, the physician who treated her with the gonadotoxic therapy should be consulted as to whether it is safe for the patient to attempt a pregnancy.

In case of transplantation of ovarian tissue, this can be done either orthotopically (at the remaining ovary or at the site of the removed ovary) or heterotopically to other sites such as the abdominal wall. It takes approximately 20 weeks from the time of transplantation for the tissue to become active again as demonstrated by the return of menses and oestradiol production. Hence, restoration of fertility is combined with the restoration of the patient's endocrine environment. Although the primary reason for the use of stored reproductive material may be a future desire for a child, the restoration of endocrine function could also potentially be a reason for transplanting ovarian tissue. The latter has to be con-

sidered with caution as a recent review by [30] has shown that endocrine restoration rate was 63.9 %.

Spontaneous pregnancies can occur after the follicles start maturing and the patient gets her cycle back, but sometimes IVF is needed. The duration of functionality varies from a few months to up to 10 years, with a mean of 3-4 years. If oocytes or embryos have been cryopreserved, an embryo replacement cycle must be planned. If she is menopausal, her endometrium will be prepared in a hormone replacement cycle.

It has to be emphasised that a significant percentage of women will experience a spontaneous return of ovarian function months after chemotherapy. For these women spontaneous pregnancies may occur and they may not need their frozen gametes or gonadal tissue [31].

Pre-menarchal girls who lose all their ovarian tissue due to chemo- or radiation therapy will not enter puberty spontaneously. These girls will need to be induced with exogenous hormones in order to undergo normal pubertal development. After puberty they will need to take hormonal replacement therapy for the health of their bones and for general well-being. Later on in life they can have their cryopreserved ovarian tissue transplanted in order to re-establish menstrual cycling and/or become pregnant.

28.7.2. Male fertility restoration

In most of the cases where chemotherapy and/or radiotherapy has been applied, spontaneous recovery of spermatogenesis is possible up to 10-15 years after the end of treatment; however, it cannot be accurately foreseen. Thus, regular semen analysis should be offered to patients after treatment. About 60 % of male cancer patients will face infertility problems after the end of the cancer therapy.

When cryopreserved samples are used, intracytoplasmic sperm injection (ICSI) is recommended to improve the chances of success. Before ICSI was implemented, the success rate of MAR procedures with cryopreserved semen samples (IUI or IVF) was low. When ICSI procedures are applied, the success rates using cryopreserved spermatozoa are comparable to standard IVF and ICSI procedures in infertile couples using fresh sperm.

28.8. Quality control and tissue evaluation

Quality control after tissue transplantation includes approaches aimed at decreasing the risk of cancer recurrence while maximising the tissue viability after thawing or warming.

As the autotransplantation of cryopreserved tissue could be associated with a risk of cancer cell reseeding, due to malignant cell transmission in oncological patients, different approaches to detect cancer cells are under development [32-34]. Depending on the medical reason for tissue cryopreservation and the type of disease, the ovarian cortex and testicular tissue should be ideally sampled and sent for histological examination to detect any malignant cells.

Since cryopreservation methods can also affect tissue viability, examination of tissue survival and presence of primordial follicles can be performed. However, due to an uneven distribution of primordial follicles along the ovarian cortex, the inexistence of primordial follicles in the examined tissue should not prevent transplantation. The success of ovarian cortex transplantation can be evaluated by measuring the endocrine function and fertility restoration. Recent follow up studies have revealed an endocrine restoration rate of around 60-65 % and an ongoing pregnancy rate of 38 % after natural conception [30, 35].

28.9. Biovigilance

Any adverse event or reaction should be notified, based on the general rules described in Chapter 16.

28.9.1. Female

In certain kinds of cancers, transplantation of the cryopreserved tissue is not possible due to the risk of re-introducing the original disease. This is to be considered especially in the case of leukaemia, since it is known that leukaemic cells can reside in the stroma of the cortical tissue. Women suffering from disseminated cancer with a risk of ovarian metastases should be advised against transplanting the tissue [4, 36, 37]. Since such transplantations are scarce, compared with other disciplines, limited cases of adverse event and reactions have been reported. Up to date the surgical related complications remain low (≈ 3 %). The report of a one major complication (intra-abdominal haemorrhage) has been recently published [35].

However, the reporting of serious adverse reactions and events affecting the offspring should follow the same rules used for MAR (Chapter 27).

28.9.2. Male

When cryopreserved sperm samples are used, the ICSI technique increases the number of MAR treatments that can be performed. No adverse effect on the health of the offspring has been reported from the combination of cryopreservation of semen and subsequent MAR.

A number of studies have been performed regarding sperm quality in the man after spontaneous recovery of spermatogenesis. Both cancer and its treatment are associated with sperm DNA damage, although treatment-induced DNA damage seems to be modest and transient. In a large cohort study of offspring from male cancer survivors, a modest but statistically significant increase in the risk of major congenital abnormalities was observed. This was independent of whether the sperm were cryopreserved pre-treatment and used for MAR, or if the children were conceived naturally [38, 39].

Any report of serious adverse reactions and events should also follow the same criteria used for assisted reproductive technologies (Chapter 27).

28.10. Future developments

28.10.1. Female experimental approaches

Female patients who seek FP but cannot undergo ovarian stimulation and oocyte/embryo preservation may consider using immature oocytes – either retrieved from antral follicles during the luteal phase or obtained during the ovarian cortex processing technique – to perform *in vitro* maturation to produce metaphase II oocytes to be used in ART [40]. Due to the lack of sufficient data from prospective clinical trials and meta-analyses, IVM should be considered an innovative procedure, and full information on its present value should be provided to patients.

In cases where no oocytes are existing, current investigations are carried out on the generation of oocytes derived *in vitro* from pluripotent stem cells as a promising though still incipient therapy.

Future techniques could also involve *in vitro* perfusion and hormonal stimulation of the patient's removed whole ovary(ies), where oocytes may be matured, aspirated and cryopreserved. This would enable fast oncologic treatment of the patient, as well as removing the risk of introducing malignant cells via transplantation.

28.10.2. Male experimental approaches

Development of the procedures used for the preservation of SSC and testicular tissues from boys and adolescents is far more advanced than research into the methods needed to realise the fertile potential of these cells. In principle, fertility restoration strategies in laboratory practice will include autotransplantation of a suspension of SSC by injection into the testis to restore spermatogenesis or autotransplantation of frozen-thawed testicular grafts back into the testis or an ectopic site. Should any risk of re-introduction of malignant cells exist via the transplant, then the only option is to grow and mature the SSC *in vitro*.

SSC transplantation was originally described in the mouse and is now an established research tool. SSC are infused through the efferent ducts into the *rete testis*, a technique which has been successfully applied in a number of species, including humans. The procedure is best performed under ultrasound guidance and presents a relatively non-invasive strategy for stem cell transfer. However, the colonisation efficiency after infusion of enzymatically digested testicular cells remains low. For future clinical applications, SSC need to be isolated, enriched and propagated *in vitro* before they can be autotransplanted in the numbers required to efficiently recolonise the testis and reinstate spermatogenesis. Nonetheless, the principle of the procedure has been shown and offspring have been generated from transplanted spermatogonia in a number of species, including primates [7]. While the demonstration of functional donor spermatogenesis following SSC transplantation in primates is an important milestone towards using SSC to restore human fertility, it remains vitally important to prove that the epigenetic programming and stability of SSC are not compromised following cryopreservation, culture and transplantation in humans.

Grafting of fragments of testicular tissue provides an alternative strategy to the use of cryobanked immature testis tissue. This approach maintains the SSC within their non-exposed natural niche, thus preserving the interactions between the germ cells and their supporting somatic cells. This procedure was successfully applied to retrieve sperm from ectopic and intra-testicular allografts, and insemination studies using ICSI have demonstrated that the spermatozoa were able to support full-term development of the progeny. This procedure is now tested in a number of species.

The major hurdle which must be overcome in patients with a haematological malignancy is the risk

of re-introducing residual malignant cells via the testicular tissue. Sorting protocols using magnetic activated cell sorting (MACS), fluorescence activated cell sorting (FACS) or differential plating have been found to have variable efficiency when used to enrich human SSC. The risk of re-introduction of malignant cells via the graft may be circumvented by *in vitro* spermatogenesis. *In vitro*-derived spermatozoa that are free from residual disease can then be used to inseminate oocytes using ICSI. Various strategies – including standard 2D cultures, 3-dimensional culture of testicular cells or organ culture – have been tested and showed some promise [41]. Although encouraging results have recently been obtained regarding the genetic and epigenetic stability of human SSC during long-term culture, the fertility of *in vitro*-derived sperm has still to be established before the clinical value of this type of experimental approach can be fully assessed.

Similarly as in the case of oocytes, when no germ cells are available in the initial testis biopsy, an alternative option may be the *in vitro* derivation of sperm cells from the patient's somatic cells, such as skin fibroblasts, by induced pluripotency or transdifferentiation of these cells. This approach is, however, still in its infancy.

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Related material

- Appendix 11. Example of consent form: female (NHS, UK)
- Appendix 12. Example of consent form: female (CNPMA, Portugal)
- Appendix 13. Example of consent form: male (NHS, UK)
- Appendix 31. Informed legal consent for cryopreserving and storing semen from a minor

Part C. Developing applications

Chapter 29. Introduction of novel processes and clinical applications

29.1. Introduction

Advances in basic science, technology and medicine create opportunities for the development of novel tissue or cell graft-preparation processes (including changes to donor selection, procurement, processing, storage and distribution methodologies) or clinical applications. There are several key elements that must be observed to ensure the quality, safety and efficacy of novel processes and applications and, thus, the safety of donors and recipients:

a. Clinical need should be the predominant driver for the development of novel processes and applications for tissues and cells.

b. The involvement of, and close co-operation between, three groups – tissue establishments (TEs), clinicians representing organisations responsible for human application (ORHA) and Health Authorities – is essential to ensure that the principles of safeguarding quality, safety and efficacy are addressed. A clear structure identifying the responsibilities of each party, and how the different parties interact with each other, must be established and documented.

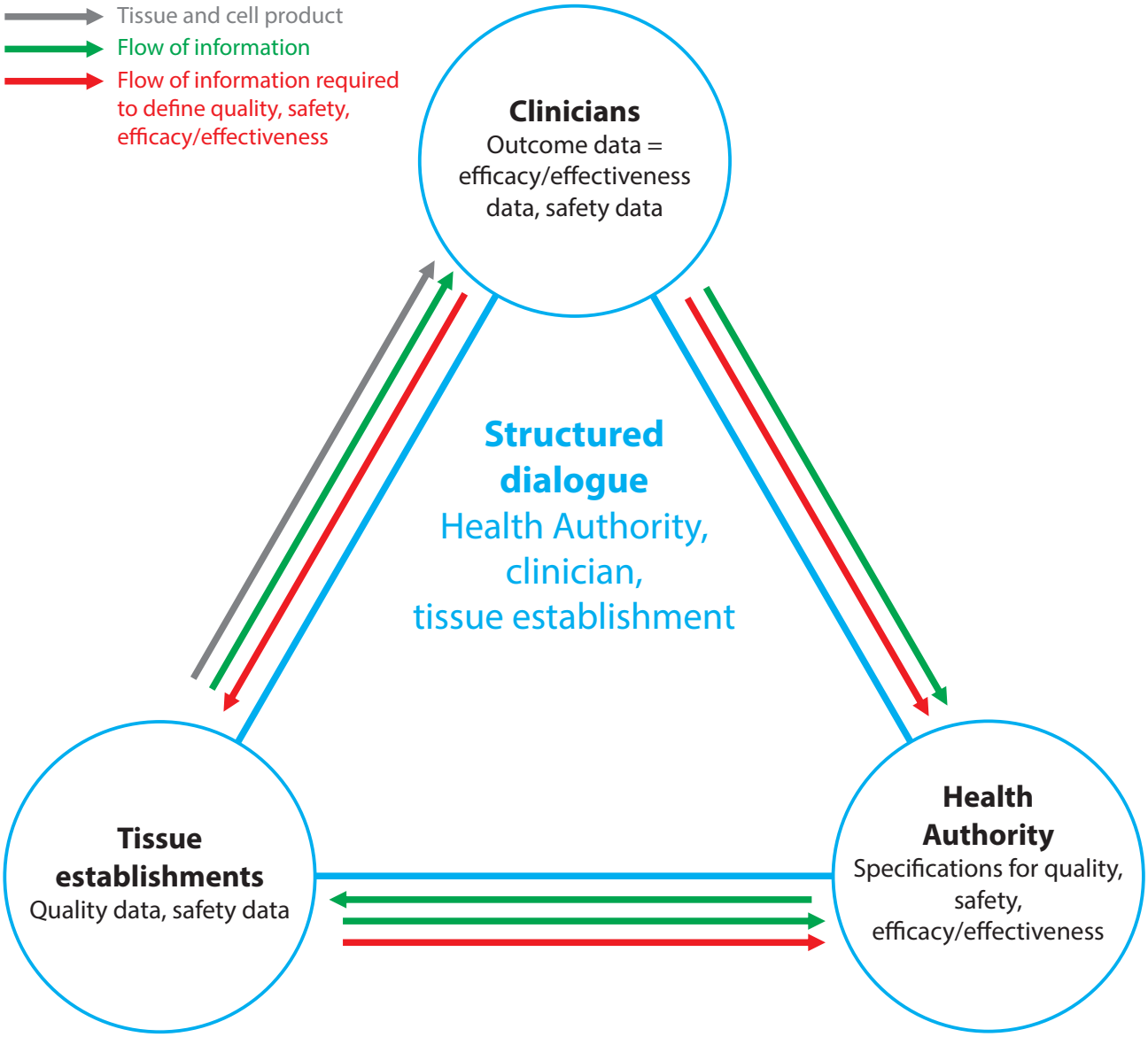
c. Comprehensive risk analysis should underpin the development and evaluation of novel processes and applications. This risk analysis should consider both the risks and potential clinical benefits of the novelty. Evaluation may comprise *in vitro*, *in vivo* and, where indicated, clinical follow-up studies according to the level of risk identified.

For established processes and applications, Part D of this Guide includes monographs appropriate to different processes and applications for tissues and cells. Monographs are useful tools for TEs and Health Authorities, providing the minimum criteria to ensure the quality of different types of tissues and cells; they are tools that can be used by TEs to design appropriate validation studies for new processes.

29.2. Regulatory considerations

When a TE is developing a novel preparation process, or if it plans to provide tissues or cells for a new clinical application, it should consider whether the process or therapy might lead to a regulatory classification of the tissues or cells as medicinal products, or as advanced therapy medicinal products (ATMPs) or medical devices. If this is the case, the regulatory framework for the authorisation of the relevant product type will be applicable in the EU (for further information, see Chapter 30). However, if the starting material for the ATMP is a tissue or cell, the regulatory requirements regarding donation and traceability may have to be applied according to the EU Tissues and Cells directives. TEs should engage with their Health Authority at an early stage of the product development cycle, in order to establish in advance which is the correct legal (regulatory) framework. This chapter addresses tissues and cells that are regulated under Directive 2004/23/EC in the EU.

Figure 29.1. **Flow of information between tissue establishments, clinicians and Health Authorities**



This chapter is largely based on the guidance developed as part of the EU Joint Action VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation), which proposed regulatory principles for Health Authorities for the appraisal and approval of clinical evaluation protocols for blood, tissues and cells prepared with new processing methodologies [1]. These VISTART principles aim to guide stakeholders in the development and implementation of novel preparation processes or clinical applications, ensuring compliance with regulatory and technical requirements, and they propose an approach whereby the degree of risk associated with the novelty is linked to an appropriate clinical follow-up plan. This chapter has also built on the EuroGTP II project, which developed good-practice guidance for TEs for the evaluation of the safety, quality and efficacy of tissue and cellular therapies and products [2].

29.3. Interaction between key stakeholders

When introducing novelties in the field of tissue and cells, three main stakeholders should interact closely. These are the TEs, the clinicians in the ORHA and Health Authorities. They each contribute their specific expertise to establish a structured, safe and efficient approach to the introduction of new grafts and clinical applications. As consideration of the risks, both to the product effectiveness and to the safety of the patient are vital, consultation with all of these stakeholders is essential. There should be a formal agreement between the TE and the clinicians/ORHA clearly specifying their respective roles and responsibilities when setting up clinical evaluations. Figure 29.1 describes schematically a structured flow of information between TE, clinicians/ORHA and Health Authorities.

29.3.1. Responsibilities of the tissue establishment

The TE is responsible for ensuring that the quality and safety of tissue and cell products meet the regulatory requirements and technical specifications necessary for release for clinical application. In this context, quality and safety result from the donor selection, the procurement of tissues and cells, the testing and the preparation processes. Preparation processes must be performed in compliance with the tissue and cell Good Practice Guidelines (Part E) and the EU Tissue and Cells directives requirements.

Two different perspectives currently apply to the meaning of the term 'quality' within the field of tissue and cell product processing and both should be addressed by the set of quality-control parameters used to characterise the tissues and cells resulting from the preparation process: quality may be seen as the fulfilment of a specific set of standards, characteristics and requirements as predefined by the preparation process, i.e. compliance of the tissue or cell product with its specifications (tissue and cell monographs). Quality may also be seen as an indicator of the safety and efficacy of the tissue or cell product. The critical parameters for novel tissue or cell preparation processes should cover both quality perspectives.

The safety profile of tissues and cells covers biological (infectious, immunological), physical (e.g. morphological appearance, integrity, elasticity) and/or chemical (e.g. toxicological, residual traces of reagents) influences. The safety of novel preparation processes or clinical application results from a careful and comprehensive risk analysis. Reference methodologies to perform a risk-based analysis of tissue and cells preparation processes and clinical applications are proposed by the EuroGTP II project (Good Practices for demonstrating safety and quality through recipient follow-up) [2], taking into account risks related to donor characteristics, procurement process and environment, preparation process and environment, reagents, reliability of microbiology testing, storage conditions, transport conditions, the presence of unwanted cellular material and the complexity of the preparation/application method.

Risk analysis is based on current evidence derived from known preparation processes, processing steps and products that are comparable to the tissues and cells obtained with novel preparation methods and/or new clinical applications. In circumstances where evidence is lacking, due to the grade of novelty and uncertainty, an analysis should be done to estimate the risks. The determination of risk levels – including a proposal for mitigation strategies – may be facilitated by supporting tools, such as the ones developed by the Euro GTP II Project, which provide a standardised methodology and an interactive assessment tool for risk analysis in the tissue, haematopoietic progenitor cell (HPC) and medically assisted reproduction (MAR) sectors.

Ultimately, the safety and efficacy of the tissue and cells product can only be confirmed by clinical outcome data. However, the TE should use *in vitro* and *in vivo* testing to investigate and mitigate any potential risks prior to clinical application.

29.3.2. Responsibilities of the clinicians/ organisations responsible for human application

The perspective of the clinician focuses on product safety and efficacy in the context of clinical application of tissue and cell products. The clinician is responsible for obtaining appropriate patient consent for application, and for collecting clinical outcome data from patients. Clinical outcome data should be gathered from a well-defined patient cohort to demonstrate clinical efficacy and safety of the novelty.

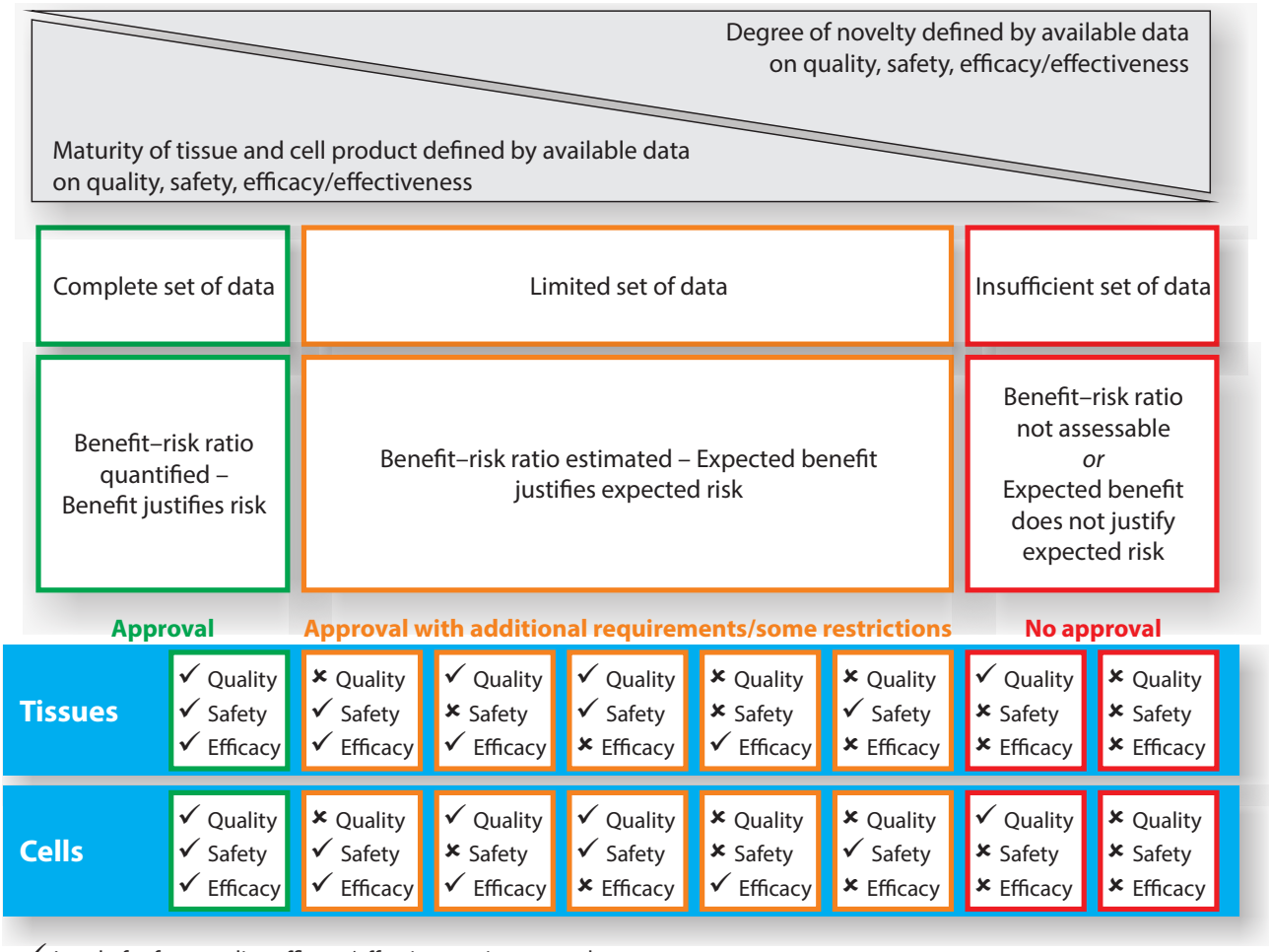
The extent to which clinical outcome data are required to verify the safety and efficacy of novel tissues or cells depends on the level of risk of the novelty. A systematic analysis of literature evidence may be used to define the extent of clinical follow-up. In this case, the methodology used must be documented. Clinical follow-up studies should be designed to generate statistically significant data. The principles of Good Clinical Practice and the Declaration of Helsinki must be integral to the design and performance of clinical evaluation. The clinical outcome data must be shared between the clinician and the TE and forwarded to the Health Authorities by means of a clearly structured process. This is in addition to the routine biovigilance reporting procedures, which are mandatory.

If long-term clinical outcome data are required for the demonstration of clinical efficacy/effectiveness, then national, European or international registries might be a useful tool to obtain sufficient clinical information.

29.3.3. Responsibilities of the Health Authorities

Regulation of tissue and cells by independent bodies, e.g. Health Authorities, is important to ensure quality, safety, efficacy/effectiveness of tissue and cell products [1]. Regulation focuses on two key elements:

Figure 29.2. **Regulatory models of risk assessment for novel preparation processes and clinical applications**



✓ Level of safety, quality, efficacy/effectiveness is accepted.

✗ Level of safety, quality, efficacy/effectiveness does not meet the required criteria.

- data-driven, risk–benefit assessment of tissues and cells, based on well-established specifications ;
- risk-based decision-making on the approval of preparation processes or clinical applications by the Health Authorities.

As novel tissue or cell products, inherent to the definition, have limited clinical data relating to quality, safety and efficacy, it can be challenging to assess their benefits and risks. Health Authorities can only approve tissue and cell products for routine clinical use based on sufficient data relating to safety and quality.

In the case of innovative preparation methodologies or new applications, the normal authorisation procedure might need to be enhanced with associated clinical follow-up requirements, depending on the assessed risk. This approach will allow regulatory requirements to be balanced with timely access for patients to novel tissues and cell therapies and is in line with the new regulatory principles described by the VISTART Joint Action [1]. For example, limited authorisation of a preparation process might be issued with additional requirements – for example, that the TE and clinicians provide novel tissue and cell therapies for clinical application only to limited numbers of selected recipients, possibly in the context of an ethically approved clinical evaluation, or only to a limited number of named clinicians – so that the data needed to guarantee a comprehensive assessment of safety and quality can be systematically generated and reported by the TE to the Health Authority. At that point, if deemed appropriate, the Health Authority can issue a full approval. These regulatory approaches are being further explored in a new EU GAPP Joint Action [3] involving tissue and cell Health Authorities from multiple EU member states.

A final proof of the quality, safety and efficacy of tissues and cells can only be provided by favourable clinical outcome data. Consequently, data resulting from clinical evaluation constitute the basis for a fully data-based, risk-dependent decision whether full approval of the novel preparation process or clinical application may be granted.

Figure 29.2 summarises models of authorisation for novel preparation processes and clinical applications for tissues and cells

29.4. Life-cycle management of novelties and registries of consolidated practices

Because novel tissues and cells are typically prepared, regulated and applied in the context of a continuously evolving 'state of the art', effective and efficient life-cycle management is of high importance. Life-cycle management comprises management of knowledge, change and uncertainty at the interface of the TE, clinician and the Health Authority. Thus, clinical follow-up of patients, i.e. the analysis of clinical outcome data and adverse reactions, is essential. Life-cycle management of tissues and cells also comprises a close regulatory oversight of the entire donation–distribution–application chain, requiring a close interaction between Health Authority, ORHA and TE.

Life-cycle management of novel preparation processes or new clinical applications requires a broad and comprehensive data-based framework. Continuous review of preparation processes, clinical monitoring of recipients and availability of updated clinical information all contribute to product quality, safety and efficacy/efficiency profile. Such data-based frameworks require consideration of geographic, temporal and technical aspects: instead of local or regional overview of products, a much broader approach should be considered, utilising the tissue and cell monographs included in Part D of this Guide and by accessing European databases, e.g. the European Cornea and Cell Transplantation Registry (ECCTR), the European Society for Blood and Marrow (ESBM) Transplant Patient Registry and European IVF Monitoring (EIM), whenever possible.

29.5. References

1. Vistart Joint Action (Vigilance and Inspection for the Safety of Transfusion Assisted Reproduction and Transplantation): Deliverable 5.4, "Principles for Competent Authorities for the evaluation and approval of clinical follow-up protocols for blood, tissues and cells prepared with newly developed and validated processing methodologies", available at https://vistart-ja.eu/home, accessed 4 January 2019.
2. EuroGTP-II Project. Good Practices for demonstrating safety and quality through recipient follow-up, available at www.goodtissuepractices.eu/, accessed 4 January 2019.
3. GAPP Joint action. Facilitating the Authorisation of Preparation Process for blood, tissues and cells, available at www.gapp-ja.eu, accessed 4 January 2019.

Chapter 30: **Developing cell technologies**

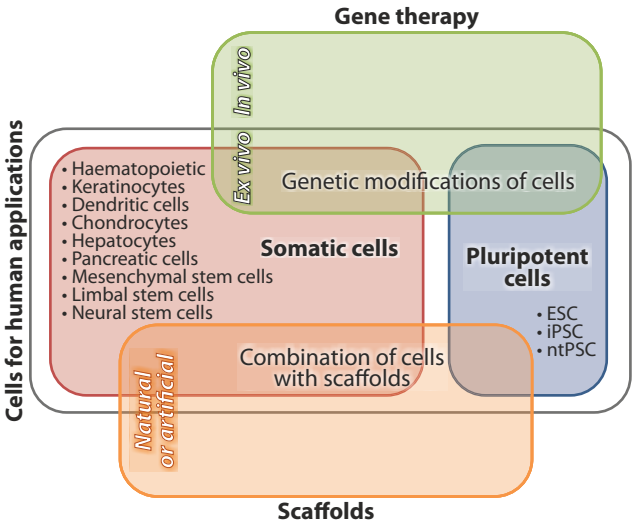
30.1. Introduction

Therapies based on tissues and cells, such as those described in Part B of this Guide, are already well established in medical practice. In recent years, increasing numbers of tissue establishments have expanded their activities, providing starting materials or engaging in the preparation of more complex products based on human tissues and cells. This chapter provides an overview of this developing field and addresses technical considerations for professionals in tissue establishments who may be interested in developing such activities. An overview of these novel therapies involving the use of cells of human origin is presented in Figure 30.1.

It is important to note that, in different countries, these therapies may fall under different regulatory frameworks, including those for transplantation, medicinal products or medical devices. Irrespective of the content of this document, any operator active in the field should carefully consider the legal requirements that apply to the activities they are undertaking and it is advisable that, before starting any activities, they consult with the relevant authorities to understand the regulatory environment and seek any licence/authorisation that may be required.

In the European Union (EU), the use of cells falling under the definition of advanced therapy medicinal products (ATMPs) is governed by specific requirements and procedures, including prior authorisation by the Health Authority. Specific GMP guidance is provided for ATMPs in the EU [1].

Figure 30.1. **Novel therapies involving human cells**



Note: ESC, embryonic stem cells; iPSC, induced pluripotent stem cells; ntPSC, nuclear transfer pluripotent stem cells.

30.2. Cell types being used in developing new technologies

Many tissues are sources of different cell types that are currently being used or researched for the development of new therapeutic options. Due to their undifferentiated nature, stem cells can be expanded *in vitro* and differentiated into various tissue-specific cells. This unique characteristic can be exploited to support the development of new therapies for the treatment of a number of conditions. However, before considering a new cell-based therapy, it is necessary to understand the physiological properties of

each stem cell or progenitor cell type. In addition, in some countries the use of these stem cells may not be permitted, and this must be taken into consideration. Table 30 .1 summarises some of the cell types currently used in developing novel cell-based therapies.

The methods most commonly used to obtain pluripotent stem cells (PSC) are shown in Figure 30.2. Haematopoietic progenitor cells (HPC) and multipotent and lineage progenitor cells are discussed in further detail in Chapter 22 and Chapter 32 respectively.

30.3. Processing cells for human application

In the preparation of cells for human application, different levels of processing may be required in order to deliver cells with the required characteristics and functionality. Some cells can be transplanted without *in vitro* expansion (e.g. HPC procured from bone marrow, HPC procured from peripheral blood, HPC procured from cord blood, hepatocytes, beta cells), whereas others need to be cultured *in vitro* and differentiated into more restricted cell types and, finally, some others undergo many manipulations to render the final product suitable for clinical application. In this section some of the most commonly used processing methods are discussed.

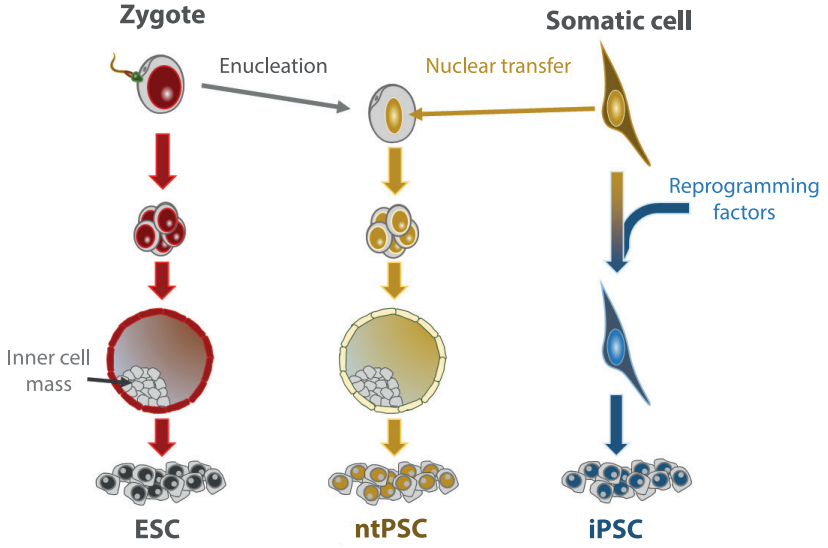
In the EU, many of the following processing methods are considered as substantial manipulations and thus the resulting products are governed as ATMPs under the medicines rules, which include specific requirements that must be respected when the activity is undertaken in the EU or if the resulting cells are intended to be used in the EU (see section 30.5 for further details).

30.3.1. Culturing cells

The origin and procurement of the starting material to isolate cells for therapeutic use is considered critical for the yield and identity/purity of the final cell population. Processing steps – such as derivation and/or expansion of cells, addition of cryoprotectants and all steps involved in the culture of cells – must be undertaken in an environment that is fully compliant with good manufacturing practices (GMP) for ATMPs. The initial procurement of tissue or cells from a human should always follow the regulations and guidelines related to human tissue/cell donation, procurement and testing, and universal precautions must be applied to minimise the risks of contamination, infection and pathogen transmission (see Chapter 4 and following).

Culture conditions are critical during expansion and differentiation of cells in culture [2]. The culture conditions for a specific cell type should be defined to control the number of cellular duplications and to achieve an adequate balance between number of passages and duplications. Antibiotics are not usually included in cell-culture media because they may mask the presence of a low level of bacterial con-

Figure 30.2. **The most common methods used to obtain pluripotent stem cells**



Note: To obtain embryonic PSC (ESC), embryos are developed until the blastocyst stage, when the inner cell mass is isolated and cultured to derivate the cells. Zygote can be enucleated, and a somatic cell nucleus is then transferred into it. The nuclear-transfer zygote is then developed until the blastocyst stage and the inner cell mass is isolated and cultured to obtain the nuclear-transfer PSC (ntPSC). Somatic cells can also be reprogrammed into induced PSCs (iPSC) by using a transcription factor cocktail.

tamination and, therefore, have the potential to cause infections in some recipients. Microbiological testing is required when culturing cells, which should be done at different critical steps and before cell release (see Chapter 10).

Validation of the preparation process should be carried out with respect to maintaining genetic stability and the relevant biological properties, as well as avoiding malignant transformation.

The impact of raw materials of biological origin used in the production of cells for therapeutic use has to be evaluated by risk assessment. Guidance is provided by the *European Pharmacopoeia (Ph. Eur.)* general chapter 5.2.12 on raw materials of biological origin for the production of cell-based and gene therapy medicinal products [3]. A safety issue when culturing human cells is the use of materials of animal origin, such as media or growth factors. The use of this xeno-material should be avoided as much as possible by using human-derived factors, for example by obtaining serum from the intended recipient. If animal materials cannot be avoided, specification and verification of both source and method of preparation of the material is required (for example, quality requirements for bovine serum can be found in *Ph. Eur.* monograph 2262). Culture media and other reagents derived from animals must be evaluated for the risk of contamination with micro-organisms, particularly viruses (see *Ph. Eur.* 5.1.7 for viral safety) and transmissible agents of human pathologies such as transmissible spongiform encephalopathies (TSE) *(Ph. Eur.* 5.2.8).

Documentation that demonstrates the application of appropriate quality-assurance measures by suppliers of media of animal origin, including origins and veterinary certificates for the animals used in the preparation of the material (e.g. bovine serum albumin), must be obtained. Certificates must be supported by audit trails for collection, pooling, shipping and final formulation by the third-party supplier. The use of raw materials and processing materials that are supplied with a TSE certificate from the European Directorate for the Quality of Medicines & HealthCare (EDQM) minimises the risks of infection from TSE [4]. In the EU, the requirements for sourcing/donation, procurement and testing are set out in GMP for ATMPs and in the guideline on xenogeneic cell-based medicinal products. For further guidance on cell culture, refer to the report (by the second Task Force of the European Centre for the Validation of Alternative Methods) on good cell-culture practice [5].

At the point where the cells will not be expanded any further, the downstream process begins. This includes the final collection and subsequent process steps of concentration or volume reduction of the collected cells, washing or clarification of the collected cells, formulation of the cells into an appropriate medium for preservation and then filling their final container with the formulated cells for cryopreservation and storage, or for direct delivery to patients. When a cell-based product is delivered for use, it must be shipped under appropriate conditions to the clinical site, prepared for application to the patient and then administered by a physician or trained healthcare professional.

30.3.1.1. *Microbiological testing when culturing cells*

Culturing cells includes a broad range of procedures that differ, depending on the cell type, in many aspects such as source material, finished product, culture reagents, time in culture, expansion, differentiation, quality-control analysis and storage procedures. All these differences make it difficult to establish a general rule for microbiological testing. Therefore, for each procedure, Quality Risk Management (QRM) should be applied to determine the quality-control strategy to be followed through the whole process and to identify critical steps to reduce the possibility of contamination and cross-contamination.

As a general recommendation, both the starting material and the finished product should be tested. For the starting material, microbiological tests should be carried out on the cells (or the cell source if the cells of interest are in too low numbers) and the culture reagents to ensure the absence of bacterial, fungal or mycoplasma contaminations. It is recommended to use the methodology described in the *Ph. Eur.* (2.6.7 for mycoplasma, 2.6.1 and 2.6.27 on microbiological examination of cell-based preparations). If other methods are used, they must always be validated.

When the starting material, including the cells themselves, cannot be stored in quarantine until the results of the microbiological analysis are obtained (for reasons of cell instability), the processing steps can start without the results being available. However, it would be necessary to study the potential risks of using this 'non-tested material' and document it, following the principles described in the QRM protocol.

In principle, the microbiological analysis should be done either in intermediate-cell products or finished product. However, when the number of cells is scarce, it may be acceptable to perform the

analysis with samples of washing media, supernatants or cell-culture media. If any microbiological contamination is detected, it is necessary to identify the strain and to investigate its source. In this case the product should not be used for clinical application unless a risk–benefit analysis indicates that it is the best option for the patient.

The finished product should be analysed for its microbiological quality before it can be released. Absence of bacteria, fungi and mycoplasma should be demonstrated. However, in specific cases when this approach is not feasible, the release of the finished product before the microbiological results are available may be justified. In this context, implementation and documentation of adequate processing – processing that provides sufficient assurance of the microbiological quality of the product when released – is essential. This will include in-process microbiological tests that have been established on the basis of risk analysis, usually including sterility testing of the culture media and of samples from the intermediate product at critical steps. It is recommended to use tests as described in *Ph. Eur.* 2.6.1 and 2.6.27 [6], the results of which are available in 7-14 days, depending on the method used. Alternative methods have been developed in recent years, and some of these methods have shown potential for real-time or near real-time results (*Ph. Eur.* general chapter 5.1.6). Tests for absence of mycoplasma (*Ph. Eur.* 2.6.7) and bacterial endotoxins (*Ph. Eur.* 2.6.14 and *Ph. Eur.* 5.1.10) should also be performed at given critical steps. More extensive details on microbiological testing may be found in Chapter 10.

When cells are allogeneic it is recommended to include the analysis of adventitious viruses, both in cell source material and in the finished product, in order to avoid transmission to the patient (see *Ph. Eur.* 5.1.7 for viral safety). Adventitious viruses can be analysed by different methodologies but polymerase chain reaction (PCR) assays are the most commonly used (indications for validation described in *Ph. Eur.* 2.6.21). In order to establish the virus that should be tested, it will also be necessary to do risk analysis [7]. Some examples of adventitious viruses that can be included are: *adeno*-associated virus, *Adenovirus*, *Bunyavirus*, *Cytomegalovirus*, Epstein–Barr virus, *Flavivirus*, hepatitis A virus, hepatitis B virus, hepatitis C virus, human *Herpes* virus 1 (HHV1), HHV6, HHV7, HHV8, human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2), human *papilloma* virus, human *rotavirus* (HRV), human T-cell leukaemia virus type-1 (HTLV-1), HTLV-2, influenza, measles, Norwalk virus, *parvovirus* B19 (Parvo-B19), rubella and *Enterovirus* (polio).

Table 30.1. **Some cell types being used to develop novel cell-based therapies**

	Cell type	Source	Processing
Pluripotent	Embryonic stem cells (ESC)	Obtained from the inner cell mass of blastocyst which have been cryopreserved and are no longer to be used for fertility treatment.	Cultured embryos (maximum 14 days) are dissociated and the inner cell mass is removed and cultured for a few more days. Then inner cell mass outgrowths consisting of potential ESC are isolated and expanded to create stem cell lines.
	Reprogrammed stem cells	Somatic cells which are reprogrammed to an embryonic stem cell-like state.	Reprogramming is achieved by introducing into adult cells (e.g. epithelial cells) a defined and limited set of transcription factors (e.g. stemness transcription factors) giving rise to induced pluripotent stem cells (iPSC). Cell reprogramming can also be achieved by nuclear transfer, giving rise to nuclear-transfer pluripotent stem cells (ntPSC).
Multipotent (lineage-restricted)	Somatic stem cells	Found in various tissues and may be isolated from extra-embryonic tissues, foetal specimens and adult tissues. Generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, neural stem cells, etc.)	Specific protocols have been developed, depending on the cell type. For details see Chapter 32.
Lineage-committed	Progenitor cells, e.g. haematopoietic progenitor cells	Somatic cells that are committed to a specific mature cell fate and can divide only a limited number of times.	Can be transplanted without *in vitro* expansion (e.g. bone marrow, peripheral blood, cord blood), or cultured *in vitro* and differentiated into more restricted cell types for clinical application.

30.3.1.2. *Considerations for quality control of other parameters for cultured cells*

It is important to demonstrate that the cell-based product meets specifications before release. For this reason, quality controls should include not only cell viability (*Ph. Eur.* 2.7.29) and genomic studies, but also cell identity, testing of biological activity, and other microbiological controls. Fluorescence-activated cell sorting (FACS) is one available method to analyse the presence or absence of specific cell-surface antigens (CD) and quantify the purity of the culture (*Ph. Eur.* 2.7.24). For example, haematopoietic progenitor cells should be positive for CD34 (*Ph. Eur.* 2.7.23) whereas mesenchymal stem cells (see Chapter 32 for nomenclature clarification) are negative for the antigen CD45 but positive for CD105, CD73 and CD90 (see Chapter 32 for specific information on cell-surface antigens of several somatic stem cells). However, in many cases the short time from cell culture to release and application is challenging, and these tests must be performed during processing.

The selection of appropriate markers is fundamental in the standardisation of isolation conditions and to identify cell populations, heterogeneity and yield. However, in many cases there are no known specific surface antigens for stem cells, which makes their purification difficult.

Control of genomic stability is also critical before releasing the cells for clinical application. Telomerase activity, proliferative capacity and senescence are also quality controls of relevance for human pluripotent stem cells.

30.3.1.3. *Master cell banks, working cell banks and cell stock*

Some cells, including stem cells, are used to produce master cell banks, which are then banked for future use as starting materials to manufacture cell-therapy products. A cell line is established from a single clone and this cell line is used to make up the master cell bank. This master cell bank must be characterised and extensively tested for contaminants such as bacteria, fungi and mycoplasmas. In addition, sterility and endotoxin testing, as well as PCR testing for viruses, may be required.

Cell lines used for patient treatment are placed in cryovials, and cryoprotectants are added before the cells are frozen and stored in the vapour phase of liquid nitrogen. The use of cryoprotectants and the control of cooling and warming rates during freezing and thawing are essential to minimise cell death and to increase cell attachment after thawing. This is of special importance during cryopreservation of human pluripotent stem cells [8]. The temperature inside the liquid nitrogen tank should be continuously monitored in order to ensure that the cells are stored under stable conditions.

It is recommended that master cell banks are stored in two or more widely separated areas within a production facility, and also at a distant site, in order to avoid loss of the cell line. Dual-site storage of all cells is accomplished with on-site storage and off-site storage.

Quality controls should be the same as described above for cell release and should be carried out before cryopreservation and after thawing to ensure that cell proliferation and viability, genome stability and purity of the culture have not been affected by the freezing process.

The working cell bank is a pool of expanded well-characterised cells derived from the master cell bank. The working cell bank is prepared from a single homogeneously mixed pool of cells. One or more of the working cell bank containers is used for each cell expansion [9, 10]. The characterisation and quality controls carried out for the working cell bank after cell thawing must be exactly the same as is done for the master cell bank, and the same specifications should be maintained.

Where cell-based products are generated from a cell stock obtained from a limited number of passages, and the stock does not cover the total life-cycle of the ATMP, it may be necessary to collect extra cells from new donors. The impact of these changes should be assessed and validated. It is recommended that cell stocks be handled in accordance with the principles outlined above for cell banks in regard to handling, storage and release.

Establishment of new cell banks/new cells stocks should be done in accordance with GMP. In exceptional and justified cases, the use of cell stocks/cell banks that were generated prior to the entry into force of Regulation 1394/2007 without full GMP compliance may be acceptable. In these cases, a risk analysis should be conducted to identify the testing requirements necessary to ensure the quality of the starting material; within the EU, approval from the competent authorities prior to use should be obtained [1].

30.3.2. Genetic modifications of cells

The genetic engineering of human, animal, plant and microbial cells in the laboratory became established biotechnology practice in the latter part of the 20th century. Techniques have been developed to allow the addition, removal and editing of gene sequences within cells, with ever greater precision,

and the reliable expression of the products from such modified genes *in vitro* and *in vivo*. New therapeutic products, such as monoclonal antibodies, have been manufactured using such genetically modified cells grown *ex vivo* in bioreactors.

There is now much interest in using the techniques developed for *in vitro* genetic modification of cells for direct therapeutic use *in vivo* for the treatment of a variety of diseases. This area of medicine is known as gene therapy. Modern gene sequencing has allowed specific genes associated with certain diseases to be identified and new techniques allow those genes to be synthesised and modified in the laboratory. In order to have a therapeutic effect, the gene needs to gain access to the target cells and be appropriately processed within the cell. This is achieved using a gene carrier or vector.

In the last decade, gene therapy has been a fast-moving area, and numerous issues related to the quality requirements of these complex, patient-specific therapies are arising as the field is advancing [11].

30.3.2.1. *General considerations in gene vectors for genetic modification of human cells*

The starting point for most gene vectors is a recombinant DNA plasmid. This contains the therapeutic gene sequence flanked by suitable sequences to allow its appropriate replication and expression in the target cell and by further sequences which allow the manufacture of multiple copies of the therapeutic gene in laboratory cell culture in order to have sufficient to transfect the target cells. Plasmids are relatively small, circular forms of DNA and several techniques have been developed that enable these plasmids to gain entry to cells *in vitro*. Most are engineered to allow multiple copies to be manufactured in bacterial cell culture, then extracted and purified before being used for gene therapy.

Techniques for transfecting cells with plasmids cannot generally be used *in vivo*, although muscle cells have been induced to take up plasmids following intramuscular injection and respiratory tract epithelia using liposomal delivery, with transient expression of the therapeutic genes. They are therefore more commonly used to transfect human cells *ex vivo* as a means of genetic manipulation before the cells are used therapeutically. For example, plasmids have been used as suitable vectors for delivering the genes to *in vitro* somatic cell cultures required to derive iPSC (see §30.2).

However, because of low transfection efficiency and generally transient nature in human cells, plasmids are not the vector of choice for *ex vivo* genetic manipulation of human cells or *in vivo* gene therapy. Instead, they are used to manufacture synthetic viral gene vectors in laboratory cultures of human or animal cell lines. Two or more plasmids are usually used to transfect the cell line. The plasmids contain not only the therapeutic gene and required flanking sequences, but also genes coding for important viral proteins, so that multiple viral particles will be generated with the therapeutic genes appropriately packaged within each particle for delivery to the target cell.

Viral vectors are based on naturally occurring viruses, selected for their particular characteristics, such as whether they selectively transfect certain tissue types and whether they integrate their genes into the host cell chromosome, or express them extra chromosomally. Specific packaging cell lines have been produced that allow the necessary replication of the vector in culture, but do not allow viral replication genes to be packaged in the viral particles produced, rendering the vector replication non-competent. A number of viruses have been used as the basis for human gene therapy, including *retrovirus*, *Adenovirus*, *lentivirus*, *Herpes simplex* virus, *vaccinia*, pox virus and *adeno*-associated virus.

Because of the potential risk of harmful infection using viral gene vectors and the unintended side-effects of genetic manipulation, appropriate experts in designing or selecting a vector for use in genetic manipulation of cells for human application must be consulted. It is also essential that the vector is manufactured under highly controlled conditions, with rigorous quality control [12, 13]. Specialist GMP manufacturers should be involved for this purpose.

30.3.2.2. *Post-genetic modification processes*

Extensive characterisation of the genetically modified cells must be performed, including establishing the number and location of integration events, sequencing of integrated sequences to establish the integrity of the molecular construct, removal of the transgene (if needed), investigation of the possibility of vector replication and viral reactivation, and confirmation of the genetic stability of the cells [14].

When the transgene is not intended to modify the cells or induce differentiation, a large number of experiments should be performed in order to demonstrate that modified cells have the same morphology/phenotype, genetic profile and functionality as the (pre-modification) parental cells. Unexpected changes in cell morphology, function and behaviour should be at least investigated and documented, and (depending on the magnitude of the changes) genetically modified cells must be discarded.

If the genetically modified cells are intended to have replacing activity, chromosomal integrity should be shown. Similarly, when genetic modification is performed to induce differentiation, the efficacy of such a process must be characterised and documented. When using pluripotent cells, any undifferentiated cells should be removed or killed to avoid teratoma formation after grafting. The purity criteria should be established and be within determined limits.

When genetic modification is performed for *ex vivo* production of secreted proteins of interest, pharmacokinetic studies should be designed in order to address not only expression, distribution and persistence of the transgene, but also dosage of protein release per cell and stability under *in vitro* and *in vivo* conditions. Toxicological studies should also be performed in order to avoid any unexpected effects. Similarly, when the cells that produce the gene product are encapsulated in biocompatible material, the appropriate secretion activity and potential toxicity should also be characterised and reported.

30.3.2.3. *Transient expression*

When transient genetic modification is intended, for example to induce cell differentiation, all genetic constructs must be removed from the final cell product. For this purpose, it is important to design not only the plasmid sequence but also the molecular strategy to verify that any traces of the plasmid used have been removed, to avoid future expression of genes or aberrant constructs.

30.3.2.4. *Purity and cell selection*

The purity of genetically modified cells is related to the efficacy of the transfection/transduction method used. In addition, when choosing the genetic modification method to be used, a selection marker can be introduced to increase the purity and consistency of the gene delivery method. The selection method, such as fluorescent or magnetic, should be chosen on the basis of the intended use. Cell selection is an important step to separate genetically modified cells from those that were not successfully modified. A complete description and a system of monitoring of the method used for the selection and/or purification is mandatory. The consistency of the method must also be demonstrated in different cell preparations.

The homogeneity and genetic stability of the modified cells should be characterised, including ascertaining that all cells in the purified population contain the intended genetic modification. The testing methods used for this should be cell type and vector-specific as necessary.

Furthermore, any observable change in morphology, function or behaviour of the purified cell population – whether caused by the genetic modification, the process of genetic modification or the purification process – should be documented. Special attention should be paid to the proliferation and differentiation properties of the modified cells and how they compare to the original unmodified cells.

30.3.2.5. *Cell banking of genetically modified cells*

After genetic modification of the cells, they can be cultured, selected and/or frozen. When expression of the gene is permanent, the production of a master cell bank (see §30.3.1.3) with modified cells is recommended. The master cell bank will give the opportunity to make the appropriate quality controls without excessive passaging of the cells.

A complete description of all post-modification steps should also be registered and appropriately monitored.

30.3.2.6. *Dosage*

It is critical to select the appropriate dose of the product when the cells are used to secrete a functional protein to produce a paracrine effect. The final dose will depend on several parameters, including the level of expression, the number of vector copies per cell and the number of cells grafted. Where possible, the number of grafted cells should be adjusted to administrate the desired protein dose. Pre-clinical studies may help to set the correct dose, but the results obtained in animal models cannot always be extrapolated to human beings since the expression of the gene may change depending on the niche of the area of application. For this reason, applying a single dose in clinical trials is not feasible in most cases. Thus, applying at least two doses, the minimal effective dose and the maximum tolerable, will provide important information for future clinical trials.

30.3.3. Tissue decellularisation and combination of cells with natural scaffolds

Decellularisation of donated tissues is a technique commonly performed in tissue establishments with the purpose of producing a cellular-neutralised parenchyma that may have several uses. These extracellular matrices (ECM), also known as 'scaffolds', may in some instances be used directly for human application (e.g. heart valves, large vessels or dermal matrices) providing structural benefits while re-

ducing immunological rejection and the risk of contamination. Alternatively, cells can be combined with these ECM and used in patients to improve or replace biological tissues.

Decellularisation and combination of ECM with cells are addressed in more detail in Chapter 31.

30.4. Safety considerations when applying cells to patients

Many of the early clinical successes using intravenous infusions of cell-based products have been seen subsequently in the treatment of systemic diseases such as graft-*versus*-host disease and sepsis. However, it is becoming more accepted that diseases involving peripheral tissues, such as cartilage repair, may be better treated by application of concentration of cells locally. Direct injection or placement of cells into a site for tissue repair may be the preferred method of application, as vascular delivery suffers from a 'pulmonary first-pass effect', where intravenous injected cells are sequestered in the lungs.

Cells may suffer substantial functional changes resulting not only from culturing, *ex vivo* activation or genetic manipulation, but also as consequence of their human application. When cells are applied, the environment changes considerably and these changes can modify the morphological and functional characteristics of the cells; therefore, evaluation of tumourigenicity should also be integrated when cells are implanted into the patient. Short-term and long-term post-grafting follow-up of each patient is critical in autologous and allogeneic applications. Details of vigilance requirements of both recipient and donor of tissues and cells are addressed in Chapter 16. Pharmacovigilance requirements in the EU are defined in Regulation EU 1235/2010 and Directive 2010/84/EU, and specific guidance for ATMP issued by the European Medicines Agency is available for consultation [15].

Cells are not classical drugs and need specific requirements when manipulated. It is important that the administering professionals have some basic knowledge about cells. Some clinical trials failed because of a lack of training of the professionals who manipulated the cells before and during human/clinical application. Detailed instructions should be given that include cell manipulation and tissue pre-treatment, to avoid cell death or modification of the biological properties of the cells. Key requirements include having adequate pre-clinical data, independent oversight and peer review, fair subject selection, informed consent, research subject monitoring, auditing of study conduct, and trial registration and reporting.

30.5. Legal framework for the development of advanced therapy medicinal products in the European Union

To provide a common framework for the marketing of ATMPs in the EU, Regulation No. 1394/2007/EC of the European Parliament and of the Council on advanced therapy medicinal products (hereafter 'the ATMP Regulation') was adopted in 2007. Specifically, cells used in human application that have been subject to substantial manipulation, and/or cells that are used for an essential function or functions in the recipient different from their function in the donor, are regulated as medicinal products in the EU.

According to Article 1 (a) of the ATMP Regulation, an ATMP is any of the following medicinal products for human use:

a. a gene therapy medicinal product;
b. a somatic cell therapy medicinal product;
c. a tissue-engineered product.

A gene therapy medicinal product is a biological medicinal product that fulfils these two characteristics:

a. it contains an active substance that contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;
b. its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products must not include vaccines against infectious diseases.

A somatic cell therapy medicinal product is a biological medicinal product that has two characteristics:

a. it contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor;
b. it is presented as having properties for (or is used in humans with a view to) treating, preventing or diagnosing a disease through the

pharmacological, immunological or metabolic action of its tissues or cells.

For the purposes of point (a), the manipulations listed in Annex I to Regulation 1394/2007/EC are not considered as substantial: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing, cryopreservation and vitrification. Note that this list is non-exhaustive and that other processes may also fall outside the scope of Regulation 1394/2007/EC. Thus, based on scientific considerations, other manipulations may also be judged 'non-substantial' (e.g. manipulations that have been used in clinical practice in a hospital setting over many years).

A tissue-engineered product is a product that:

a. contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor;

b. is presented as having properties for (or it is used in humans with a view to) regenerating, repairing or replacing human tissue.

A tissue-engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, bio-molecules, biomaterials, chemical substances, scaffolds or matrices. Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, are excluded from this definition.

The cornerstone of the ATMP Regulation is that a marketing authorisation must be obtained before the marketing of ATMP. In turn, the marketing authorisation can only be granted if, after a scientific assessment of the product's quality, efficacy and safety profile, it is demonstrated that the benefits outweigh the risks. The Committee for Advanced Therapies (CAT) is a specialised and multidisciplinary committee at the European Medicines Agency (EMA) responsible for assessing the quality, safety and efficacy of ATMPs that follow the centralised procedure for marketing authorisation and it assists in the preparation of any documents related to fulfilment of the objectives of Regulation 1394/2007, where relevant. The application for a marketing authorisation must be submitted to the EMA and the final decision is taken by the European Commission.

Developers of products based on genes, tissues or cells can seek advice from the CAT on whether a specific product is an ATMP. The procedure is intended as an incentive for developers, who can ascertain at an early stage of development if their product must comply with the requirements that apply to ATMPs [16].

Where ATMPs contain human cells or tissues, Directive 2004/23/EC applies to the donation, procurement and testing of the tissues and cells.

The ATMP Regulation gives member states the power to authorise the use of custom-made ATMPs prepared on a non-routine basis in the absence of a centralised marketing authorisation, provided that the product is used for individual patients in a hospital and under the professional responsibility of a medical practitioner. This 'hospital exemption' requires the application of national requirements on quality, traceability and pharmacovigilance equivalent to those required for authorised medicinal products.

It is important to stress that, in the EU, ATMPs are medicinal products. It follows that the overall regulatory framework governing medicines (including, but not limited to, rules on manufacture, distribution, packaging, labelling, evaluation of risks and benefits, determination of the data needed to demonstrate efficacy and safety, pharmacovigilance and advertising of medicines) apply to ATMPs. Furthermore, use of ATMPs in an investigational setting is also subject to EU rules on clinical trials.

However, flexibility in the development of ATMPs is important to anticipate the rapid evolution of science and technology in the field. To facilitate the process, the EU introduced the risk-based approach [17], which is based on the identification of various risks associated with the clinical use of an ATMP and risk factors inherent to the ATMP with respect to quality, safety and efficacy.

Additional information about the EU regulatory framework for ATMPs can be found at the EMA and EC Internet websites [18].

It is important to emphasise that, in Part C of this Guide, the term 'cells' or 'cell therapy' can be used to refer to situations that may be regulated as ATMPs in the EU. This guidance is not intended to affect the scope of the EU rules on medicines, and any operator who intends to process, store, distribute or use cells in humans should first seek advice from national authorities on the appropriate, applicable legal framework.

30.5.1. National competent authorities

In EU member states, human cells for human application can be subject to different regulatory frameworks (depending on the intended use, mode of action and degree of manipulation). Advice on the classification of a specific cell therapy can be sought from the national competent authorities or from the CAT [19]. In some countries, the regulatory body is the same for all cell therapy products whereas, in others, those classified as ATMP are regulated by a different body/agency from those classified as cells for transplantation.

The ethical and legal position on the use of human stem cells or progenitor cells, as well as the regulatory oversight, differs in countries around the world. In the EU, each member state is able to make decisions on the use of progenitor stem cells for basic research. However, they must be compliant with the requirements stated in the ATMP Regulation if they are to be used for the manufacture of ATMP for treating patients.

Some countries have national legislation on paediatric donors that should be taken into account when cells from children are considered.

30.5.2. Independent ethics committees

Local/regional/national independent ethics committees (IECs) are important bodies designated to approve and review biomedical and behavioural research involving humans, including the scientific rationale for the clinical application of a new therapy. For the latter, IECs should consider the irreversible nature of some gene/cell therapies and address the acceptability of exposing a donor to a research protocol for the benefit of the recipient, in particular where the donor is a relative of the subject, especially a parent or a sibling, to be included in the trial. IECs should also check appropriate traceability and guarantees regarding subject data protection and confidentiality. Written informed consent for receiving a cellular therapy is considered a prerequisite, as in any clinical trial.

The International Society for Stem Cell Research (ISSCR) guidelines recommend that special emphasis be placed on the risks of stem cell-based clinical research during the informed consent process. The risks include tumour formation, immunological reactions, unexpected behaviour of cells, unknown long-term health effects and sensitivities around the source of cellular products [20].

30.5.3. Considerations on conduct of clinical research

Clinical research, including trials of experimental interventions, is essential in translating cell-based applications and it requires the participation of human subjects, whose rights and welfare must be protected [18]. All people involved, including sponsors, investigators, oversight bodies and regulators, must be responsible for ensuring the ethical conduct of clinical trials. As with all clinical research, clinical trials must follow internationally accepted principles governing the ethical design and conduct of clinical research and the protection of human subjects [21, 22]. In the EU, a specific directive regulates clinical trials [23].

30.6. References

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Chapter 31: **Preparation of natural scaffolds**

31.1. Introduction

The state of the art on tissue manipulation is in constant growth and evolution, due to the diversity of its clinical applications. Decellularisation of tissues is a reality, as this process is currently being applied in clinical practice with different type of tissues (details related to technical procedures can be found in Chapter 8 and Appendix 32). Despite this, decellularisation processes are continuously improving and adapting in order to obtain new tissues and/or clinical applications.

This chapter aims to provide a general overview of the potential new uses of scaffolds obtained through different decellularisation processes, addressing its main advantages and challenges. Moreover, this chapter present a general overview of the challenging process of bioprinting, which is now in a period of exponential growth, with no consolidated application yet.

Decellularisation of substances of human origin (SoHO) must maintain an equilibrium between eliminating cellular content and maintaining the mechanical and biological properties of the extracellular matrix (ECM). It is challenging to develop a decellularisation process that fully retains the essential properties for its final clinical applications. In this context, a variety of investigation lines are currently open with the aim to improve the properties of the SoHO and give solutions to medical conditions that at the moment do not have an appropriate therapy, or where improvements are still needed.

The major goal of a scaffold is to create the *in vivo* micro-environment, which is mainly provided by the ECM. Apart from the particular criteria needed for specific applications, an ideal scaffold designed for clinical use should fulfil a set of technical requirements. Firstly, biocompatibility and biodegradability are required, to allow scaffold replacement by proteins synthesised and secreted by native or implanted cells [1-3]. However, some applications require a compromise between complete degradation and mechanical support; biomaterials have been successfully used to constrain the post-myocardial-infarction failing heart, preventing it from further remodelling and dilatation [4-5]. The degradation products must be non-toxic and readily removed from the body to minimise inflammatory and immunological response, avoiding further tissue damage [3]. Moreover, since cell-degradation products are toxic to other cells, it would be important for the scaffold to allow host macrophages to infiltrate and remove cellular debris [6]. Finally, material production/procurement, purification and processing should be easy and scalable [7, 8].

Biological scaffolds composed of ECM have been shown to facilitate the constructive remodelling of many different tissues in both preclinical animal studies and human clinical applications [9]. Specifically, decellularised tissue reproduces more accurately the structure of the ECM. However, its composition strongly depends on the specific origin as well as on the isolation, decellularisation and purification procedures [3, 10]. Bioprinting would over-

come this obstacle, providing reliable biomaterials in every batch.

The methodologies described in this chapter can be considered to be the fundamentals for regenerative medicine and/or medical device purposes, but nevertheless the scope of this chapter is not the development of such procedures. Instead, the following sections aim to provide an overview and a description of the state of the art of the possibilities for the preparation and clinical application of natural materials.

31.2. Decellularisation

31.2.1. Opportunities

Decellularised tissue is used with the aim of developing biological substitutes to restore, replace or regenerate damaged tissues [11-13]. A variety of tissues could be used to produce decellularised ECMs, such as heart valves [14-17], blood vessels [18-20], skin [21], nerves [22, 23], skeletal muscle [24], tendons [25] or ligaments [26]. These ECMs may be used directly for tissue implantation or can be modified, depending on the desired application. The resulting scaffolds have several advantages for the effective treatment and restoration of unhealthy, missing or damaged tissue. In addition, the absence of constituent donor cells can help to create a micro-environment more conducive to recipient recellularisation and revascularisation *in vivo*. In most tissues, except tissues with an immune-privileged niche, such as cartilage or eye, the removal of components represents a significant improvement of graft compatibility and enhances transplantation outcomes through the reduction of immunogenicity [27].

Additional manipulations of ECM are outside the scope of this Guide, but they include, for example:

- scaffold recellularisation, in cases where it is necessary to combine manipulation of ECM with cellular therapy,
- use in conjunction with a chemical molecule (drug, growth factor or protein) in cases where localised delivery is a must,
- chemical treatment, in cases where it is needed to change the biomechanical properties of the tissue.

In the EU, whenever the manipulation of ECM leads to a substantial manipulation, or when cells and tissues are not intended to be used for the same essential function or functions in the recipient as in the donor [28], the ATMP Regulation applies (see Chapter 30). Legal requirements for the development of ATMPs are not addressed in this Guide. Information on regulations and requirements applicable to development of cell technologies can be found in Chapter 28.

The resulting scaffold from decellularisation is composed of ECM molecules – secreted by the resident cells of each tissue – which provide biological properties and are organised in a three-dimensional (3D) arrangement that confers mechanical and structural properties. The ECM is an interconnected network composed of proteins, lipids, proteoglycans and, in some cases, inorganic salts such as occur in the bone matrix [29]. The recellularisation of the scaffold can be produced by cells surrounding the tissue or by cells that have been seeded previously *in vitro*. These cells will be responsible for generating the new ECM, promoting tissue regeneration [30].

Decellularisation procedures usually represent a greater complexity than the traditional processes developed in tissue establishments. Therefore, before implementing decellularisation techniques, special attention should be given to activities planning because decellularisation procedures may have a significant impact on daily activities, since they often require the use of classified areas for a period of several days.

Table 31.1. **Biomaterials used now or under study to be used as scaffolds for different applications [31]**

Tissue	Biological
Bone	autologous grafts, alginate, chitosan, collagen, fibrin, gelatine
Articular cartilage	alginate, agarose, chitosan, collagen, fibrin, gelatine, hyaluronan
Heart	alginate, agarose, chitosan, collagen, elastin, fibrin, gelatine, hyaluronan, laminin, platelet gel, silk, starch, vitronectin
Pancreas	alginate, agarose, chitosan, collagen, laminin
Vasculature	alginate, agarose, chitosan, collagen, elastin, fibrin, gelatine, hyaluronan
Skin	collagen, glycosaminoglycans, hyaluronan
Nerve	collagen, fibrin
Ocular	collagen, fibrin, amniotic membrane

31.2.2. Challenges

The structures of any scaffold should incorporate the appropriate biophysical, biomechanical and biochemical cues that guide cell proliferation, differentiation, maintenance and function [8]. The complexity lies in the fact that each product should focus on the specific clinical application, and therefore different scaffolds with different properties need to be delineated [32, 33].

Regarding physical signalling, the scaffold internal structure – e.g. size and interconnectivity of pores – determines not only the transport of nutrients, metabolites and regulatory molecules [32, 34], but also the accommodation of the cells and their organisation into functional tissues. For instance, it has been largely demonstrated that extremely large pores could avoid vascularisation (endothelial cells are not capable of bridging pores larger than their diameter), but pores smaller than 100 µm can influence diffusion. Therefore, from the point of view of the cells, both the micro-environment and the macro-environment should be taken into account. From the chemical perspective, the scaffold has an important role in cellular attachment and differentiation. Specifically, it has been reported that properties such as wettability, charge, chemistry, roughness and stiffness play an essential role in determining whether cells are able to adhere. All these properties must be taken into account in the biomaterial selection or can be achieved through specific modifications.

31.2.2.1. *Evaluation of the decellularisation set-up*

During the set-up of a decellularisation process there are many different parameters to take into account and analyse, with the aim of assuring the maintenance of the biological and mechanical characteristics of the tissue. These parameters are analysed during the set-up of the protocols and their validation, but not all of them will be transferred and implemented as quality controls of the released tissues. Moreover, the set of parameters to be analysed should be defined for each decellularised tissue, depending on the final specifications of the product.

As the aim of decellularisation is to preserve the native properties of the tissue while eliminating the cell remnants that could elicit a host immune response, it is necessary to evaluate such parameters as the removal of cells, the elimination of genetic material, quantification of ECM protein content and the mechanical properties in order to assess the quality of the decellularisation protocol [35, 36]. Furthermore, it is necessary to ensure that toxicity resulting from the implantation of SoHO scaffolds is not a risk for the host.

Although complete decellularisation (with the elimination of 100 % of cell material) may be impossible to achieve, it is convenient to evaluate the effectiveness of the decellularisation protocols with some minimal criteria, such as cell removal and the accepted residual DNA [35-39].

31.2.2.2. *Microbiological assessment of natural scaffolds*

Sterilisation techniques may be applied to provide a higher level of safety, since scaffolds contain no living cells; however, classic sterilisation methods, such as ethylene oxide exposure [40-42], gamma irradiation [43-47] and electron-beam irradiation [48-50], are known to alter ECM structure and mechanical properties, which are critical for ECM functionality [51, 52]. Additionally, ethylene oxide treatment can cause undesirable host immune responses that impair proper functioning of the biological scaffold after implantation. Therefore, sterilisation by ethylene oxide is only acceptable when there is no other suitable alternative (*Ph. Eur.* 5.1.1).

ECM can be sterilised by simple treatments with acids or solvents, but such methods may not provide sufficient penetration within the tissue depending on thickness and matrix components. Also, as explained above, these methods are aggressive and (depending on time and temperature of the procedure) may damage key ECM components. There are significant advantages to combining the decellularisation and sterilisation processes to ensure a clinically safe ECM for the recipient without affecting its ECM structure. At the moment, paracetic acid has been shown to be the best solution to minimise bacterial, fungal and spore contamination [53]. Tributyl phosphate organic solvent has viricidal properties [54].

Supercritical carbon dioxide is under investigation as a method for the sterilisation of natural ECM [55, 56]. This agent reduces the bacterial and viral loads, with minor changes in mechanical properties relative to other sterilisation methods.

Absence of bacteria and other microbial contaminants, which is achieved by a correct process of sterilisation, is not the only condition required to avoid an undesired patient response caused by pathogens. Endotoxins and other pyrogens in the ECM may induce strong biological responses in the patient, fever being the main manifestation (see Chapter 10 for detailed endotoxin testing guidance). Because pyrogens are often difficult to remove from ECM, inactivation or destruction by depyrogenation agents may be preferable [41, 42, 57]. However, careful evaluation should be made to avoid affecting the mechanical or biological properties of the ECM.

31.2.2.3. *Quality control of decellularised tissues*

The quality control and acceptance criteria for tissues that will be decellularised should be adapted, taking into account the purposes of the decellular-

isation. For instance, tissues with low levels of microbial contamination may be considered acceptable for decellularisation, as long as the process can be validated to remove such contamination. When the donor meets all the criteria, but any human pathogen is detected in the tissue sample at any stage, further processing must be undertaken, even if the tissue is sterilised throughout the process. These measures should be based on an validated risk-assessment algorithm.

The quality control of ECM scaffold after the decellularisation process should consider the following:

a. effective removal of cells and cellular components (see Chapter 8);
b. effective removal of microbial contamination (see Chapter 10) and any potentially toxic microbial products (e.g. endotoxins);
c. effective removal of undesirable and potentially toxic reagents;
d. maintenance of desired ECM structural characteristics.

31.3. Bioprinting

31.3.1. Opportunities

The ability to procure an effective custom-made graft, with specific shape, size, porosity and mechanical properties, is of great interest for personalised medicine to treat specific patient pathologies. Ideally, the composition of this *de novo* built graft should accomplish certain specific needs, such as the induction of new tissue formation and regeneration, without activating the immunological response [58]. Several studies show that decellularised ECM from different tissues can promote regeneration in damaged areas, such as demineralised bone [59], amniotic membrane [60], nerve [61] and skin [62, 63]. In recent years, due to the biological composition of ECM, the use of decellularised tissues as starting material has been considered for the generation of an ECM-based biomaterials pool to develop bionics in 3D bioprinting. The main hypothesis is that, if decellularised ECM promotes tissue regeneration, so the ECM pool from digested tissue could be used as the starting materials in a 3D bioprinting technique to develop *de novo* personalised grafts.

Bioprinting is an emerging methodology that allows the generation of 3D structures by controlled disposition of biological materials and functional elements as biochemicals or living cells. The technique itself arranges these components layer-by-layer with a particular spatial placement of functional components [64]. The final aim of the technique is to mimic, to the maximum extent, the natural environment of live tissues at both structural and cellular levels. Importantly, the combination of natural biomaterials (that possess the appropriate biochemical and biomechanical signals) with bioprinting (which can design complex structures with specific shape and size at macroscopic and microscopic levels) opens the door to obtaining a new set of tissues with defined and specific characteristics.

Nowadays, 3D bioprinting is being explored in the regenerative medicine field as a way to tackle the need for tissues and organs suitable for transplantation. Importantly, recent advances have enabled 3D bioprinting of biocompatible materials, cells and supporting components to generate and transplant complex 3D functional living tissues. For example multilayered skin, bone, vascular grafts, tracheal splints, heart tissue and cartilaginous structures have been implanted [64]. Moreover, this technique offers the opportunity to combine different natural materials to obtain hybrid scaffolds, in order to enhance bioactivity in the implantation site.

Decellularised ECM has been used for bioprinting [54-67], as well as several ECM components such as collagens [68], fibrin [69] or gelatine [70]. The advantage of using natural polymers is that they fully satisfy the biochemical requirements of the tissue in terms of composition and biochemical signalling. Nowadays, the natural polymers used are mainly collagen and gelatin obtained from tendons and ligaments from tissue banks. This approach allows clinicians and patients to benefit from the biological properties of human tissues that have been increasingly used in recent decades, and gives the opportunity to tissue establishments to integrate the new 3D bioprinting technologies for more personalised grafts.

31.3.2. Challenges

3D bioprinting involves different levels of complexity, such as the choice of biological or synthetic materials, cell types, or growth and differentiation factors, and technical challenges related to the sensitivities of living cells and the rheology of the raw materials for the construction of the tissues. Dealing with these complexities requires a combination of different fields of expertise, from engineering and biomaterials science to cell biology, physics and medicine [64].

The process of bioprinting is composed typically of various steps: imaging of the native tissue or organ, design approach, material and cell selection, bioprinting itself and application [64]. Clinical trials have been made with bioprinting technology for the

regeneration of tracheal [71] and craniofacial defects [72].

But it must be repeated that these approaches have not yet been deeply studied for human application. Therefore, a lot of effort is still needed in this field to improve the procurement of tissues and organs useful for transplant.

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Related material

• Appendix 32. Decellularisation

Chapter 32: **Somatic cells in clinical use**

32.1. Introduction

Advances in medical research and the developing field for clinical applications using somatic cells for autologous or allogeneic therapies hold great promise for patients with a wide range of serious diseases. These therapies rely on a supply of cells of appropriate safety and quality. This chapter provides guidance for tissue establishments (TEs) on quality and safety aspects, not only of donation, procurement and testing of the starting material but also in the further processing, storage and release of the cells. The general quality and safety demands in Chapters 1 to 16 (Part A) apply, but some specific considerations for these cells are also relevant. The chapter also aims to provide an overview of some of the cellular therapies used in the clinic but still under further development. The special considerations for donor selection, procurement, certain quality criteria, biovigilance, storage and administration are described in these sections, and an overview of the different steps is provided in Table 32.1 and Table 32.2.

> *In the EU, some of the cell preparations described in this chapter fall under the definition of an advanced therapy medicinal product (ATMP). Such cell preparations are governed by specific requirements and procedures, including prior authorisation by the competent authority, i.e. the medicinal product agency. When ATMP preparation takes place in the EU, or where products meeting the ATMP classification are intended to be used in the EU, there are specific requirements. Acceptance criteria for cells used as starting materials for the manufacture of ATMPs, such as minimal cell number, viability or cell composition – besides viral (as per Commission Directive 2006/17/EC) and microbiological controls – should be established, as minimum requirements. Processing, quality control, storage, packaging, release, distribution, traceability use and pharmavigilance must be done in accordance with medicinal product legislation, specifically EC Regulation 1394/2007. In these cases, donation, procurement and testing of such cells must comply with the requirements in Directive 2004/23/EC. For all other requirements, full Good Manufacturing Practice (GMP) must be applied. In the EU, there is specific guidance on GMP for ATMP [1].*
>
> *Any operator intending to process, store, distribute or use cells which might be considered to be ATMPs should seek advice from their national competent authority. In case of doubt whether a specific cell-processing activity is regulated under the ATMP regulation, a recommendation for classification from the Committee for Advanced Therapies (CAT) can be requested. For further details of EU legislation for ATMPs (including their manufacture,*

storage, distribution, labelling, advertising, traceability and use), see Chapter 30.

The following chapters of this Guide all apply to these cells and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

In an attempt to offer to the reader a more comprehensive approach to the different cell therapies included in this chapter, they have been split into two parts:

a. somatic cells employed to restore or produce immunological functions in patients:
  i. antigen-specific T-cells,
  ii. natural killer cells,
  iii. dendritic cells,
  iv. mesenchymal stem cells,
  and
b. somatic cells employed to restore organ- or tissue-specific functions:
  i. mesenchymal stem cells,
  ii. chondrocytes,
  iii. keratinocytes,
  iv. limbal stem cells (ocular surface),
  v. stromal vascular fraction from adipose tissue.

Sections 32.2 to 32.5 examine group a: somatic cells employed to restore or produce immunological functions in patients. Sections 32.6 to 32.10 examine group b: somatic cells employed to restore organ- or tissue-specific functions.

32.2. Antigen-specific T-cells

32.2.1. General introduction

Autologous or allogeneic antigen-specific T-cells directed to pathogens or tumour cells may be obtained either through enrichment by cell culture in the presence of a specific antigen or by direct selection. Technology is available for the capture and isolation of cells based on the affinity of cell-surface receptors for specific proteins or peptides immobilised on a suitable insoluble matrix. This technology can be used to isolate donor T-cells from peripheral circulation with specific affinity for pathogenic or other antigens that can then be transplanted to elicit a beneficial immune response in the recipient (adoptive immuno-therapy).

For example, viral infection in immunocompromised patients after haematopoietic stem cell or solid organ transplantation is a frequent cause of morbidity and mortality. It has been possible to reconstitute the anti-viral immunity of the recipient against specific viruses – e.g. *Cytomegalovirus*, Epstein–Barr virus and *Adenovirus* – through isolation and adoptive transfer of autologous (solid organ) or donor-derived virus-specific T-cells. Also, pre-established virus-specific T-lymphocytes from allogeneic HLA-typed third-party donors may be used to treat virus-induced disease after HLA-mapping and selection of a suitable HLA-match [2]. If such banked virus-specific T-cells are to be used, an assessment for the risk of graft-*versus*-host disease (GvHD) or graft rejection, and an assessment for efficacy, based on the degree of HLA-(mis)match, must be considered by a qualified specialist in immunology and allogeneic stem cell transplantation.

T-cells can also be modified, using gene-transfer technology (see Chapter 30), to express high-affinity natural T-cell receptors or antibody-like receptors to selected antigens. The latter are synthetic proteins normally consisting of single-chain variable fragments (scFv) of an antigen-specific antibody fused with other proteins to ensure that it is displayed on the surface of the T-cell with appropriate transmembrane activity and effector properties in response to the desired target. Because these synthetic receptors consist of a fusion of different proteins, they are known as chimeric receptors, and T-cells modified in this way are called chimeric antigen receptor-T (CAR-T) cells [3]. Because of the need for appropriate expression and processing of the chimeric proteins in the host cells, integrating retroviral or lentiviral gene vectors (see Chapter 30) are commonly used for gene transfer to create CAR-T-cells, although phys-

ical methods based on electroporation have also been successfully employed. This approach is used for cellular immuno-therapy of cancer when sufficient naturally occurring antigen-specific T-cells cannot be isolated from an individual, or to overcome the consequences of immune tolerance on endogenous tumour-specific T-cell repertoire.

Clinical trials with such engineered CAR-T-cells represent a promising development of specific anti-tumour responses targeting diverse antigens in blood cancers as well as in solid tumours; for a brief overview, see Table 32.1 [3, 4]. Until 2018, two CAR-T-cell therapies had been approved by the Food and Drug Administration and the European Medicines Agency, one therapy for the treatment of children with acute lymphoblastic leukemia and the other for adults with advanced lymphomas. Now there are also pre-clinical advances in CAR design that argue favourably for the advancement of CAR therapy to tackle other haematological malignancies as well as solid tumours. [5].

32.2.2. Donor selection

Donors should be tested for transmissible diseases in accordance with Chapter 5; in addition, the presence of circulating antibodies against the specific target antigen should be determined before assays for specific T-cells are initiated. In cases of autologous use, a donor viral positive test result may not be an exclusion criterion for the manufacture of a product (see §5.5.4).

The patient's own cells are normally used for CAR-T-cell therapy, but stem-cell donors or third-party donors are also employed. As well as the transmissible disease testing just mentioned, consideration should be given both to the possible presence of a wild-type virus of the same type as the basis of the gene vector employed and to the likelihood of the formation of a replication-competent virus.

32.2.3. Procurement

Mostly, antigen-specific therapeutic T-cells are selected from an apheresis product following the specifications described previously (see Chapter 22). In the case of selection by culture, a sample of heparinised venous blood (50-60 mL) from a stem cell donor may be sufficient to obtain T-lymphocytes specific for viruses that elicit high-frequency memory T-cells (Epstein–Barr virus, *Cytomegalovirus*).

For CAR-T-cells, a sample of heparinised venous blood may be enough, since the CAR-T-cells are then expanded *in vitro* and *in vivo*. However, the mononuclear cell fraction isolated through an apheresis process is normally used as starting material to ensure a higher dose of T-cells for cell culture.

32.2.4. Quality control

The specific requirements for release include potency assays to determine antigen-specificity according to pre-established criteria (i.e. IFN-γ production that can be quantified using ELISpot assay or flow cytometry; or lytic activity that can be quantified by chromium-release assay or by flow cytometry) and identity by phenotype using flow cytometry. Evaluation of contaminants by flow cytometry should also be included. Expected viability after thawing should also be established if the specific T-cells are stored frozen for repeated *in vivo* transfer. For example, for CAR-T cells, quality control should include identity, purity, potency, impurities, cell viability (post-thaw), cell number/dose, percentage of transduced cells (in cases of *ex vivo* approaches) and vector/plasmid copy number per cell. Where T-cells expanded in cell culture are used for cancer treatment, dosing may have to be synchronised with chemotherapy and the cells may need to be available freshly prepared. This may require a planned two-phase release (see Chapter 27).

32.2.5. Storage and distribution

Cultured and released cells can be cryopreserved < – 140 °C in liquid or vapour-phase nitrogen, or deep-frozen with liquid nitrogen back-up. Stability testing and data are required for the cells used as starting materials, for intermediates (if any) and finished products.

Precautions should be taken to prevent cross-contamination of infectious agents if stored in the liquid phase of nitrogen, including the use of double containers.

32.2.6. Traceability

Records, with all information from procurement to *in vivo* administration, should be kept by the TE.

32.2.7. Biovigilance/pharmacovigilance

Whenever adverse events occur during the processing – or adverse reactions during application – of

the T-cells, this should be documented and reported (see Chapter 16).

The most common adverse effects and complications of this cell therapy are during cell application. Some of the adverse effects and complications are very serious and life-threatening, and require specific attention and urgent reporting, e.g. cytokine release syndrome, severe neurological toxicity and insertional oncogenesis hypogammaglobulinaemia [6].

32.3. Natural killer cells

32.3.1. Introduction

Natural killer (NK) cells were described, first in mice, and later in humans as non-B, non-T lymphoid cells with a non-major histocompatibility complex (MHC)-restricted cytotoxic activity against transformed or virally infected cells. The 'missing-self' theory by Kärre *et al.* [7] and the identification of killer Ig-like receptors (KIR) acting as inhibitory or activating signals have contributed to the understanding and better design of clinical trials. NK cells are bone-marrow-derived from $CD34^+$ progenitors, and migrate upon differentiation to lymphoid organs and peripheral blood. Their development and homeostasis are dependent on IL-15, and they express the adhesion molecule CD56 but lack the T-cell receptor and CD3. NK cells can be divided into at least two subpopulations according to their surface density of CD56 expression:

a. $CD56^{bright}$ in a resting stage are considered to be regulatory NK cells that produce high levels of cytokines and are more proliferative, but poor mediators of NK cell cytotoxicity;

b. $CD56^{dim}$ in a resting stage are potent cytotoxic cells mediating NK cytotoxicity as well as antibody-dependent cytotoxicity through CD16 (FcγRIII).

NK cells can be activated by several cytokines and they produce a wide variety of cytokines and chemokines: granulocyte-colony stimulating factor (G-CSF), tumour necrosis factor (TNF)-α and TNF-β, IFN-γ, tumour growth factor (TGF)-β, macrophage inflammatory protein 1-beta, and regulated on activation, normal T-cell expressed and secreted (RANTES). It is still not clear whether the *in vivo* effect of NK cells is a result of direct killing or indirectly through cytokine production, engaging other parts of the immune system. NK cell-based immuno-therapies against malignancies involve using either the autologous NK cells *in vivo*, by cytokine stimulation, or by adoptive transfer of autologous or allogeneic NK cells. There are many different protocols and clinical studies using NK cells against malignancies, as reviewed by Cheng *et al.* [8], but not covered in this chapter.

In addition to T-cell-mediated immuno-therapy, the unique biology of NK cells makes them a valid tool for immuno-therapy. In contrast to T-cells, CAR-modified NK cells show less severe side-effects, such as GvHD, because donor NK cells usually do not attack non-haematopoietic tissues such as liver, kidney, muscle and lung. A number of clinical trials have shown that NK cell infusion has less severe GvHD than does T-cell infusion.

Currently, CAR-modified NK cell lines are used as effector cells for various cancer treatments (acute lymphoblastic leukemias, glioblastoma, neuroblastoma, breast and prostate cancers, multiple myeloma) and also as immuno-therapy for serious infectious diseases such as HIV [9].

32.3.2. Donor selection

Depending on the clinical protocol, autologous NK cells can be used, either by activation of the presumed NK cells *in vivo*, or by *ex vivo* selection and/or activation.

Increasing knowledge of MHC recognition (classical or non-classical) and interaction, and of the haplotypes of KIR, suggests that donor selection for protocols with adoptive transfer of allogeneic NK cells could include MHC typing for HLA-C, HLA-E, and possibly also HLA-F and HLA-G, as well as KIR. The aim would be to select a donor whose ligands for the inhibitory signals were absent in the recipient. For adoptive transfer of *ex vivo*-activated NK cells with additional *in vivo* activation, special care should be taken to reduce the possible toxic effects of cytokines, depending on the underlying disease and the dose of the cytokine(s).

32.3.3. Procurement

NK cells can be procured from peripheral blood, by apheresis carried out by experienced personnel, as described in Chapter 22 (see §22.3.2.2).

Smaller amounts of NK cells to be expanded *in vitro* are obtained from 30-50 mL anti-coagulated venous blood. *In vitro* expanded NK cells can also be obtained from $CD34^+$ stem cells from cord blood after *in vitro* differentiation and maturation with defined cytokines.

32.3.4. Quality controls/release criteria

In addition to microbiological testing as described in Chapter 10, release criteria should include the defined level of phenotypic NK cells as established by flow cytometry, and possibly also a functional assay (cytokine production or cytotoxic assay).

32.3.5. Packaging and distribution

The processed and released NK cells are aspirated into a syringe for direct administration to the patient, with labelling containing the recipient identification and, if applicable, the dose of NK cells. Attached documentation should contain additional information to prevent errors if other patients or clinical trials are ongoing in the clinic (see Chapter 11 and Chapter 14).

32.3.6. Traceability

Records, with all information from procurement to *in vivo* administration, should be kept by the TE.

32.3.7. Biovigilance/pharmacovigilance

Adverse events during the procurement or processing that should be documented could involve, for example, less NK cell recovery than expected after a cell-separation step or a lack of functional activity measured *in vitro* as a quality control. Adverse reactions that should be documented could involve unexpected side-effects related to the administration of NK cells or to the additional activating cytokines, all of which should be documented.

32.4. Dendritic cells

32.4.1. General introduction

Tumour vaccines based on dendritic cells (DC) are a form of immuno-therapy which is being tested in a large number of trials internationally [10]. DC have the capacity to activate tumour-specific T-cells to attack and eliminate the patient's tumour. There are several subtypes of DC vaccines, but most are derived from monocytes that are cultured in a cytokine mixture composed of GM-CSF and IL-4 and then induced into mature DC by various maturation factors. See Table 32.1 for an overview.

32.4.2. Donor selection

The majority of DC vaccines are autologous and derived from monocytes purified from the blood circulation of the patients. As described in Chapter 5, donor testing includes assays for transmissible diseases; and, although their presence is not an exclusion criterion, it should be documented and care should be taken to avoid cross-contamination of other cells or personnel. Medical evaluation of the patient should take into account the burden of collecting large amount of leukocytes using apheresis. Depending on the method used to obtain monocytes from the collected leukocytes, a predetermined level of circulating monocytes may be relevant.

32.4.3. Procurement

As a starting material, apheresis-derived leukocytes are normally used. Apheresis should be performed by professionals specialised in apheresis, with the precautions mentioned in this chapter for DLI collection (Chapter 22).

The blood volume processed, in order to obtain a sufficient number of monocytes for further processing, depends on the patient's peripheral blood counts and should be calculated to avoid unnecessary apheresis time with the increasing risks of serious adverse reactions.

32.4.4. Quality controls/release criteria

Prior to freezing, the mature DC are tested by flow cytometry for their expression of a number of cell-surface markers which are characteristic for mature DC. These include markers such as low CD14 expression and high expression of CD80, CD83, CD86, CD1a, HLA-DR, DC-SIGN, ILT-3 and CCR-7. Secreted markers, e.g. IL-12, can be tested by ELISA assays.

32.4.5. Packaging and distribution

Matured and aliquoted DC can be used fresh or frozen with cryoprotectant in cryo-tubes and stored at −140 °C in a freezer or in the vapour phase of liquid nitrogen. Frozen cells are distributed on dry ice or a dry-shipper.

32.4.6. Traceability

Records for the complete process, from donor selection to clinical use, should be kept by the responsible TE.

32.4.7. Biovigilance/pharmacovigilance

Any adverse event during procurement of leukocytes, cell separation, culturing, cryopreservation or distribution, or any adverse reactions during administration, should be documented as described in Chapter 16. An example of such an adverse event might be lower numbers of monocytes than expected when collected by apheresis or during the cell-separation procedures. The route of administration of DC may involve adverse reactions that should be recorded and if possible avoided, or at least managed according to a risk–benefit assessment.

32.5. Mesenchymal stem cells

32.5.1. General introduction

Mesenchymal stem cells (MSC), also referred to as mesenchymal stromal cells [11], are multipotent stem cells with immuno-regulatory and regenerative properties. They can differentiate into a variety of cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells) and adipocytes (fat cells). This phenomenon has been documented in specific cells and tissues in living animals and their counterparts growing in tissue culture.

While the terms MSC and bone-marrow stromal cells (BMSC) have been used interchangeably, neither term is sufficiently descriptive, as discussed below.

Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into haematopoietic and connective tissue, whereas MSC do not differentiate into haematopoietic cells.

Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSC, the term fails to convey the (relatively recently discovered) roles of MSC in the repair of tissue.

The term MSC, used by many labs today, can encompass multipotent cells derived from other non-bone-marrow tissues, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp of deciduous baby teeth; see also Table 32.1 and Table 32.2.

The International Society for Cellular Therapy encourages the scientific community in all written and oral communications to adopt this uniform nomenclature (MSC) when cells meet specified stem cell criteria. It defines the specific MSC criteria thus:

> The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*. [11]

These criteria will probably change as new knowledge unfolds. MSC are characterised morphologically by a small cell body with a few cell processes that are long and thin. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance. The cells, which are long and thin, are widely dispersed; and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils.

The immuno-regulatory and regenerative properties of MSC make them an attractive tool for the development of treatments of autoimmunity, inflammation and tissue repair [12, 13]. MSC do not induce alloreactivity but generate a local immunosuppressive micro-environment by secreting cytokines. However, MSC interfere with dendritic cell activation, and they suppress lymphocyte activation and T-cell function *in vitro*. They have been shown to reverse inflammation in several experimental animal models, and clinical studies indicate that MSC are immuno-suppressive also in humans as they reverse steroid-refractory GvHD and other inflammatory conditions [13, 14]. MSC are a heterogeneous population of cells, with functions depending on both source and *in vitro* culturing conditions. MSC are also investigated for tissue-engineering purposes, mainly for osteo-articular diseases: bone and cartilage regeneration. The mechanisms behind their tissue-regenerating ability and their immuno-modulating capacity, and the extent to which the two processes interact, require further elucidation. In view of the increasing interest in using MSC for human application, the safety and quality aspects to bear in mind are mentioned in this section.

32.5.2. Donor selection

Under resting conditions, MSC express HLA class I but not class II alloantigens. When cultured *in vitro* with allogeneic lymphocytes, MSC do not stimulate immune responses. Based on these findings, it has been assumed that MSC can be transfused across HLA barriers; and therefore cells from HLA-identical

siblings, HLA-haplo-identical relatives or third-party HLA-mismatched healthy volunteer donors have been used in clinical protocols. However, MSC that are to be used for their regenerative capacity should preferably be autologous.

Donors should be evaluated for their own safety, and for the safety of the recipient, according to the criteria described in Chapter 4.

32.5.3. Procurement

MSC can be isolated from haematopoietic tissues, such as bone marrow, peripheral blood and umbilical cord blood, but also from parenchymal non-haematopoietic tissues, such as muscle, fat or liver. The youngest, most primitive MSC can be obtained from the umbilical cord tissue, namely Wharton's jelly. Umbilical cord-derived MSC have more primitive properties than other, adult MSC obtained later in life, which might make them a useful source of MSC for clinical applications.

The two main sources of MSC presently used for human application are bone marrow- and adipose-derived. The latter is one of the richest sources of MSC: there are about 500 times more stem cells in 1 gram of adipose tissue than in 1 gram of aspirated bone marrow.

32.5.4. Quality controls/release criteria

At present, release criteria are mostly phenotypic, and include cultures predominately expressing CD73, CD90 and CD105, although the relevance of these remains to be clarified. Further release criteria are sterility (absence of bacteria, mycoplasma and fungi; see Chapter 10) and lineage-negativity, including exclusion of haematopoietic contaminating cells.

It is challenging to identify markers that may be predictive for the potency of a specific product with a specific indication. However, surrogate markers, such as immuno-modulating capacity or ability to reconstitute specific tissues or to secrete tissue-specific factors, according to the intended use of MSC, will be needed to address functional properties and consistency of the cells. It will therefore be necessary to define and validate the release criteria depending on the culture conditions used and the clinical protocol.

32.5.5. Packaging and distribution

When distributed to the clinic for administration to the patient, the cells should be either transferred to vials with the pre-decided dose for administration or distributed directly in a pre-labelled syringe, depending on whether the MSC are processed in the close vicinity of the clinic.

Frozen vials of MSC are often thawed at the bedside by diluting with isotonic saline solution, at least 4× volumes (to avoid toxicity by the cryoprotectant to the MSC), and administered immediately to the patient. A small portion of the thawed, diluted MSC can be used to verify cell numbers and viability.

32.5.6. Traceability

Records to ensure traceability from the donation to the recipient should be kept with the TE.

32.5.7. Biovigilance/pharmacovigilance

As indicated above, the MSC consist of a heterogeneous population; their phenotype and function are dependent on source and culture condition. Accordingly, any deviation from the expected endpoints (according to the predefined criteria) should be considered an adverse event that should be recorded.

Sections 32.6 to 32.10 (below) examine group b: somatic cells employed to restore organ- or tissue-specific functions.

32.6. Mesenchymal stem cells

Mesenchymal stem cells are mentioned again here because they can be used not only to produce immunological functions but also to restore organ- or tissue-specific functions. They have been described (above) in section 32.5.

32.7. Chondrocytes

32.7.1. General introduction

Damaged articular cartilage has a limited capacity for self-repair. Cartilage lesions are usually associated with disability and symptoms such as pain, swelling, locking and malfunction of the joint, and if these lesions are left untreated it may lead to osteoarthritis. Autologous chondrocyte implantation (ACI) is a therapy widely used for the treatment of isolated cartilage defects. The original (first-generation) technique is based on an implantation of a suspension of *in vitro* expanded chondrocytes into the defect beneath a sealed cover of periostium flap. Since the technique was introduced in 1987 by Britt-

berg *et al.* [15], over 35 000 patients have been treated worldwide.

The second-generation ACI technique includes the use of a collagen membrane instead of the periosteal flap. The use of collagen membrane simplifies the surgical procedure and reduces complications such as periosteal hypertrophy.

Further technological advances led to the development of the third-generation technique that involves both *in vitro* expanded chondrocytes and a scaffold (briefly described in Chapter 29). After the expansion in culture flasks, the cells are seeded onto a membrane or a biodegradable scaffold before implantation. The scaffold may function as a carrier for the cells or as a structure to build up the 3D environment for the cells. In most techniques only fibrin glue is used for the fixation of the graft and, since there is no need for any suturing of the periostium/collagen membrane cover, this implantation can be done arthroscopically.

For an overview, see Table 32.2.

32.7.2. Donor selection

The patient is examined by an arthroscopic procedure where the location, depth and size of the defect and the quality of the surrounding cartilage are evaluated. A typical patient is a young patient with large (> 2 cm^2) full-thickness chondral or osteochondral defects surrounded by healthy cartilage. At the present time ACI is not indicated for patients with severe osteoarthritis, active rheumatoid arthritis or active autoimmune connective-tissue diseases, or patients with concomitant malignancies [12, 16].

32.7.3. Procurement

The ACI technique includes a two-stage procedure, with an initial procurement of a cartilage biopsy, which is sent for chondrocyte culture, followed by a second-stage operation that includes the cell application. A full-thickness cartilage biopsy (about 200-400 mg) is procured from a low-weight-bearing area of the knee during arthroscopy. The biopsy is transferred to a sterile transport tube with biopsy medium. The biopsy tube and blood tubes should then be placed in an outer secondary packaging that ensures the sterility and maintenance of the temperature, and is approved for transport of biological substances (see also Chapter 14). The biopsy should be kept cold – at about 5-15 °C – during transport to ensure the quality of the biopsy specimen. Transport should be sent directly to the TE for further processing, which should start within 48 hours. For culture conditions with autologous serum instead of foetal bovine serum, up to 50 mL of autologous blood should accompany the biopsy.

32.7.4. Quality controls/release criteria

Living cells cannot be sterilised, and therefore it is very important to ensure that all handling of the product is performed under good aseptic conditions and that all material, media and reagents used are sterile and endotoxin-free (see Chapter 10). The first sterility test for release is done a few days before the scheduled implantation, at the last media change, and the second test is made during assembly of the final product. The final result of a sterility test takes normally 10-14 days and the expiry time for the final product is normally 24-72 hours, so it is common that the cells are already released and implanted before the final results are available. However, a preliminary result of the sterility test can normally be given after 24 hours and it is upon this result that the cells can be released. An indication of the viability of cells in suspension is obtained using trypan blue. It is more difficult to test the viability of cells growing in a 3D construct. There is currently no non-destructive assay available, but other release criteria specific for chondrocytes are used.

32.7.4.1. *Morphology*

The morphology of the cells can be followed easily during culture using an inverted microscope. The chondrocytes should be typical of cultured chondrocytes in appearance, and the personnel who perform this subjective judgment must have good experience in this task and should have reference pictures of cultures for comparison. The cells lose the phenotype of uncultured chondrocytes during culture and become more fibroblast-like.

32.7.4.2. *Population doubling*

The cells should undergo only a limited and defined number of population doublings, for example 4-8 population doublings. In order to ensure a proper re-differentiation of the cells, the functional capacity of the cells after maximal expansion has to be shown, and it is important to record the population doubling during culture.

32.7.4.3. *Number of cells*

The number of cells must be provided. This can be in form of either the actual number of cells in the vial or syringe or the number of cells per surface area

that have been seeded in the scaffold. Other labelling requirements are as described in Chapter 14.

32.7.4.4. *Purity*

To exclude the presence of cells other than chondrocytes (synoviocytes, bone cells) in the culture, the purity of isolated and cultured cells should be assessed. A representative batch of cells can be validated for presence of mRNA markers for chondrogenic lineage, like sox9, and lack of (or low) mRNA expression of synoviocyte-specific genes. Since cells are dedifferentiated during culture, more specific markers of differentiation are not tested.

32.7.4.5. *Chondrogenic potential*

Attempts to predict the chondrogenic potential of the cells and thus the chondrogenic repair capacity have been made [17], but currently no clinical potency marker exists. Thus, the functional properties of the cells and an appropriate surrogate marker are still to be defined, and research is needed.

32.7.5. Packaging and distribution

The first-generation product (cells in a suspension) is aseptically filled in syringes or vials. The third-generation product is aseptically packed in a sterile two-container system. The advantage of having primary packaging that is also sterile on the outside is that it can be taken directly to the operation table and be handled by sterile personnel. The product should then be placed in an outer secondary packaging that ensures the sterility and temperature and that should be approved for transport of biological substances. Implantation is normally done within 48 hours, depending on the expiry time for the product. For the accompanying information sent to organisations responsible for human applications, see Chapter 12.

32.7.6. Traceability

Records with all information, from procurement to implantation, must be kept. Reference samples may also be archived.

32.7.7. Biovigilance/pharmacovigilance

Several adverse events that affect the growth and differentiation of chondrocytes may occur during the relatively long *in vitro* expansion period. In addition, if for example the degree of contaminating cell types unexpectedly exceeds the release criteria, this should be recorded and managed as described in Chapter 16.

32.8. Keratinocytes

32.8.1. General introduction

The current 'gold standard' of burn care [18] is early tangential excision of eschar, i.e. necrotic tissue, and autologous split-thickness skin grafting to the surgical wound areas. The skin has many crucial functions and the main goal with the treatment is to heal the patient's skin as soon as possible and thus restore its functions to make survival from the burn possible for the patient.

Procurement of skin transplants can be repeated from the same donor site after some weeks of healing but, since every procurement includes a small dermal portion, one can usually not procure more than 2 or 3 times from the same site due to the risk of creating a full-thickness skin wound. However, once 20-30 % of the patient's body surface area is burned, the access to healthy skin for transplantation starts to be limited. Therefore, culture of autologous keratinocytes is often the last resort for the most severely burned patients, and a useful tool in treating significant burns.

Culture of human keratinocytes has been in clinical use since the beginning of the 1980s [19]. Cultured keratinocytes, i.e. epithelial cells, can be used both for autologous and allogeneic treatment of patients as described here (see also Table 32.2). Culture of human cells is, according to EC Regulation 1394/2007, an advanced therapy medicinal product and the manufacture of the cells must therefore follow the requirements in the medicinal product regulations. A manufacturing licence from the medicinal product agency is required.

The skin comprises approximately 5 % epidermis and 95 % dermis (even though sometimes part of the subcutaneous fatty tissue is regarded as part of the skin). The dermis is responsible for the skin's strength and pliability. Cultured skin in most applications today is composed only of epidermal cells (keratinocytes), which restore a new epithelium (epidermis) on the patient, thus closing the wound and contributing to the survival of the severely burned patient. Depending on the depth of the burn (i.e. deep dermal or full-thickness burn), the amount of remaining dermis varies greatly. As a result, the quality of the healed skin, after transplantation of cultured epithelial autografts, varies equally greatly

(in structure, function and cosmetics), depending on the residual amount of dermis.

Today there are very few therapies to restore the dermis through ordinary cell culture; this is due, *inter alia*, to the morphological appearance of the dermis, with a substantial extracellular matrix in a three-dimensional structure. Extensive research is needed to develop a tissue-engineered skin consisting of both dermis and epidermis that is usable for wound care. Meanwhile, when the options for skin substitutes are limited, biological acellular allogeneic dermis (prepared from donated skin) or synthetic dermal regeneration templates can be used for reconstruction of the dermis. By applying a 3D biological degradable matrix to surgical wounds, the host's cells will migrate and populate the matrix, starting to develop autologous extracellular matrix while degrading the applied matrix, thereby restoring the dermal part of the skin and improving the quality of the reconstructed skin [20] (see also Chapter 19).

Cultured keratinocytes can be used in two ways, either for permanent skin cover in an autologous manner, or contributing to healing in an allogeneic situation through the natural growth-stimulating properties of the cells.

Autologous keratinocytes were until recently cultured and guided into stratified growth, rendering keratinocyte sheets, which can be grafted in the same manner as split-thickness skin grafts [21]. Today a common practice is to culture keratinocytes in multiplicity and mix the cell suspension with tissue glue, to be sprayed onto the wound in a single-cell suspension [22, 23]. Epidermal cell suspensions without culturing, containing keratinocytes, melanocytes and fibroblasts, can also be applied as spray to restore epithelialisation *in vivo* (see §19.11.1).

In the allogeneic use of cultured keratinocytes on full-thickness wounds, the cells make up a temporary skin that will eventually be replaced by the patient's own skin (either split-thickness skin grafts or cultured epithelial autografts). With the use of allogeneic keratinocytes on superficial dermal wounds, the healing time can be shortened and – since the wound is superficial – the possibility of spontaneous re-epithelialisation is good.

32.8.2. Donor selection

For autologous use, donor-site selection and timing are important. To get access to as many adult progenitor cells as possible, the donor site should preferably be in a hair-bearing area of healthy skin. The sooner (after trauma) the skin biopsy is taken, the better because the patient (and tissue) will be contaminated by microbes, which will affect the subsequent cell culture. However, the skin areas available for donor-site selection are principally determined by the extent and location of the burns.

For allogeneic application, donor selection must include – apart from general donor evaluation criteria (see Chapter 4 and Chapter 5) – the tissue-specific criteria defined in Chapter 19. The transplanted allogeneic keratinocytes will be a temporary wound coverage, stimulating wound healing, and thus human leukocyte antigen (HLA) typing or ABO (ABo) blood grouping are not necessary.

32.8.3. Procurement

When procuring the skin for culture of keratinocytes, it is essential that the site for the biopsy is located in an area with healthy skin as remote from the burned skin as possible. The biopsy can be either full-thickness or split-thickness. A full-thickness skin biopsy is preferred because of the amount of progenitor-cell-like keratinocytes in the appendages (hair follicles, sweat glands, etc.). The procurement should yield as many non-differentiated keratinocytes as possible.

The biopsy site should first be cleaned properly with disinfectant ethanol (70 %) with no additives and, after the site has dried, given a second wash with sterile saline solution (9 mg/mL) before the biopsy is procured (with e.g. a scalpel). Local anaesthetics can be used *ad lib*. Immediately after procurement, the biopsy is placed in a medium suitable for transportation to the TE (basal culture medium with the addition of 10 % foetal bovine serum – or similar – and antibiotics in normal cell-culture concentrations). The primary container should be sterile, closed and appropriately labelled. Transport the biopsy to the culture facility and initiate the cell-isolation process as soon as possible, < 24-48 hours after surgical removal of the biopsy. Minimising the time will increase the likelihood of the successful culture of cells.

It is crucial to keep in mind that handling and culturing the skin biopsy/keratinocytes *in vitro* opens up a risk of contributing (microbiological) contaminants to the cells, both from the skin itself and from culture conditions. Those risks can only be avoided by adequate facilities with controlled environment (see Chapter 9), and skilful, excellent handling techniques by the staff (see Chapter 27).

32.8.4. Quality controls/release criteria

The quality controls needed, besides microbiological controls (see Chapter 10), include continuous

surveillance: of keratinocyte morphology, mode of growth and expansion rate. It is important to have cellular expansion that corresponds to the patient's need for cultured keratinocytes, but it is equally important not to exceed the cells' capacity to proliferate. The possibility of proliferation within the keratinocytes is determined by different biological aspects, including the age and healthiness of the patient (prior to the trauma) and the biopsy site.

32.8.5. Packaging and distribution

The packing procedure is determined by the mode of delivery, i.e. sheets or suspension. Sheets are attached to an inert synthetic carrier membrane and placed one by one in transport liquid consisting of a basal cell-culture media (e.g. Leibovitz 15). Keratinocytes delivered for spray application are enzymatically detached from the culture vessel, washed repeatedly in basal cell-culture media and finally diluted in a minimal amount of basal cell-culture media, just enough to be in a solution. Further dilution is performed with the tissue glue to reach the appropriate cell concentration at the surgery room, just before application of the cells. The extent of dilution depends on the number of cells needed to cover the burned area of the patient.

The keratinocytes, either in sheets or in suspension, must be applied as soon as possible (or within a maximum of approximately 6 hours) after preparation.

32.8.6. Traceability

Records to secure traceability from donor to recipient, and all steps in between, are the responsibility of the TE, as described in Chapter 15.

32.8.7. Biovigilance/pharmacovigilance

As described in Chapter 16, adverse events and adverse reactions must be documented and reported. One can foresee that any event in the laboratory affecting the culture conditions, and thus reducing the amount of cells or the size of cell layer expected on the day of transplantation, should be considered as an adverse event.

32.9. Limbal stem cells (ocular surface)

32.9.1. General introduction

In the normal human eye, the epithelial cells of the cornea and conjunctiva are responsible for the continuing regeneration and homeostasis of the ocular surface. Cells with progenitor characteristics have been identified in the corneoscleral limbus (the transitional zone between the cornea and sclera) and these have the capability to continually repair and replace the epithelial surface of the cornea [24]. These cells are known as limbal stem cells (LSCs). Limbal stem cell deficiency, a term which covers acquired pathological deficiencies such as chemical burns and inherited ones such as aniridia, may lead to ocular surface disease, including persistent epithelial defects with chronic inflammatory conditions, vascularisation and scarring of the cornea and conjunctiva, and corneal conjunctivalisation [25]. These anomalies can be painful and are very difficult to treat because of the significant impairment of the patient's vision which, in most cases, progresses to legal blindness. LSCs have the characteristics of undifferentiated multipotent stem cells and are capable of undergoing proliferation and differentiation into the corneal epithelium. They may also be genetically modulated for therapeutic purposes [26].

Currently, approaches to the treatment of many ocular surface diseases focus mainly on three strategies: transplantation of portions of keratolimbus (Kenyon technique), either from the healthy fellow eye or from a cadaveric donor; *ex vivo* LSC expansion from a limbal explant; *ex vivo* expansion of isolated LSCs and *in vitro* culture. Achieving effective control of the underlying inflammatory process and preventing tissue rejection are the key objectives [25, 26]. In addition to LSCs, corneal endothelial cells [27] derived from the corneoscleral disc are being used for treatment of corneal endothelial disease; see Chapter 17 and Table 32.2. Recently, retinal pigment epithelial cells [28], induced to differentiate from different sources of pluripotent stem cells, have been investigated for the treatment of age-related macular degeneration; see Table 32.2 for an overview.

32.9.2. Donor and tissue selection

Donors should be tested for infectious diseases as described in Chapter 5, although in the case of autologous transplantation a positive test result may not be an exclusion criterion.

The treatment of unilateral LSC deficiency involves *ex vivo* expansion of a tissue explant or isolated LSCs from the unaffected limbal region of a patient's healthy eye. The autologous cell population is isolated and the final aim is to expand the limbal epithelial cells for transplantation into the affected eye. Human amniotic membrane or human fibrin gel are typically used as a scaffold for supporting the *ex vivo* expansion of LSC and used as a carrier for the transplantation of the cells (see Chapter 31). Bilateral LSC deficiency, on the other hand, is a devastating pathological condition affecting both eyes; in this case, autologous limbal tissue or cells cannot be sourced from the same patient since both eyes are affected. Alternative sources of transplantable tissue include the culture of epithelial cells lining the autologous oral mucosa. Allogeneic sources of transplantable tissue, from deceased donors, may be an option for restoring the function of the ocular surface, although procedures with autologous healthy tissue are always preferable to those using heterologous sources [29, 30].

32.9.3. Procurement

Extraction as well as *ex vivo* expansion/*in vitro* cultures must be carried out in strict laboratory conditions.

The explants (autologous or allogeneic) are obtained by a biopsy of healthy ocular tissue from the limbal region and maintained in sterile culture medium until processed. The most widely applied technique, with minimal manipulation of tissue, is the explant culture system, in which the specimen obtained by biopsy is directly placed in the centre of the amniotic membrane.

Epithelial cells (LSCs) grow out from the biopsied tissue and proliferate to form a multi-layered epithelial construct.

An alternative approach, with additional manipulation of the tissue, is to obtain single epithelial cell suspensions by enzymatic isolation (a one- or two-step enzymatic method) from limbal specimens.

32.9.4. Quality controls and release criteria

Microbiological tests that simulate normal processing conditions by replacing the cells with culture media are essential. Three simulations of all the critical stages of the process should be performed before the implementation process [31].

Conventional microbiological (bacteria and fungi) assays should be performed at baseline, during the process and in the final product before clinical use. Specific tests for the presence of *Mycoplasma* spp. are conducted after handling the final product (see Chapter 10). Morphological control of culture cells and bioengineered products can be achieved with the use of a phase-contrast microscope. Basic cell-culture techniques can be applied, such as cell viability tests with vital dyes and techniques for cell counting using an automated cytometer or cell-counting chambers.

In the explant culture system the final product requires less handling, and adequate microbiological control of the final product (LSCs on amniotic membrane) is needed to obtain approval for clinical use. Further release criteria are to be established during the pharmaceutical and clinical development of the cell-based product. In the enzymatic approach, in addition to the controls mentioned, the cell product may be tested for the presence of holoclones (clonogenic assay), high expression of the transcription factor deltaNp63alpha or ABCB5 and negative or very low expression of markers both for differentiated epithelial cells CK3 and CK12 and, where used as a feeder layer for LSC expansion, markers or PCR-based assays for the presence of 3T3 murine fibroblasts [29, 30].

32.9.5. Packaging and distribution

The purpose of packaging and labelling operations is to protect the product and to provide identification and information for the user. Packaging and labelling are key elements in the quality management system to ensure that appropriate standards are maintained during a product's life cycle [31].

The final cell product should be conditioned in a suitable sterile container immersed in unprocessed complete cell-culture medium and maintained at an appropriate and controlled temperature until arrival at the transplant centre (see also Chapter 14). A maximum period should be set for delivery and it is recommended that the product is implanted on the same day as its release for clinical use, unless longer storage is adequately justified. As described in Chapter 12, the essential information about usage of the cells should be included in accompanying documentation to the organisation responsible for human application.

32.9.6. Traceability

Special consideration should be given to the reagents that come into contact with tissues and cells, and which may be left as residues when cells are applied in the recipient. The following links in the process are particularly important: between the donor and donation at the tissue centre; between donation and the final cell product at the manufacturing

site; and between the final product and the recipient at the transplant centre. All records and information should be kept and stored at the TE(s) or according to national legislation.

32.9.7. Biovigilance/pharmacovigilance

As described in Chapter 16, deviations from standard operating procedures, from donation to clinical application, should be recorded and documented, as well as adverse reactions after application.

32.10. Stromal vascular fraction from adipose tissue

32.10.1. General introduction

Adipose tissue is a source of stromal cells similar to those identified in bone marrow. The so-called stromal vascular fraction (SVF) isolated from fat is a heterogeneous cell population that includes endothelial cells (10-20 %), haematopoietic lineage cells (25-45 %), stromal cells (15-30 %) and pericytes (3-5 %), as well as adipose stromal/stem cells (1-10 %). SVF may be used either directly or as a source material to isolate regenerative cells for treating various clinical conditions including musculoskeletal, neurological, immunological, cardio-pulmonary and immunological disorders, as well as soft tissue defects [31-33].

32.10.2. Donor selection

Commonly, SVF cells are used autologously. Donor testing includes assays for transmissible diseases as described in Chapter 5; although their presence is not an exclusion criterion, it should be documented and special actions should be taken to avoid cross-contamination to other cells and to ensure the safety of personnel.

32.10.3. Procurement

The SVF can be isolated from either resected adipose tissue or aspirated adipose tissue using tumescent liposuction. Although a common procedure is still lacking, in general minced adipose tissue is digested by enzymes including collagenase, dispase, trypsin or the like [33]. However, mechanical procedures have also been reported [34]. After neutralising the enzymes, the released elements defined as SVF are separated from mature adipocytes by differential centrifugation.

32.10.4. Quality controls/release criteria

Currently, as there is no single marker to identify SVF cell subpopulations, the use of a combination of fluorochrome-labelled antibodies to surface antigens is suggested. The following markers or marker combinations should be considered for identifying the stromal cells within the SVF: $CD45^-/CD235a^-/CD31^-/CD34^+$, $CD13^+$, $CD73^+$, $CD90^+$, $CD105^+$. Alternative positive stromal cell markers, including CD10, CD29 and CD49, can be applied. Viability is recommended to be > 70 % to allow for good cell expansion. A fibroblastoid colony-formation unit assay and testing of the differentiation potential of the adipose stromal/stem cells within the SVF might enhance information on the quality of the cellular product [33].

However, it is necessary to define and validate release criteria that are specific to the chosen clinical setting.

32.10.5. Traceability

Records to ensure traceability, from the donation to the recipient, should be kept with the TE.

32.10.6. Biovigilance/pharmacovigilance

Although recent literature supports a paracrine role for SVF cells in regenerative settings, these same secreted cytokines may have adverse effects in the presence of tumour cells, e.g. recruiting the homing and promoting the proliferation of cancer cells following transplantation [33]. Hence, the safety of SVF treatment has to be evaluated carefully.

Tables appear on the following pages.

Table 32.1. **Somatic cells employed to restore or produce immunological functions in patients**

	Starting material	Processing steps	Parameters for specificity (quality control)	Storage	Transport/distribution/ administration	Ref.
Antigen-specific T-cells (see §32.2)	Blood from heparinised sample or apheresis product Autologous or allogeneic origin	Selection of virus-specific or tumour-specific T-lymphocytes: 1. Direct T-cell selection from starting material based on i. phenotype with peptide-HLA-multimers or ii. Interferon gamma (IFNγ) production after stimulation with specific peptides and antigen-presenting cells with or without subsequent expansion by culture 2. Selection by *in vitro* culture	Phenotype ($CD3^+$, $CD4^+$, $CD8^+$) Specificity for the virus measured by • IFNγ production (ELISA or flow cytometry assay) or • virus-directed lysis (chromium-release assay or flow cytometry assay)	≤ – 140 °C	Administered fresh or Frozen vials thawed at bedside	1, 4
CAR-T-cells (see §32.2)	Blood from heparinised sample or apheresis product Autologous or allogeneic origin	Preparation of CAR-T-cells: 1. T-cell selection 2. Insertion of the modified gene for the chimeric receptor 3. *In vitro* expansion	Phenotype ($CD3^+$, $CD4^+$, $CD8^+$) Specificity for the antigen measured by • Expression and functionality of insert by cytokine production • Lysis of tumour cells by cytotoxicity assays (chromium-release assay or flow cytometry assays)	≤ – 140 °C	Administered immediately or Frozen vials thawed at bedside	2, 3
Natural killer cells (see §32.3)	Blood from heparinised sample, apheresis product or cord blood Autologous or allogeneic origin	Selection of functional NK cells: 1. Obtain mononuclear fraction 2. Positive and/or cell selection using phenotypes: i. T-cell depletion ($CD3^-$) and/or ii. NK cell enrichment ($CD56^+$) 3. (Optional) activation and expansion *in vitro*	Phenotype Function measured by cytokine production and cytotoxicity	≤ – 140 °C	Administered immediately or Frozen vials thawed at bedside	7
Dendritic cells (see §32.4)	Leukocytes collected by apheresis Autologous origin	Isolation of monocytes: 1. Physical isolation (counterflow centrifuge elutriation) or Affinity column separation based on surface markers (CD14) 2. Culture with growth factors for differentiation into dendritic cells 3. Maturation step with required specificity	Phenotype ($CD80^+$, CD83, $CD86^+$, $CD1a^+$, $HLA\text{-}DR^+$, $CD14^-$) IL-12 production	≤ – 140 °C	Transport at low temperature Administration: immediate after thawing at bedside	9-11
MSC (see §32.5)	Bone marrow, peripheral blood, umbilical cord blood, adipose tissue, muscle, liver and umbilical cord tissue (Wharton's jelly) Allogeneic or autologous origin (depending on source)	Generation of functional MSC: *In vitro* culture with growth factors (culture conditions depend on source of cells)	Phenotype: depends on the protocol for *in vitro* culture, but usually $CD73^+$, $CD90^+$ and $CD105^+$	≤ – 140 °C	Preparation of non-cryopreserved cells in syringe or Frozen vials thawed at bedside	10-13

MSC: mesenchymal stem cells. SVF: stromal vascular fraction.

Table 32.2. **Somatic cells employed to restore organ- or tissue-specific functions**

	Starting material	Processing steps	Parameters for specificity (quality control)	Storage	Transport/distribution/ administration	Ref.
MSC (see §32.6)	Bone marrow, peripheral blood, umbilical cord blood, adipose tissue, muscle, liver and umbilical cord tissue (Wharton's jelly) Allogeneic or autologous origin (depending on source)	Generation of functional MSC: *In vitro* culture with growth factors (culture conditions depend on source of cells)	Phenotype: depends on the protocol for *in vitro* culture, but usually $CD73^+$, $CD90^+$ and $CD105^+$	≤ – 140 °C	Preparation of non-cryopreserved cells in syringe or Frozen vials thawed at bedside	10-13
Chondrocytes (see §32.7)	Cartilage biopsy Autologous origin	Isolation of chondrocytes: 1. Mechanical and enzymatic 2. Culture for 2-3 weeks as adherent cell layers (expansion) and/or Culture for 4-5 weeks expansion and attachment to scaffold for 3D support	Morphology Limited population doubling (max. 8 ×) Purity of the population (mRNA)	Not to be stored	Chondrocytes are suspended into syringes/ampoules for administration within 48 h	14-16
Keratinocytes (see §32.8)	Biopsy Autologous or allogeneic origin	Isolation of keratinocytes: 1. Mechanical or enzymatic in combination with mechanical 2. Culture with or without serum and feeder layer	Morphology Expansion rate	Ideally, use fresh. If necessary, store at ≤ – 140 °C Short period of culture is needed after thawing, before distribution	Keratinocytes in sheets attached to synthetic carrier membrane and kept in culture media or Keratinocytes in suspension as spray – administration: immediate	17-22
Limbal cells (see §32.9)	Biopsy from the limbal region of the eye Autologous or allogeneic	Generation of limbal cells: Biopsy is cultured on a feeder layer (irradiated) or Directly attached to amniotic membrane	Morphology Clonogenic assay (holoclones) Transcription factors Phenotypes for epithelial cells ($CK3^+$, $CK12^+$) The parameters assessed will depend on the culture system	No storage	2-8 °C during transport to the clinic Administration the same day as released	23-26, 28, 29
Retinal pigment epithelial cells (see §32.9)	Embryonic stem cells, pluripotent stem cells, cord blood, foetal brain or bone marrow	Generation of retinal pigment epithelial cells: *In vitro* culture with growth factors	Immuno-histochemistry Phagocytosis Melanin content Up-regulation of retinal pigment epithelial cell-specific genes	≤ – 140 °C	Thawed and resuspended cells distributed to operating room at 2-8 °C	27
Corneal endothelial cells (see §32.9)	Corneo-scleral disc Allogeneic origin	Isolation of corneal endothelial cells: 1. Mechanical, released during medium storage 2. Expanded *in vitro*	Morphology Expression of ZO-1 and Na^+/K^+-ATPase	No storage	Distributed to operating room at 2-8 °C	28
SVF (see §32.10)	Adipose tissue Autologous or allogeneic origin	Isolation of SVF: Enzymatic and/or mechanical; no culture	Phenotype (heterogeneous populations)	≤ – 140 °C	Administered immediately or Frozen vials thawed at bedside	30-33

MSC: mesenchymal stem cells. SVF: stromal vascular fraction.

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Chapter 33: **Breast milk**

33.1. Introduction

In the European Union (EU), human milk (HM) may fall within different national legal frameworks (see §33.1.1 below) for which appropriate quality and safety requirements need to be applied, e.g. food, tissues and cells [1]. It is essential that the ethical principles described in Chapter 1 of this Guide are respected so that donors are not exploited and the human body itself is not the subject of trade.

Regardless of the regulatory status of HM, ensuring safety and quality requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide. Thus, the guidance on selection and testing of donors, quality management and traceability described in the generic section (Part A) of this Guide can be applied usefully to the banking of HM to provide an appropriate framework for safe and effective services to patients.

HM contains essential nutrients and bioactive components that promote the growth and development of the newborn. HM not only covers the nutritional needs of healthy term infants but it also facilitates the process of maturation of various organs such as the gut and the brain. It is highly nutritious and contains a complex combination of immunological and anti-infective constituents that promote health, protect against infection and support a baby's immune system. For these reasons, a mother's own milk is universally accepted as the optimal feeding choice for neonates and infants but is considered as vital for preterm infants.

Unfortunately, not all preterm infants can receive milk from their mothers and some mothers are unable to provide enough milk for their infants. When this is the case, official bodies such as the World Health Organization (WHO), the American Academy of Pediatrics and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, as well as many scientific associations, consider donated human milk, obtained and processed in HM banks (HMB), to be the clinically preferred option in the absence of sufficient maternal milk [2, 3, 4, 5]. Donor HM should not be substituted for a mother's own milk if available. Given the significant impact that HMBs can have on infant health outcomes, the WHO has asked member countries 'to promote the safe use of donor HM through HMBs for vulnerable infants' [2].

HMBs are institutions that collect, process, store and distribute HM prescribed for babies that are medically fragile, preterm babies, those with low birth weight, babies from mothers with delayed lactation, infants recovering from serious gut complications and surgery, and newborns affected by feeding intolerance, malabsorption syndromes or infectious diseases. The first HMB was established in Vienna in 1909. Since then, HMBs have been established in many countries: currently 224 HMBs exist in Europe [6], more than 300 in South America (217 in Brazil) and 26 in North America. The numbers and activities of HMBs are growing, driven by studies indicating that premature infants show better development if they are fed HM rather than formula. With donated HM, there is a lower rate of late-onset sepsis, a lower

rate of necrotising enterocolitis and a better tolerance of enteral feeding, and better long-term psychomotor development [7].

In addition, it has been shown that where new HMBs are established there is an increase in the rates of breastfeeding on discharge from hospital of these babies [8, 9]. It is now widely accepted that HMBs and the availability of donor HM encourage and support breastfeeding.

Theoretical microbiological risks associated with feeding with donor HM are similar to those in the food industry (i.e. contamination or cross-contamination with bacteria or fungi, with subsequent recipient infection). The food industry's standard quality assurance tool is HACCP (Hazard Analysis Critical Control Points) and it is widely recommended for HM banking. However, microbiological risks are also equivalent to those associated with transfusion and transplantation (i.e. transmission of viruses and other infective agents).

The following generic chapters (Part A) of this Guide all apply to milk banking and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 8: Processing,
g. Chapter 9: Storage and release,
h. Chapter 10: Principles of microbiological testing,
i. Chapter 12: Organisations responsible for human application,
j. Chapter 13: Computerised systems,
k. Chapter 14: Coding, packaging and labelling,
l. Chapter 15: Traceability,
m. Chapter 16: Biovigilance.

33.1.1. Legal status of breast milk

Following a Competent Authorities expert group meeting on substances of human origin in June 2014, the European Commission addressed the questions related to the legal status of breast milk in the EU and, despite confirming that Article 168 (4) of the Treaty on the Functioning of the European Union provides a legal basis for future regulation of these substances of human origin in terms of their quality and safety, it was clarified that member states are free to decide on the most suitable framework either by creating a specific regulatory framework at national level or by applying an existing national legislative framework, including the tissues and cells quality and safety requirements, to these substances [1].

33.2. Donor recruitment

The donation of HM must be voluntary and unpaid. In the case of reimbursement of expenses it should be in accordance with national regulation.

Promoting HM donation is carried out through a variety of different channels: written material (e.g. in prenatal clinics, paediatric surgeries, pharmacies, shops for maternity products), media, social networks, associations for breastfeeding programmes or educational guidance, and direct contact with pregnant women by their physicians and midwives.

A woman should be of legal age and lawfully competent to take this decision in accordance with national regulations: she should be nursing her own baby, who, if appropriate, should be adequately fed, before giving milk to a HMB. Bereaved mothers should be made aware of the possibility of donating their previously expressed and stored milk as well as continuing to lactate for the purposes of HM donation if desired. This has been shown to be of benefit to grieving mothers [10, 11].

Because HM donation is carried out frequently over a period of a few months, it must be stressed to the donor that certain health-related conditions – like infectious diseases or drugs (including nicotine and alcohol) – during the donation period would make her unsuitable as a HM donor. HMB staff have a duty of care to those who offer to donate HM, including a duty to those whose milk is not accepted because of, for example, medication or tobacco use. A mother who is unable to donate for whatever reason should be reassured that this should not affect her choice to feed her own baby – assuming that is the case. HMB staff should take responsibility for ensuring that she understands the reasons for her deferral and how this affects or does not affect her own infant [12]. The value of breast milk and of breastfeeding her own infant in accordance with WHO guidance should be highlighted in all communications between HMB and prospective donors.

33.3. Donor evaluation

HMB processing cannot guarantee complete elimination of toxic substances and potential infectious elements that may be contained in the milk. For this reason, HM, which has not undergone

any treatment, should be as safe as possible from the point of its origin.

In addition to the general contraindications for donation specified in Chapter 4, the following conditions contraindicate the donation of milk [13, 14, 15, 16]:

a. Donor's behavioural risks
   i. smoking tobacco, use of snuff or use of nicotine-containing products to help stop smoking; wait for 7 days from the last exposure;
   ii. drug abuse or use of methadone;
   iii. daily consumption of beer (≥ 200 mL), wine (≥ 100 mL) or spirits (≥ 30-40 mL); occasional consumption may be accepted if milk collection is avoided for 12 hours;
   iv. consumption of high quantities (> 300 mg) of substances containing caffeine (coffee, tea, cola or cacao) should be avoided; occasional consumption may perhaps be accepted;
   v. if there is the suspicion of low vitamin B12 level (vegans or strict vegetarians, without vitamin B12 supplementation), the donor can be accepted if an adequate level of vitamin is verified in a blood test.

b. Donor's treatments
   i. the use of drugs or other pharmacologically active substances (including herbal products) must be evaluated since most will be secreted into breast milk; the concentration and potential toxicity vary substantially depending on the substance and the dose (relevant information can be accessed at: www.e-lactancia.org);
   ii. women immunised with attenuated live virus should not donate milk for 4 weeks after the immunisation;
   iii. the transfusion of blood and blood components, treatments with acupuncture needles that are not properly sterilised or disposable, endoscopic examinations or treatments made with flexible instruments (e.g. colonoscopy or bronchoscopy) and transplantation of organs, should exclude donation (organ transplantation is usually followed by long-term anti-rejection medication which excludes donation).

c. Donor's medical history
   i. acute infections and diseases must be evaluated, depending on the type of infection for the appropriateness of temporary exclusion and the exclusion time itself;
   ii. women who have recently been in contact with infectious patients (e.g. chicken pox, mumps, measles) unless they have been immunised; if they have not been immunised, they should be excluded for a period equivalent to the incubation period or, if not known, for 4 weeks;
   iii. women with sexual contact with patients with viral hepatitis B or C should be excluded [13];
   iv. women with mastitis or fungal infections of the nipple or areola should be excluded temporarily;
   v. women with reactivation of *Herpes simplex* (HSV) or varicella–zoster infections in the mammary or thoracic region should be excluded;
   vi. women with a history of malignancy, including haematological malignancies, must be excluded since viruses have been shown to play a role in the development of some types of tumour. However, women with the following conditions can be accepted as donors:
      - cervical *in situ* carcinoma and localised skin tumours (basocellular carcinoma and squamous carcinoma) if they are removed and the donor has recovered;
      - some childhood solid tumours such as neuroblastoma, Wilms tumour and retinoblastoma; these are considered cured if the diagnosis was made before the donor was 5 years old and there has been no recurrence.

33.4. Milk donor testing

In addition to the tests outlined in Chapter 5, testing for HTLV I/II is recommended in most of the guidelines for HMBs; at least it should certainly be performed for donors living in or originating from high-prevalence areas or with known sexual partners originating from those areas, or if the donor's parents originate from those areas.

The risk of transmission of *Trypanosoma Cruzi* through breastfeeding has not clearly been established. Pasteurisation destroys *T. Cruzi* [14]. If there is a suspicion of this disease during the health interview, serological screening for *T. Cruzi* should be done.

Theoretically, it is not necessary to repeat donor testing during the period of milk donation if there is no change to the donor's risk status, When careful evaluation of the change in donor's risk status is not feasible in practice, donors should be tested every 3 months.

Seropositivity for *Cytomegalovirus* (CMV) is not considered a contraindication as long as the milk is pasteurised.

33.5. Procurement

The milk can be expressed by hand or with a breast pump. Good breast hygiene should be encouraged and at least once daily washing of the breasts is recommended. Additional washing prior to expressing has also been shown to reduce bacterial contamination of the milk, together with cleaning and disinfecting all the components of breast pumps [16].

Most recommended containers are rigid plastic, made from a variety of food-contact materials such as polyethylene or polypropylene. It is recommended that containers should be sterile and single-use. Reusing containers requires cleaning and disinfection. The use of containers sterilised with ethylene oxide is regulated at the European level, and users should ascertain that manufacturers respect this EU regulation (or users should avoid devices sterilised with ethylene oxide, as shown on the label). Glass containers can be used; however, they should be purpose-designed and sold as intended for breast milk storage, including freezing and high temperatures, and made from glass that is chosen to resist breakage [17].

Some milk banks use plastic bags of polyethylene as an alternative to rigid containers; but these bags can easily rupture with the risk of loss of milk and contamination. Use of a double bag is therefore recommended if bags are used.

If the milk is to be frozen, the container should not be filled completely. The containers must be labelled with a donor code (or donor's given name and family name), and the date of collection.

33.6. Temporary milk storage and transportation to the milk bank

Collected milk should be kept at room temperature for the shortest possible time. After collection, the container should be sealed and cooled in order to avoid bacterial growth and degradation of the milk [10, 18].

It can be stored at 4 °C for 24 hours and then frozen at ≤ – 20 °C. Some HMB accept the pooling of milk of different collections from the same mother as long as the milk is kept in the fridge, but the new milk should first be cooled before being added to previously collected milk [19, 20].

If a woman has accumulated milk before being accepted as a donor, medical and behavioural history must be evaluated retrospectively for suitability, and the milk can be accepted only if it has been appropriately preserved and identified.

HM for donation should be transported frozen to the milk bank. Dry ice may be used as the refrigerant during transport; however, frozen cool packs to fill any spaces within the insulated transport containers are generally sufficient for short journeys. It is recommended that the milk bank is responsible for the transportation of milk. If third parties are used, there must be a formal agreement in place, with the milk bank covering transport conditions to ensure the safety and quality of the milk.

The transport process must be validated or temperatures monitored during transport to ensure the milk is kept under appropriate conditions.

Evidence of the integrity of the containers on arrival at the milk bank must be documented.

33.7. Processing

It is common practice in HMBs to increase safety and reduce the risk of contamination by pasteurising the milk.

However, in Norway raw (i.e. non-pasteurised) milk from CMV-negative donors is used in very specific contexts [21], and the donors who are suitable for milk banks are not a high-risk group for any of the viral diseases screened before donation (HIV, hepatitis, HTLV etc.). Furthermore, in Norway there is bacteriological screening of all donated milk, and samples with a bacterial count of more than 10^4 and less than 10^5 colony-forming units/mL are pasteurised [22]. However, the use of raw donor HM in Norway is constantly under review [21].

Recent guidelines for pasteurisation recommend a temperature of 62.5 °C for 30 minutes, the so-called Holder pasteurisation [23]. It is recommended that the heated milk should be cooled to 25 °C within 10 minutes, although a final temperature of 10 °C or lower is preferable [17].

It has been shown that pasteurisation does not affect the macronutrient composition (protein, carbohydrates and lipids, including polyunsaturated fatty acids) of milk and that HM oligosaccharides are preserved by freezing and pasteurisation. However, new treatments are under development to provide the same level of safety without affecting the other biological components of HM [23-27].

The pasteurisation process begins with the thawing of milk either slowly, overnight in a refrigerator, or quickly by immersion in a water bath with stirring at a controlled temperature, not higher than 37 °C [13]. The milk should be refrigerated as soon as it has thawed, to prevent bacterial proliferation, and pasteurised within 24 hours of thawing.

Table 33.1. **Criteria for the discard of milk before pasteurisation**

NICE (UK) Guidelines	Total bacteria > 10^5 CFU/mL	*Enterobacteriaceae* > 10^4 CFU/mL	*Staphylococcus aureus* > 10^4 CFU/mL
Italian Guidelines	Total bacteria > 10^5 CFU/mL	*Enterobacteriaceae* > 10^4 CFU/mL	*Staphylococcus aureus* > 10^4 CFU/mL
French legislation	Total (aerobic) flora > 10^6 CFU/mL		*Staphylococcus aureus* > 10^4 CFU/mL
Australian Guidelines	Confluent bacterial growth > 10^5 CFU/mL	Any *enterobacteriaceae*, *enterococci* or potential pathogens capable of producing heat-stable enterotoxins	
Indian Guidelines			*Staphylococcus aureus* > 10^4 CFU/mL
Swedish Guidelines	Total bacteria > 10^4 CFU/mL	*Enterobacteriaceae* > 10^7 CFU/mL Any pathogenic bacteria	*Staphylococcus aureus* > 10^7 CFU/mL
North American Guidelines	No testing		

Milk must be processed under hygienic conditions. Individuals handling open containers of milk must wear a hair covering, gloves and a clean gown, apron or lab coat to prevent contamination of the milk. Although it may not be considered strictly necessary, an increasing number of HMBs handle the milk in a laminar-flow or bio-safety cabinet, which must be qualified (including regular analyses of the particulate and microbiological contamination of the cabinet).

After thawing, some HMBs combine or pool milk from multiple donors. This practice of pooling may increase uniformity in the product and provide more consistent nutrient content; however, if there is contamination of pooled milk, it may be difficult to trace the source of the contamination. If milk pooling is practised, the HMB must decide whether pooling will be allowed between different donors, and the maximum number of donors whose milk may be pooled.

Before pasteurisation, a sample of milk from each batch should be taken for microbiological testing. This sample may also be used for a macronutrient analysis. The pasteurisation process can be carried out in a shaker water bath, or equipment specifically designed for pasteurising milk may be used. The equipment used for pasteurisation must be calibrated at least every 12 months [6]. Regular qualification of pasteurisers is needed to optimise pasteurisation and milk quality. A few criteria have been proposed: temperature plateau of 62.5-64.5 °C, duration of the plateau between 30 and 35 minutes, exposure time over 58 °C < 50 min and exposure time from 62.5 °C to 6 °C ≤ 1 h [26, 27].

During pasteurisation, bottle caps must remain above water level to prevent contamination, unless caps and equipment designed for submersion are used, in which case additional checks should be in place to ensure seals are effective and end users should be cautioned to discard containers with an incomplete seal and to notify the milk bank immediately. A control bottle containing the same amount of milk or water as the fullest container of milk in the batch must be fitted with a calibrated thermometer to record milk temperature during pasteurisation. The control bottle should follow the same process as the rest of the batch at all times. In addition to the milk temperature, the water bath temperature must be monitored and recorded.

At the end of the process, a sample of the pasteurised milk should be taken for microbiological testing. It is advisable to keep a sample of each batch of pasteurised milk for further tests should the need arise.

33.8. Quality control

The first control for donated milk, before processing, is the evaluation of its appearance. Human milk varies widely in colour and may be clear (early colostrum), yellow (colostrum and early transitional milk), creamy white and white tinged with blue or green, depending on the age of the baby, the extent to which the breast has been emptied and diet. If the milk exhibits unusual colouring or if it contains any foreign bodies or visible impurities, these should be noted. This information may help final decision making. If it contains foreign bodies, it should be discarded.

33.8.1. Microbiological evaluation

The microbiological evaluation of donated HM includes the testing of each batch before and after, to identify unusual or heavy contamination and pathogens. However, there is no consensus within internationally published guidelines of the optimal microbiological control both before and after pasteurisation [6, 7, 9]. A similar level of discrepancy is observed in the criteria of acceptability of the milk when bacteriologic analysis is performed, as is shown in Table 33.1. The criterion for discarding pasteurised milk should be any microbial growth.

33.9. Labelling and packaging

In addition to the information about labelling set out in Chapter 14, labels for packaging pasteurised milk may contain information about the nutritional value, such as the concentrations of protein, fats and carbohydrates, and the energy content.

The volumes of the final storage containers may vary depending on the needs of the recipient. The most common volumes are 50, 100 and 200 mL.

33.10. Preservation/storage

The most common method of milk preservation is freezing ≤ – 20 °C. Devices used for freezing should be qualified and the temperature recorded and controlled using calibrated probes.

Milk that has not been frozen is acceptable for use (or for freezing) only if kept for less than 24 hours from collection.

Despite its advantages, very few milk banks use lyophilisation after pasteurisation as a method of preservation. Donor milk that has been lyophilised after pasteurisation can be stored at ambient temperature, as a powder, for up to 18 months, *versus* 6 months after pasteurisation alone. It is used to supply donor human milk to French overseas territories. However, the HMB of Bordeaux-Marmande uses Holder pasteurisation followed by lyophilisation and processes more than 12 000 litres of milk per year [28].

33.10.1. Expiry date

There is no unanimous agreement about the expiry date for milk. In most European countries, it is accepted that milk should not be kept more than 4 months at – 20 °C before pasteurisation whereas the USA and Canada accept storage for up to 12 months. After processing, milk may be stored for between 3 and 12 months, depending on the country [17].

In the case of milk stored at – 80 °C, no more than 12 months' storage both before and after pasteurisation is the recommended maximum [16].

33.10.2. Storage temperature

According to the basic principles of freezing, frozen foods at – 18 °C are safe indefinitely from bacterial contamination, although enzymatic processes inherent in food could persist, with possible changes in milk quality [29]. Freezing milk at – 20 °C/– 30 °C before and after processing slows down but does not stop lipolysis. However, it has been recently reported that storage of human milk at – 20 °C for 9 months was associated with preservation of key macronutrients and immunoactive components [30].

When human milk is preserved at – 80 °C, lipolysis is stopped. Long-term storage at – 80 °C has been proposed but it has been recently reported that freezing at these temperatures significantly decreases the energy content of HM, both from fat and carbohydrates [20, 28, 32].

For pasteurised donor human milk, most guidelines recommend storage in freezers at – 18 °C to – 20 °C, for a maximum duration of 3 to 6 months [33-36].

33.11. Distribution and transport conditions

Distribution of milk must be so conducted as to ensure product traceability between donor and recipient, as described in Chapter 15 of this Guide. The HMB must keep records of the documentation of each donor, the processing pool, qualification, storage and final destination (distribution, disposal, expiry date), and the hospital must document how the milk is used.

During transport, milk must remain frozen, and dry ice may be used for this purpose. The freezing point of milk is at a lower temperature than that of water and, consequently, use of water ice as a coolant during transportation can result in partial melting of the milk. The use of validated, easily cleaned insulated transport containers is recommended.

The transport procedure should be validated, and the temperature of the transport container should be monitored at all times during transportation. In the case of transport by third parties, a formal agreement is required with the milk bank to ensure appropriate transport conditions are maintained.

33.12. Biovigilance

As described in Chapter 16, deviations from the standard operating procedure (SOP), from donation to the administration of human milk, should be recorded and documented, as well as adverse reactions after application [37].

In addition to milk banks, hospitals should also have appropriate SOPs for the storage, thawing and handling of milk containers to avoid degradation of the quality of the milk and the possibility of adverse reactions in recipients.

Milk should not be thawed in a microwave oven, since that significantly reduces the amount of vitamin C, the total IgA content and lysozyme activity [38].

Milk thawed in the refrigerator can be kept at 4 °C for up to 72 hours if the container has not been opened. Once opened, the package should be consumed within 30 hours. Furthermore, thawed milk must not be re-frozen.

33.13. New techniques for processing

Several techniques have been investigated to eliminate pathogens in milk without affecting its biological properties. These include:

- high temperature short time (HTST) or ultra-high short time (UHST),
- ultra-pasteurisation or ultra-high temperature (UHT).

There are also methods for reducing micro-organisms in food that do not use heat. While not technically pasteurisation, they achieve the same effect and are known as cold pasteurisation. These include:

- high-pressure processing (HPP) or pascalisation,
- ultraviolet (UV) irradiation,
- ultrasonication,
- high intensity pulsed electric field (PEF).

Even if such techniques are shown to be effective and preserve important bioactive components of HM better than Holder pasteurisation, they may be difficult to translate into practice, given the lack of appropriately scaled equipment for use in HMBs. Furthermore, these developing devices need to be validated in real conditions, with milk volumes that are currently treated in milk banks, and to be compared with Holder pasteurisation performed with qualified pasteurisers. When testing new technologies, precise description of the process and recording of the process parameters are necessary.

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Chapter 34: **Faecal microbiota**

34.1. General considerations

The regulatory classification of faecal microbiota is challenging because they do not fit clearly into one group of therapies or another, whether the criterion applied is anatomical origin, method of application, mode of action or complexity of processing.

Hence, a wide variety of approaches to regulation exists in Europe, and in some cases faecal microbiota are banked outside any regulatory framework. Following a Competent Authorities expert group meeting on substances of human origin in June 2014, the European Commission addressed the questions related to the legal status of faecal microbiota in the EU and, despite confirming that Article 168 (4) of the Treaty on the Functioning of the European Union provides a legal basis for future regulation of these substances of human origin in terms of their quality and safety, it was clarified that member states are free to decide on the most suitable framework (e.g. medicinal products, tissues and cells) either by creating a specific regulatory framework at national level or by applying an existing national legislative framework, including the tissues and cells quality and safety requirements, to these substances. Several countries have introduced some national rules and others require compliance with Directive 2004/23/EC on the quality and safety of tissues and cells (see also §34.3 below).

Given the significant commercial interest in developing these services on a for-profit basis, it is essential that the ethical principles described in Chapter 1 of this Guide are respected so that donors are not exploited and the human body itself is not the subject of trade. Promotion of altruistic unpaid donation of faecal microbiota by means of advertisement or public appeal may be undertaken in accordance with domestic regulations.

Regardless of the regulatory status of faecal microbiota, ensuring their safety and quality requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide. Thus, the guidance on selection and testing of donors, quality management and traceability described in the generic section (Part A) of this Guide can be applied usefully to the banking of faecal microbiota to provide an appropriate framework for safe and effective services to patients.

34.2. Introduction

Faecal microbiota transplantation (FMT) is the transfer of biological material containing a minimally manipulated community of micro-organisms from a human donor to a human recipient (including autologous use) with the intent to restore the diversity of gut microflora. FMT may confer protection against toxigenic *Clostridium difficile* [1, 2]. FMT was first reported in 1958, by Eiseman *et al.*, to treat a case of pseudomembranous colitis [3]. Since then, a large body of evidence, including randomised controlled trials, systematic reviews and meta-analyses, has proved clear evidence that FMT is a highly effective treatment against recurrent *Clostridium difficile* infection unresponsive to repeated antibiotic treatments (rCDI) [4-10]. Due to the rising prevalence,

severity and mortality of this infection, the therapeutic role played by FMT is therefore important to save human lives and to decrease the economic burden on healthcare systems [11-14]. Based on these data, both the European Society for Microbiology and Infectious Disease and the American College of Gastroenterology recommend FMT as a treatment for recurrent *Clostridium difficile* infection [15, 16]. A 2017 European consensus conference report strongly recommends the implementation of FMT centres for the treatment of *Clostridium difficile* infection [17].

FMT has also been investigated in the treatment of other disorders associated with the alteration of gut microbiota. In particular, studies in humans include randomised controlled trials [18-21] with systematic review and meta-analysis, in patients with IBD, especially ulcerative colitis UC [22-24], and case series reports in patients with metabolic syndrome [25, 26], hepatic encephalopathy [27] and graft-*versus*-host disease [28].

The following generic chapters (Part A) of this Guide all apply to FMT and must be read in conjunction with this chapter:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 3: Recruitment of potential donors, identification and consent,
d. Chapter 4: Donor evaluation,
e. Chapter 5: Donor testing – markers for infectious diseases,
f. Chapter 6: Procurement,
g. Chapter 7: Premises,
h. Chapter 8: Processing,
i. Chapter 9: Storage and release,
j. Chapter 10: Principles of microbiological testing,
k. Chapter 11: Distribution and import/export,
l. Chapter 12: Organisations responsible for human application,
m. Chapter 13: Computerised systems,
n. Chapter 14: Coding, packaging and labelling,
o. Chapter 15: Traceability,
p. Chapter 16: Biovigilance.

34.3. Donor evaluation – exclusion criteria

Limited trials have evaluated the outcomes of FMT as related to donor or FM characteristics (see §34.10). Current criteria are based on expert opinion, guidelines and rules from other domains (e.g. blood donation) [29, 30]. Published results from studies demonstrate that limited percentages of donors screened met the criteria for donation [31-34].

The selection of donors for FMT has two main aims. The first aim is to prevent adverse events potentially associated with the infusion of faecal material, while the second aim is to avoid the transmission of impaired microbiota that could be not useful (or harmful) to the recipient.

According to the recommendations of the European FMT Working Group [17], potential donors should undergo four different steps to be selected, as follows:

a. written questionnaire;
b. general clinical examination;
c. blood and stool testing;
d. further questionnaire (the day of the donation).

First, potential donors should complete a written questionnaire to assess their medical history and lifestyle habits. This approach is particularly important to rule out issues not detectable by laboratory testing.

Usually subjects younger than 60 years old are preferred, as older individuals are more likely to suffer from other diseases. However, this suggestion should not be mandatory.

The questionnaire should be designed both to exclude the risk factors for infectious diseases, as required by the European Commission to select allogeneic living donors of human tissue transplants (Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells) and to identify subjects who have gastrointestinal (GI) disorders or who take drugs, which can alter the donor microbiota [35, 36] (see Table 34.1).

Candidates who are suitable for donation on the basis of the questionnaire should then undergo blood and stool testing (Table 34.2), which should be done no longer than eight weeks before donation [17]; after this term, testing exams should be repeated before further donations. The tests should be validated [29, 37] and carried out in accordance with the specification in Chapter 5.

Finally, the last assessment of selected donors on the day of the donation should be done by questionnaire to rule out any newly onset issue that could impair the donation (see Table 34.3). If the centre has been authorised for (or has the expertise in) the management of frozen stools, blood tests for HBV, HCV and HIV should be repeated before storage in order to check for possible infections occurring in the

window phase (nucleic acid testing assays should be performed due to the substantial decrease in window period comparing with routine serological tests).

Table 34.1. **Exclusion criteria for stool donors to be addressed in the written questionnaire**

- history of, or known exposure to, HIV, HBV or HCV, syphilis, human T-lymphotropic virus I and II, malaria, trypanosomiasis, tuberculosis
- known systemic infection not controlled at the time of donation
- use of illegal drugs
- risky sexual behaviour (anonymous sexual contacts; sexual contacts with prostitutes, drug addicts, individuals with HIV, viral hepatitis, syphilis; work as prostitute; history of sexually transmittable disease)
- previous reception of tissue/organ transplant
- previous (< 12 months) reception of blood products
- recent (< 6 months) needle stick accident
- recent (< 6 months) body tattoo, piercing, earring, acupuncture
- recent medical treatment in poor hygienic conditions
- risk of transmission of diseases caused by prions
- recent parasitosis or infection from *rotavirus*, *Giardia lamblia* and other microbes with GI involvement
- recent (< 6 months) travel in tropical countries, countries at high risk of communicable diseases or traveller's diarrhoea
- recent (< 6 months) history of vaccination with a live attenuated virus, if there is a possible risk of transmission
- healthcare workers (to exclude the risk of transmission of multidrug-resistant organisms)
- individual working with animals (to exclude the risk of transmission of zoonotic infections)
- history of IBS, IBD, functional chronic constipation, coeliac disease, other chronic GI disorders
- history of chronic, systemic autoimmune disorders with GI involvement
- history of, or high risk for, GI cancer or polyposis
- recent appearance of diarrhoea, haematochezia
- history of neurological/neurodegenerative disorders
- history of psychiatric conditions
- overweight and obesity (body mass index > 25)
- recent (< 3 months) exposure to antibiotics, immunosuppressants, chemotherapy
- chronic therapy with proton pump inhibitors

Source: European FMT Working Group consensus [17]

Table 34.2. **Blood and stool testing for donor selection**

General blood testing

- *Cytomegalovirus*
- Epstein–Barr virus
- hepatitis A
- HBV
- HCV
- hepatitis E virus
- syphilis
- HIV-1 and HIV-2
- *Entamoeba histolytica*
- complete blood cell count with differential
- C-reactive protein and erythrocyte sedimentation rate
- albumin
- creatinine and electrolytes
- aminotransferases, bilirubin, gamma-glutamyltransferase, alkaline phosphatase

Blood testing to be performed in specific situations

- human T-lymphotropic virus types I and II antibodies
- *Strongyloides stercoralis*

General stool testing

- detection of *Clostridium difficile*
- detection of enteric pathogens, including *Salmonella, Shigella*
- *Campylobacter*, *Escherichia coli* O157 H7, *Yersinia*, vancomycin-resistant *Enterococci*, methicillin-resistant *Staphylococcus aureus*
- Gram-negative multidrug-resistant bacteria
- norovirus
- antigens and/or acid fast staining for *Giardia lamblia* and *Criptosporidium parvum*
- protozoa (including *Blastocystis hominis*) and helminths
- faecal occult blood testing

Stool testing to be performed in specific situations

- detection of *Vibrio cholera* and *Listeria monocytogenes*
- antigens and/or acid fast staining for *Isospora* and *Microsporidia*
- calprotectin
- *Helicobacter pylori* faecal antigen
- *rotavirus*

Source: European FMT Working Group consensus [17]

Table 34.3. **Issues to be addressed by questionnaire on the day of the donation**

- newly appeared GI signs and symptoms (e.g. diarrhoea, nausea, vomiting, abdominal pain)
- newly appeared illness or general signs (e.g. fever, throat pain, swollen lymph nodes)
- use of antibiotics or other drugs that may impair gut microbiota, new sexual partners or travels abroad since the last screening
- recent ingestion of a substance that may result in harm for the recipients
- travel in tropical areas
- contact with human blood (sting, wound, showing, piercings, tattoos)
- sexual high-risk behaviour
- diarrhoea (more than three loose or liquid stools per day) among members of the entourage (including children) within 4 weeks of donation

Source: European FMT Working Group consensus [17]

Current evidence does not support the superiority of related donors over unrelated ones, at least when FMT is administered to cure *C. difficile* infection [38]. For other indications, definitive data are still not available. The use of anonymous healthy donors may be useful in large centres to allow clinicians to satisfy the need for FMT to treat *C. difficile* infection. The recruitment of potentially interested donors could be organised through existing pathways (e.g. blood donors). The procurement must be authorised only after informed consent procedure. According to the European directives mentioned above, donation is voluntary and unpaid, a factor which may contribute to high safety standards and therefore to the protection of human health [35].

34.4. Preparation of donors

There is no specific preparation of the donor. The donor can be advised to avoid food that can be allergic for the recipient, such as peanuts, other nuts and shellfish within the 5 days prior to donation. Consider the use of a gentle osmotic laxative the night before donation [38, 39].

34.5. Procurement, transportation to the processing facility, processing of faeces and storage

Faeces will, most likely, be collected by the donor at home. The travel distance to the processing facility is important as it is generally believed that a high viability of bacteria in stools increases the chance of successful FMT. The processing facility should provide sterile faecal containers in order to prevent contamination.

The data related to the donation must be handled with respect for confidentiality of any health-related information provided to the authorised personnel, the results of tests on their donations, and details of traceability from donor to recipient and vice versa.

Faeces have traditionally been processed for immediate 'fresh' use. More recently evidence has accumulated that the use of frozen FMT is as effective as fresh FMT. Frozen FMT has different advantages, mainly from the logistical point of view (selection and screening of donor, quality of stool etc.) [6, 40, 41].

34.5.1. Stool handling and fresh faeces preparation

To protect anaerobic bacteria, the storage and preparation should be as brief as possible. The stool should be processed, following safety requirements, in Class II biosafety cabinets. Protective gloves and facial masks should be used during preparation. Until further processing, the stool sample can be stored at ambient temperature. 'Ambient' is rather ill-defined and comparable to 'room temperature'. A minimum amount of 30-50 g of faeces should be used [10, 42-44]. Anaerobic storage and processing should be applied if possible; and a dedicated space, disinfected using measures that are effective against sporulating bacteria, should be used. Faecal material should be suspended in saline [45], using a blender or manual effort, and sieved in order to avoid the clogging of infusion syringes and tubes. Fresh stool should be processed and used preferably within 6 hours after donation [4, 6, 10, 38, 41, 42].

34.5.2. Preparation and defrosting of frozen faecal material

At least 30 g of donor faeces and 150 mL of saline solution should be used. Similarly to fresh samples, preparation of frozen faecal suspensions

under normal air or under oxygen-free atmosphere yielded a similar resolution rate [6, 42, 46]. Before freezing, glycerol should be added up to a final concentration of 10 % [47, 52]. Inulin would seem to be an equivalent alternative for glycerol [48]. The final suspension should be clearly labelled and traceable, and stored at – 80 °C. The labels should include a unique donor code, suspension number, production and expiration date, volume and storage instructions.

The frozen faecal material should be stored in dedicated freezers since the faecal material in itself is contaminated and further (cross-)contamination should be avoided. Information regarding length of storage is limited: 5 to 6 months should be acceptable, but probably longer too. On the day of faecal infusion, the faecal suspension should be thawed in a warm (37 °C) water bath and infused within 6 hours from thawing. After thawing, saline solution can be added to obtain a desired suspension volume. Since microbial cells are sensitive after defrosting, repeated thawing and freezing should be avoided [49].

34.5.3. Quality control

Samples of donor faeces before the processing and before the administration to recipients should be stored for possible microbiological evaluation and qualitative and quantitative characterisation for safety reasons. The frozen samples should be stored in adequate facilities (or, when specifically regulated, by authorised facilities) and should be clearly labelled with the code of the donor and the date of donation.

34.6. Basic requirements for implementing a faecal microbiota transplantation centre

Stool banking and centres for the treatment of *Clostridium difficile* infection should be implemented in hospitals with appropriate expertise and facilities [17]. The dissemination of the FMT procedure and the establishment of FMT services in clinical settings could be useful practices to reduce the *Clostridium difficile*-related healthcare burden [44, 50-54]. The development of an FMT centre service would ensure the optimal standardisation of the FMT process. The availability of several facilities (including endoscopy service, clinical ward and outpatient clinic) is essential to implement an FMT centre. FMT can be performed in either an inpatient or an outpatient setting.

FMT centres need to have access to, or be part of, a facility that allows safe processing of human samples (biosafety level 2) including aliquoting, storage and preparation of faeces [55]. In fact, one of the key functions of the FMT centre is the management of blood and faecal samples from either donor or recipients. *C. difficile* is a pathogen with a biosafety level 2 (cabinet with high-efficiency particulate air filter) and safety requirements and recommendations need to be implemented accordingly [55]. Material processing (fresh faeces, banks of frozen and stored faeces) and safety precautions have to adhere to basic principles for safe preparation of human material, including: rigorous protocols in securing the materials; maintenance of standard operating procedures for the processing; use of certified laboratory testing; definition of quality-control tests and standards for the release of the final product.

Finally, management of the records related to the FMT procedure should be regulated by a local health organisation. FMT procedure and donors' and recipients' records should be stored for at least 10 years. According to Directive 2004/23/EC, data required for full traceability must be kept for a minimum of 30 years after clinical use [35]. This may differ from local requirements, and longer storage time could be needed. The records of the FMT centre will provide access to the long-term safety data.

34.7. Preparation of recipients

Patients with *C. difficile* infection should be pre-treated with vancomycin or fidaxomicin, at least for 3 days and until 12–48 hours before FMT. This pre-treatment aims both to reduce bowel movements (allowing a longer persistence of the infusate in the bowel of the recipient) and to provide a bridging therapy to recipients while they are waiting for the procedure. In case of emergency, antibiotic pre-treatment can be avoided if donor samples are quickly available. For other indications beyond *C. difficile* infection, there is no reliable evidence to recommend any pre-treatment before FMT [17].

However, recipients should undergo bowel lavage by polyethylene glycol before FMT, at least when it is administered by upper route or by colonoscopy [17], to reduce the *C. difficile* load in the intestines. Inadequate bowel preparation has been identified as a risk factor for failure of FMT [56]. However, current evidence of enema FMT shows high cure rates without bowel lavage before the infusion [6], so no indications for bowel preparation have been suggested for this route of delivery.

34.8. Delivery of faecal material

FMT can be performed through different routes of delivery, including colonoscopy, upper endoscopy, nasoduodenal/nasojejunal tube, enema or capsule. For each route of delivery, faecal infusions can be repeated if a single one fails to cure *C. difficile* infection. Risk factors for failure of a single faecal infusion include inadequate bowel preparation, severe *C. difficile* infection [56] and hospitalisation during FMT [57].

Several systematic reviews and meta-analyses found that colonoscopy provided higher resolution rates of *C. difficile* infection than other routes and a similar safety profile [7-9, 58].

During colonoscopy, the faecal material should be administered into the right colon of the recipient, when possible. In patients with severe *C. difficile* infection, it can also be infused in the left colon, for safety reasons.

FMT via enema showed satisfactory results in treating *C. difficile* infection, and repeated infusions could increase its success rates [6]. Enema may be useful for several reasons, including poor invasivity and costs, and wide availability.

Moreover, before starting the infusion through upper routes, by gastroscopy, by gastroduodenal or nasojejunal tube, or by gastrostomy tube, recipients must be positioned 45 ° upright, and keep this position for at least 4 hours after it, to reduce the risk of aspiration [17].

The ideal volume for instillation has not been established. However, smaller volumes (e.g. 25-50 mL) could be used for delivery via a nasoduodenal tube or nasogastric intubation; larger volumes (e.g. 250-500 mL) could be used for instillation via colonoscopy [41, 42].

34.9. Monitoring of patients in biovigilance

Recipients should be monitored for the occurrence of possible acute complications related to the procedure. Infection-control practices for patients with rCDI should be performed according to disease severity and comorbidities. The need for hospitalisation of patients with other underlying diseases depends on the diagnosis and clinical condition. When repeated faecal infusions are necessary, provided that the patient's condition is good, further applications can be performed in an outpatient setting [17]. The duration of the observation period has not been defined yet, as it depends on the route of delivery, the underlying diseases and the general condition of the patient.

Periodicity and length of follow-up for long-term adverse events and reactions are not determined. Follow-up should include clinical and analytical data.

Adverse events and reactions are not rare and should be carefully monitored throughout FMT [59]. The vast majority are mild, self-limiting and gastrointestinal in nature [60]. However, severe adverse reactions (such as death, viral and bacterial infections, transient relapse of IBD), were reported in several studies [59], but in a number of cases the association with FMT was not established due to the lack of controlled data.

These findings mandate the creation of registries – at the local (hospital) level and/or at the level of regional, national or international competent authorities – recording well-defined and standardised data. In order to trace possible causality linking FMT and newly developed diseases, keeping appropriate registries would be a wise process to trace and learn about potential long-term side effects.

34.10. Recent developments

FMT has also been investigated in the treatment of other disorders associated with the alteration of gut microbiota. In particular, studies in humans include ulcerative colitis [18-24], patients with metabolic syndrome [25, 26] or hepatic encephalopathy [27] and graft-*versus*-host disease [28]. FMT has been also thought to treat various diseases, including Parkinson, multiple sclerosis, fibromyalgia and chronic fatigue syndrome, among others [17, 61]. The United Kingdom's National Institute for Health and Care Excellence (NICE) has also published guidance on interventional procedures using FMT [62].

More recently, the composition of the intestinal microbiota has been thoroughly investigated and shown to vary among individuals and throughout development, and to be dependent on host and environmental factors [63-65]. These results are an important step towards better understanding of environment–diet–microbe–host interactions and further understanding of the role of dysbiosis, the role of FMT as a therapy and the importance of donor selection in clinical success [66, 67].

Nowadays, accumulating evidence is elucidating the relation of dysbiosis of intestinal bacteria with obesity and metabolic disorders. Certain gut microbial strains have been shown to inhibit or attenuate immune responses related to chronic inflammation in experimental models, suggesting that specific species among gut microbiota may play either a protective or a pathogenic role in the progression of obesity [68].

Moreover, capsule FMT represents a new but promising approach [69-72], which may increase the availability of FMT, both by expanding the access to treatment and by ameliorating patients' compliance with this procedure. Similarly lyophilisation (freeze-drying) could simplify FMT treatment [40, 73].

Finally, an NIH-funded study, aimed at addressing the regulation of (F)MT, adopted the following definition of MT:

> A microbiota transplantation is the transfer of biological material containing a minimally manipulated community of microorganisms from a human donor to a human recipient (including autologous use) with the intent of affecting the microbiota of the recipient. [74]

Minimal manipulation is processing that does not alter the original relevant characteristics of the transferred community of micro-organisms.

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Chapter 35: **Blood components for topical use or injection**

35.1. Introduction

Serum eye drops and platelet concentrates are examples of substances of human origin where there is wide variation in approach to regulation in Europe [1]. Platelet concentrates (PC) are defined as autologous and allogenic platelets with a concentration higher than the baseline used for transfusion. They include platelet-rich plasma (PRP), platelet gel, platelet-rich fibrin (PRF) and platelet lysate eye drops.

Within the European Union (EU), blood used for the manufacture of serum eye drops must meet the standards of quality and safety specified in Commission Directive 2004/33/EC of 22 March 2004, which implements Directive 2002/98/EC of the European Parliament and of the Council regarding certain technical requirements for blood and blood components, including donor selection. For cord blood serum, the selection criteria for living donors of tissues and cells specified in Annex I/III of Directive 2006/17/EC are applicable for EU member states. Serum eye drops *per se* and platelet concentrates may fall within different national legal frameworks in the EU for which the appropriate quality, safety and vigilance requirements need to be applied (these may include blood, tissues and cells, medicinal products).

The following generic chapters (Part A) of this Guide all apply to serum eye drops and must be read in conjunction with this chapter where applicable:

a. Chapter 1: Introduction,
b. Chapter 2: Quality management, risk management and validation,
c. Chapter 4: Donor evaluation,
d. Chapter 5: Donor testing – markers for infectious diseases,
e. Chapter 7: Premises,
f. Chapter 8: Processing,
g. Chapter 9: Storage and release,
h. Chapter 10: Principles of microbiological testing,
i. Chapter 11: Distribution and import/export,
j. Chapter 13: Computerised systems,
k. Chapter 14: Coding, packaging and labelling,
l. Chapter 15: Traceability,
m. Chapter 16: Biovigilance.

35.2. Serum eye drops

35.2.1. Introduction

Serum eye drops are prepared from the serum component of whole blood or cord blood for use by patients suffering from severe ocular surface diseases, specifically for patients who have either not responded to, or who in their clinician's opinion are unlikely to benefit from, conventional treatments. Serum eye drops can be prepared for autologous use from the patient's own serum or they can be allogeneic, prepared from blood donors or from cord blood. The serum, either undiluted or diluted in physiological saline, is dispensed in small aliquots into dropper bottles or suitable dispensers as eye drops, for application either by the healthcare professional or (for home treatment) by the patient.

Serum eye drops have a potential advantage over traditional therapies for dry eye syndrome and persistent epithelial defects because human serum not only replicates the mechanical functions of tears (lubricating the eyelid, and rinsing particles from the ocular surface), but also serves as a lacrimal substitute, containing many of the same growth factors and other biochemical components that are present in natural tears. This is the reason why serum eye drops have become a popular second-line therapy in dry eye treatment [2-4]. The Cochrane review of eye drops made from autologous serum concluded that autologous serum *versus* artificial tears might provide benefit for treatment of dry eye in the short term. However, the overall benefit seems unclear at this time, and much more research is needed in this area.

35.2.2. Donor evaluation

35.2.2.1. *Autologous setting*

In the case of autologous donation, the risks posed by blood donation must be carefully considered on an individual basis against the potential benefits from the treatment. Special attention should be paid to avoiding the development of anaemia, especially where there is repeated collection of blood to prepare serum eye drops. To overcome problems of co-existing medical conditions related to autologous donors and delays in treatment because of autologous serum eye drops preparation, the use of allogeneic eye drops can be considered. Active viral or fungal infection and certain medications that may injure the cornea are contraindications to donation for serum eye drops.

35.2.2.2. *Allogeneic setting*

Allogeneic serum eye drops can be prepared in advance and be ready for use in emergency cases, or if patients are not eligible to donate for themselves. Allogeneic serum eye drop donors must meet the same eligibility criteria as voluntary blood donors [5]. Additional selection criteria over and above these can be applied according to national requirements. As allogeneic serum eye drops are not lifesaving products, quarantining of the products for 4 months, followed by a negative nucleic acid test (NAT) and/or antibody screen on the donors' subsequent donation should be the minimum standard to enhance product safety. If the initial screening includes NAT, and if appropriate donor-referral criteria and donor-compliance monitoring are in place to cover the risk of window-period infections, this quarantine period may not be necessary.

At the end of the quarantine period, the donor should be re-tested for relevant infectious disease markers, and if the outcome of this further screening is negative, the serum can be released for clinical application. In addition to general donor selection applicable for allogeneic blood donors, specific factors such as medications that may change the physiological or immunological state of the eye or that might injure the cornea should be considered in determining donor suitability for serum eye drops.

35.2.2.3. *Umbilical cord serum*

Umbilical cord blood can be obtained during delivery, and laboratory testing of maternal blood for infectious diseases is required. Umbilical cord serum contains a higher concentration of growth factors and neurotropic factors compared with the levels in adult peripheral blood [6]. There is no definitive evidence demonstrating which components of serum are essential for serum eyedrop efficacy. In comparison to standard serum, there is limited published evidence available on the use of cord blood serum in the treatment of various ocular surface diseases that demonstrates efficacy [7, 8].

35.2.3. Processing and storage

There must be written protocols for all procedures related to blood and eye drops production. All measures should be taken to minimise the risk of microbiological contamination, including disinfection of the phlebotomy site using methods accepted for collection of blood for transfusion. Blood must be collected in a sterile container/blood bag, without anticoagulant. The collected blood volume depends on local procedure but cannot be more than for regular blood donors.

The collected blood must be allowed to clot, and the serum must be separated, following validated protocols used for preparing serum eye drops [9]. The serum can be used undiluted, or diluted with physiological saline solution to different concentrations. No standard production protocol or optimal serum concentration has been established to deliver maximal clinical benefit.

Eye-drop preparation must be carried out using aseptic technique. If the process involves open dispensing, it is required that clean rooms be used for manufacture of eye drops. It is strongly recommended to use a closed system for aliquoting. Microbiological control for each batch is mandatory (see Chapter 10). The volume of one aliquot should be adjusted to be no more than one daily dose to minimise microbiological growth in the thawed serum during

the application period. All bags that are used in the collection, processing and/or aliquots of final packaging must be properly labelled.

Eye drops must be stored frozen at <– 20 °C and transported in an appropriate container, to maintain the required temperature. Establishments must specify the shelf life of serum eye drop products at a defined temperature(s), to the best of their knowledge. This shelf life could be based on studies of the presumptive active components of serum eye drops at the designated storage temperature [10, 11].

The same principles apply to preparing umbilical cord serum eye drops. There must be a written protocol for the preparation of cord blood serum eye drops as described in this section for serum eye drops prepared from whole blood.

Until now no drug substance or mode of action has been specified to explain the beneficial effects of serum eye drops. The risk of bacterial contamination caused by donation and the manufacturing process, as well as during the application period of the thawed product by the patient, should be considered. To improve the risk–benefit ratio, precautions should be taken to avoid bacterial contamination and growth of harmful bacteria by sterile filtration of the final product before freezing the aliquots. However, filtration could also remove some of the presumptive active components of serum eye drops, and this risk should be considered. Other risk-reduction methods, such as a short shelf life, training of the patient and secure product packaging, can be implemented to reduce the bacterial contamination risk during the application period.

35.2.4. Clinical application

The patient must be given appropriate information about the blood collection and testing, and about eye-drop preparation. The patient must be provided with written instructions for storage and handling of the eye drops at home, as well as information about the risk of, and signs of, potential bacterial contamination of the product.

35.2.5. Biovigilance/pharmacovigilance

It is strongly recommended that the ophthalmologist monitors the patient's progress in a systematic way to enable collection of data regarding the benefits of using serum eye drops. Any adverse reaction that occurs during usage of eye drops should be notified to the regulatory authority following national regulations.

35.3. Platelet concentrates

35.3.1. Introduction

Platelet concentrates are used in regenerative medicine as source of growth factors and cytokines for the treatment of soft and hard tissue lesions. Each growth factor is involved in a phase of the healing process, such as inflammation, collagen synthesis, tissue granulation and angiogenesis, collectively promoting tissue restitution.

The use of platelet concentrates is an emerging field and its efficacy remains controversial. Several techniques for platelet concentrates are available; however, their applications have been confusing because each method results in a different product with different biology and potential uses. Platelet concentrates have been prepared as platelet-rich plasma (PRP), platelet gel, platelet-rich fibrin (PRF) and platelet lysate eye drops, and they vary in consistency and in composition, for example in the concentration of growth factors and cytokines. Depending on the leukocyte and fibrin content, platelet concentrates could be classified into four categories: pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure plaletet-rich fibrin (P-PRF), and leukocyte- and platelet-rich fibrin (L-PRF) [12].

They are usually used in an autologous setting and can be prepared at the time of application or in advance. When they are prepared in advance and stored, this should be done by a blood or tissue establishment. Allogeneic platelet concentrates can be collected from healthy donors or produced from umbilical cord blood.

PRP is a concentrated source of autologous platelets, and it contains several different growth factors and other cytokines, in concentrations 5 to 10 times higher than in standard plasma; PRP can be used to stimulate healing of soft tissue by injecting this concentrated plasma in the tissue where healing or effect is desired. There are primarily 3 isomers of platelet-derived growth factor (PDGF), namely αα, ββ and αβ, 2 transforming growth factors, TGF-β1 and TGF-β2, endothelial growth factor (EGF) and vascular epidermal growth factor (VEGF). PRP also contains proteins responsible for cell adhesion: fibrin, fibronectin and vitronectin [13]. The content of bioactive molecules depends on the production protocol [14]. All the products of this family can be used as liquid solutions or in an activated gel form. It can therefore be injected, for example in sports medicine, or placed during gelling on a skin wound or suture.

PRP is used to promote healing of injured tendons, ligaments, muscles and joints, and can be applied to various musculoskeletal problems. In addition to orthopaedics, other uses include dermatology, ophthalmology, plastic surgery and dentistry, including oral and maxillofacial surgery. As of 2017, no large-scale randomised controlled trials have confirmed the efficacy of PRP as a treatment for musculoskeletal or nerve injuries, the accelerated healing of bone grafts or the reduction of androgenic hair loss.

The main advantages so far identified in platelet gel derived from umbilical cord blood (CBPG), as compared with platelet gel from adult platelets, relate to a different profile of growth factor concentrations, such as a higher content of VEGF and lower content of TGF-β in CBPG. Recent developments have led to a procedure in which cord blood platelet gel can be prepared, stored in a cryopreservation bag and applied to the skin ulcer without breaking the sterility chain [15].

Platelet-rich fibrin (PRF) is a second-generation PRP where autologous platelets and leukocytes form a strong natural fibrin matrix or three-dimensional scaffold. This 'scaffolding' helps localise the growth factors, essentially increasing their concentration at the desired location to guide tissue regeneration [16]. PRF has a dense fibrin network with leukocytes, cytokines and structural glycoproteins, as well as growth factors (e.g. TGF β1, PDGF, VEGF) and glycoproteins, such as thrombospondin-1. Leukocytes that are concentrated in PRF scaffold play an important role in growth factor release, immune regulation, anti-infectious activities and matrix remodelling during wound healing. In addition, due to their elasticity and viscosity, these membranes adhere to the bone surface, acting as mechanical barriers against the penetration of the epithelium that has faster regeneration potency than connective tissues [17].

Topical application of a platelet lysate, administered as eye drops, is an alternative therapeutic option for treatment of ocular surface disorders that do not respond to standard treatment [18]. The plasma component contains proteins essential for surface lubrication, whereas platelets provide growth factors (PDGF, EGF and TGF-β) and fibronectin that can promote ocular re-epithelialisation [19]. Eye drops comprising PRP have been used to treat dry eye syndrome for patients with Sjögren disease, and ocular chronic graft-*versus*-host disease (cGvHD) [20], and are used during macular hole surgery. So far, only studies of small cases series have been published to explore the use of platelet concentrates in ophthalmology, and further large-scale studies are necessary to demonstrate efficacy.

35.3.2. Donor evaluation

In the case of autologous donation, special attention should be paid to the status of coagulation systems. The use of autologous platelet concentrates avoids the ethical and legal implications of exposing the patient to the risks (albeit low) of transmission of blood-borne pathogens, although the risk of infection related to contamination during collection and handling still remains. Disadvantages of autologous products include a larger individual variability in the quality of platelet concentrates compared with allogeneic products that are prepared from healthy blood through standardised working procedures.

35.3.3. Procurement and processing

Depending on the type of platelet concentrate, they can be prepared from whole blood, from apheresis product, or by using other methods of collection, such as small volume bags, tubes or various types of medical devices. Different blood volumes can be used, but the volume of anticoagulant must be proportional to the amount of blood collected. All manipulations during processing carried out in open system must be performed under clean-room conditions.

35.3.3.1. *Procurement and processing of platelet-rich plasma*

For the preparation of PRP, the blood is drawn with the addition of an anticoagulant, such as citrate dextrose A (ACD-A), to prevent platelet activation prior to its use. The platelets are separated from other blood cells using the two-step centrifugation method. A 30 mL venous blood draw will yield 3-5 mL of PRP, depending on the patient's baseline platelet count, the device used and the technique employed. An initial centrifugation separates red blood cells from PRP, and is followed by a second centrifugation that concentrates platelets in 3-5 mL of the final plasma volume. After the first centrifugation step, the whole blood is separated into three layers: an upper layer that contains mostly platelets and white blood cells, an intermediate thin layer that is known as the buffy coat and is rich in white blood cells, and a bottom layer that consists mostly of erythrocytes.

To produce pure PRP, the upper layer and superficial buffy coat are transferred to an empty sterile tube. The second centrifugation process should be adequate to generate the formation of soft platelet pellets at the bottom of the tube. The upper portion of the volume that is composed mostly of platelet-poor plasma is removed. Platelet pellets are re-suspended in the lower third part of plasma to create the PRP.

Many automated systems for the preparation of PRP which facilitate the preparation of ready-to-apply platelet-rich suspensions in a reproducible manner are commercially available. These systems widely differ in their ability to collect and concentrate platelets, depending on the method and time of its centrifugation. As a result, suspensions of different concentration of platelets and leukocytes are obtained. Differences in the concentrations in platelets and white blood cells influence the diversity of growth factors concentration.

35.3.3.2. *Procurement and processing of platelet-rich fibrin*

For the preparation of PRF, a sample of blood is collected from the patient in tubes without anticoagulant and the blood is immediately centrifuged. During centrifugation, the platelets are activated when the blood contacts the tube wall.

The duration of time between blood collection and centrifugation is an important factor affecting the success and clinical outcome of this procedure. The majority of PRF preparation protocols recommend immediate (within 2 minutes of collection) centrifugation after blood collection. Delay in centrifugation will result in diffuse polymerisation of fibrin leading to the formation of a small blood clot with irregular consistency. Therefore, a reproducible protocol for PRF production should be followed to obtain a clinically usable fibrin clot with substantial enmeshment of platelets.

After centrifugation, the uppermost of the three layers consists of acellular platelet-poor plasma, the PRF clot is in the middle layer and red blood cells are at the bottom of the tube. After centrifugation, the fibrin clot is removed from the tube and any attached red blood cells are scraped off and discarded.

PRF can also be applied as a membrane; the membrane can be formed in different shapes by squeezing out the fluids present in the fibrin clot using, for example, the stainless steel PRF compression device composed of two spoon-shaped parts [21].

35.3.3.3. *Procurement and processing of platelet lysate eye drops*

Platelet lysate eye drops are prepared using PRP after freezing–thawing at a final dilution of 30 %. A volume of 40 to 60 mL of peripheral blood anticoagulated with anticoagulant citrate dextrose solution A (ACD-A) is collected and centrifuged to obtain an autologous PRP. The platelet concentrate is frozen to – 80 °C for at least 60 min and then thawed to induce platelet lysis. The lysate can be diluted with sterile saline solution, and aliquoted into defined doses. A sample for microbiological control must be taken at the time the product was prepared (see Chapter 10). The final product is then frozen again at – 20 °C and stored in a freezer. Patients are usually provided with a monthly supply of doses and trained how to thaw the dose, store it for the day at 40 °C and safely instil eye drops.

35.3.4. Quality control

The quality of platelet concentrates could be evaluated according to platelet recovery and growth factor contents. Further investigations are required to define standardised protocols for the preparation of high-quality platelet concentrates suitable for different clinical applications, thus making it possible to compare results [22].

35.3.5. Biovigilance

Studies that have evaluated the topical use of platelet concentrates have shown that the application is safe, and no serious adverse events were observed [23, 24]. According to a current literature search on platelet concentrates use, there is no evidence of systemic effects that might limit the use of platelet concentrates, provided that the possible risk of infections is excluded [14]. Few randomised controlled trials have reported adverse events after injection of platelet product; where these occur, they are mostly local side-effects related to venipuncture required for blood collection or (rarely) bad scarring or calcification at the application sites after injection of platelet product.

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Part D. Tissue and cell monographs

Tissue and cell monographs

Introduction

In this 4th edition of the *Guide to the quality and safety of tissues and cells for human application* the *ad hoc* working group has worked on the elaboration of tissue and cell monographs. The role of a tissue and cell monograph is to provide information about individual tissue and cell preparations and clinical applications that are precisely defined and have been shown to be safe and effective when used in patients. Each tissue and cell monograph combines two aspects, a consolidated process and a consolidated use, in a single document that is complementary to other sections of the Guide. Part A of the Guide determines the generic requirements for donor selection, procurement, processing, storage, distribution, etc., while Part B describes the specific criteria for each tissue and cell type. Tissue and cell monographs complement and connect these Parts, linking both aspects to define specific critical properties and quality-control requirements for specific tissues and cells, prepared in specific ways, and to be used for specific clinical indications.

Data collected from EU member states in 2016 on the reporting of serious adverse reactions and events for tissues and cells (during 2015) indicated that more than 2 100 000 units of tissues and cells were distributed, to treat a wide range of clinical indications using multiple different processing methods. These processes, which include freezing, cryopreservation and lyophilisation, are very well-established methods and have been consolidated through many years of experience with the support of scientific results and publications showing the safety, the quality and the effectiveness of the tissues and cells prepared and used in these validated conditions.

A common language is necessary for tissue establishments and Health Authorities when defining the commonly accepted, relevant, validated quality criteria for each consolidated processing method applied to each specific tissue or cell type and indication. Thus, tissue and cell monographs are intended as tools for tissue establishments and Health Authorities providing recommendations on the minimum controls necessary to ensure the quality of the tissues and cells that are processed by a tissue establishment. They also allow tissue establishments to demonstrate to their Health Authorities that they have fulfilled appropriate quality requirements when producing their tissue and cell preparations. In those cases, Health Authorities can be confident and they need only check that the quality parameters for the intended clinical uses are being appropriately reached through the processes performed by the tissue establishments.

The field of tissues and cells is a dynamic one and approaches change with advances in the development of new processing methods and the emergence of new indications. It is important that these new improvements are carefully evaluated by tissue establishments and Health Authorities, notably via the process of authorisation. Chapter 29 of this guide provides generic guidance on good practice for the introduction of new or changed procedures and their subsequent authorisation.

By reference to these tissue and cell monographs, both tissue establishments and Health Authorities will know that products that do not have a matching tissue and cell monograph may need more attention (more risk assessment, validation, possibly clinical studies etc.) before they can be authorised and supplied for routine use.

In future editions of the Guide, new processes/products will eventually have their own tissue or cell monograph once they become consolidated by evidence and there is consensus on the release criteria and appropriate clinical indications.

17.1: Organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)

Tissue/cell product	Organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
Definition	Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for anterior lamellar keratoplasty
Established clinical indications	• Stromal opacities caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
Critical properties	• Absence of stromal opacities. • Viable, functioning endothelium may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) because anterior chamber penetrated. • No evidence of microbial growth.
Quality control requirements	• Stroma is clear and without scars within a 7.50 mm diameter zone. • If applicable, endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2 at end of organ culture storage or ≥ 2 200 cells/mm^2 if only measured before organ culture). • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Storage time in organ culture – Endothelial cell density, if applicable – Diameter of the central clear zone.
Special warnings	• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the quality control requirements (above).

17.2: Cold-stored corneal tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)

Tissue/cell product	Cold-stored corneal tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
Definition	Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for anterior lamellar keratoplasty
Established clinical indications	• Stromal opacities caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
Critical properties	• Absence of stromal opacities. • Viable, functioning endothelium may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) because anterior chamber penetrated. • No evidence of microbial growth.
Quality control requirements	• Stroma is clear and without scars within a 7.50 mm diameter zone. • If applicable, endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2). • Not to be used if the organ-culture medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Time in cold-storage medium – Endothelial cell density, if applicable – Diameter of the central clear zone.
Special warnings	• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the quality control requirements (above).

17.3: Organ-cultured corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)

Tissue/cell product	Organ-cultured corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)
Definition	Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for DMEK.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Viable, functioning endothelium. • No evidence of microbial growth.
Quality control requirements	• Descemet membrane is manually peeled off the corneal stroma with the endothelium attached, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy after organ culture but before Descemet membrane peeling (preferably ≥ 2 000 cells/mm^2). • Graft diameter measurement with calliper or trephine (peeled zone ≥ 9.00 mm). • Tears (damage) in Descemet membrane within 9.00 mm zone must be noted in the accompanying documentation. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of the medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with or without an osmotically active agent. • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information regarding graft placement (free roll or attached to stroma) must be provided. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Storage time in organ culture – Endothelial cell density – Graft diameter and presence of tears (damage).
Special warnings	• Not to be used for penetrating keratoplasty.

17.4: Cold-stored corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)

Tissue/cell product	Cold-stored corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)
Definition	Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for DMEK.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Viable, functioning endothelium. • No evidence of microbial growth.
Quality control requirements	• Descemet membrane is manually peeled off the corneal stroma with attached endothelium, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy before Descemet membrane peeling (≥ 2 000 cells/mm^2). • Graft diameter measurement with calliper or trephine (peeled zone ≥ 9.00 mm). • Tears (damage) in Descemet membrane within 9.00 mm zone must be noted in accompanying documentation. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information regarding graft placement (free roll or attached to stroma) must be provided. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Storage time in organ culture – Endothelial cell density – Graft diameter and presence of tears (damage).
Special warnings	• Not to be used for penetrating keratoplasty.

17.5: Organ-cultured corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

Tissue/cell product	Organ-cultured corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
Definition	Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for DSAEK.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Viable, functioning endothelium. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy after organ culture but before pre-cutting (≥ 2 000 cells/mm^2). • Central stromal thickness measurement of the graft (ultrasound or, preferably, optical coherence tomography). • Minimal variation in graft thickness from centre to periphery (≤ 50 % increase in thickness). • Graft diameter measurement with calliper (cap diameter ≥ 9.00 mm). • Replaced anterior corneal cap after pre-cutting. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be kept at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Storage time in organ culture – Endothelial cell density – Central graft thickness – Cap diameter.
Special warnings	• Not to be used for penetrating keratoplasty.

17.6: Cold-stored corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

Tissue/cell product	Cold-stored corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
Definition	Human cold-stored corneal donor tissue prepared in a tissue establishment to be used for DSAEK.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Viable, functioning endothelium. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy before pre-cutting (≥ 2 000 cells/mm^2). • No stromal opacities within a 6.00 mm central zone. • Central stromal thickness measurement of the graft (ultrasound or, preferably, optical coherence tomography). • Minimal variation in graft thickness from centre to periphery (≤ 50 % increase in thickness). • Graft diameter measurement with calliper (cap diameter ≥ 9.00 mm). • Replaced anterior corneal cap after pre-cutting. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Total time in cold storage – Endothelial cell density – Central graft thickness – Cap diameter.
Special warnings	• Not to be used for penetrating keratoplasty.

17.7: Organ-cultured corneal tissue for penetrating keratoplasty (PK)

Tissue/cell product	Organ-cultured corneal tissue for penetrating keratoplasty (PK)
Definition	Human organ-cultured corneal donor tissue prepared in a tissue establishment to be used for PK.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, stromal dystrophies, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
Critical properties	• Viable, functioning endothelium. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2 at end of organ-culture storage). • No stromal scars/opacities within a 7.50 mm diameter central zone. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Storage time in organ culture – Endothelial cell density – Diameter of the clear zone.
Special warnings	• None.

17.8: Cold-stored corneal tissue for penetrating keratoplasty (PK)

Tissue/cell product	Cold-stored corneal tissue for penetrating keratoplasty (PK)
Definition	Human cold-stored corneal donor tissue prepared in a tissue establishment to be used for PK.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, stromal dystrophies, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
Critical properties	• Viable, functioning endothelium. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy before cold storage (≥ 2 000 cells/mm^2). • No stromal scars or opacities within a 7.50 mm diameter central zone. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age – Total time in cold-storage – Endothelial cell density – Diameter of the clear central zone.
Special warnings	• None.

18.1: Amniotic membrane (AM) for biological dressing

Tissue/cell product	Amniotic membrane (AM) for biological dressing
Definition	Human amniotic membrane obtained from placenta processed in a tissue establishment and preserved for use as biological dressing and substrate for cell growth in different clinical applications.
Established clinical indications	• Ophthalmological indications (e.g. ophthalmic corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, chemical or thermal burns). • Burns (as a temporary or permanent wound dressing). • Skin lesions of different aetiologies (e.g. vascular ulcers, epidermolysis bullosa, radiation burns).
Critical properties	• Preserved structural integrity (barrier function). • No evidence of microbial growth. • Adequate graft size.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • In cases of lyophilisation (freeze drying) a residual moisture of 5-10 % (w/w) or available water (aW) of < 0.5 is recommended.
Storage and transport	• Lyophilised/freeze-dried AM can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be justified according to the known range of ambient variables that could affect the critical properties of the graft. • Frozen AM should be stored between – 15 and – 80 °C and transported at a similar temperature as was used during storage. The shelf life should be justified according to the storage temperature conditions that could impact on the critical properties of the graft. • Cryoprotected AM grafts are stored at – 80 °C (deep frozen) or in liquid or vapour phase of nitrogen at temperatures below – 140 °C (cryopreserved). Distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. Transport temperatures of cryoprotected AM above – 60 °C must be avoided to ensure the stability of the product and maximum safety for the recipient. • Glycerolised AM (preserved in glycerol 85 % solution) should be both stored and transported at 2-8 °C.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Key specifications (e.g. graft dimensions in cm^2, appropriate radiation-sensitive labels) – Instructions for appropriate rehydration/thawing – Information on the composition of antibiotic decontamination cocktail. • Orientation of amnion (epithelial or mesenchymal side on carrier material).
Special warnings	• Dried, lyophilised, frozen or cryopreserved allografts should be used within 6 hours after rehydration or thawing (in saline solution). • Rehydration time of freeze-dried grafts or thawing time of frozen grafts should be defined by originating tissue bank and should be performed under sterile conditions. • When applicable, rinse out glycerol before use (glycerolised AM). • After thawing, if applicable, rinse out cryoprotectants before use (cryoprotected AM). • Do not re-freeze thawed AM graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

19.1: Acellular dermal matrix (ADM)

Tissue/cell product	Acellular dermal matrix (ADM)
Definition	Human decellularised dermis from donor skin to be used for wound-healing procedures.
Established clinical indications	• Burn injuries. As dermal substitute in partial- or full-thickness burns when donor sites are insufficient for autograft or when patient condition is critical, to improve scar quality and to prevent post-burn joint contracture. • Reconstructive surgery. As a valid reconstructive tool for any surgical wound in general surgery (e.g. abdominal wall repair), orthopaedic (e.g. rotator cuff reconstruction), oncologic (e.g. breast-conserving surgery), ear nose and throat (ENT) surgery (e.g. myringoplasty, rhinoplasty) and bariatric surgery. • Ulcers. Uninfected, chronic ulcers and diabetic foot ulcers, to accelerate the closure and healing rate. • Full-thickness acute wounds. As a scaffold to support cell ingrowth and granulation tissue formation and to achieve durable coverage of exposed critical structures (bone, tendons). • Composite graft technique. To restore the dermal component by application of human dermis or dermal equivalents, and thin autologous grafts or keratinocytes.
Critical properties	• Absence of donor cells (and genetic material). • Graft thickness ranging 0.2-0.8 mm (thin), 0.8-1.2 mm (medium thickness) and 1.3-2.8 mm (thick). • Preserved structural integrity. • Flexibility/pliability. • Resistance to mechanical stress and suturability. • Sterility.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • Graft thickness measurement. • Biocompatibility test (cytotoxicity tests). • Mechanical resistance test. • Morpho-structure evaluation by histological staining (e.g. orcein, Masson, haematoxylin-eosin) or electron microscopy (EM). • Decellularisation process by specific histological staining e.g. DAPI, Hoersch and DNA quantification assay (ng DNA/mg dry tissue). • Residual water test (≤ 5 %) – for lyophilised ADM.
Storage and transport	• Depending on the processing methods, most dermal matrices are stored and transported at room temperature (15-25 °C) – e.g. acellular glycerol-preserved, lyophilised and/or irradiated matrices – or refrigerated at 2-8 °C.
Special labelling and accompanying information	• In the EU, grafts must be labelled with the Single European Code (SEC), as applicable. • Appropriate radiation-sensitive labels must be used for irradiated ADM. • Thickness and size.
Special warnings	• Do not sterilise irradiated dermal matrices. • Rehydration of glycerol-preserved or lyophilised allografts is recommended before use (e.g. by washing in saline solution for 20-30 min).

19.2: Deep-frozen skin allografts

Tissue/cell product	Deep-frozen skin allografts
Definition	Viable skin allografts preserved in a cryoprotective solution.
Established clinical indications	• Temporary/semipermanent covering of burns. • Temporary covering of epidermolytic diseases (e.g. toxic epidermolytic necrosis, staphylococcal scalded skin syndrome). • Treatment of hard-to-heal ulcers. • Temporary covering of wounds with exposed bone and/or tendons. • Wound-bed preparation (promoting neovascularisation).
Critical properties	• Cell viability: it is maintained by cryopreserved and, to a lesser degree, deep-frozen skin grafts; to consider a graft as viable, a minimum of 20 % of residual cell viability should be achieved. The mean percentage after 10-20 days of storage is reportedly 30-51 % (deep-frozen skin grafts) and 20-60 % (cryopreserved skin grafts) compared to that of the fresh skin, according to different viability assays. • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure). • Graft thickness ranging 0.2-0.8 mm. • Mechanical resistance. • Engraftment to the wound bed.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • Cell viability assessment (if required, depending on the intended application).
Storage and transport	• Cryopreserved skin grafts are stored in liquid or in the vapour phase of nitrogen at temperatures lower than – 140 °C. • Deep-frozen skin grafts are stored in ultra-low-temperature refrigerators, which generally maintain a temperature lower than – 80 °C. • Transport in a nitrogen dry-shipper or in dry ice (according to the processing method and storage temperature).
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation: – Graft area in cm^2 – Graft thickness – Number of sheets – Decontamination solution composition – Cryoprotective solution composition – Cell viability – Antibodies to *Cytomegalovirus* (CMV) when positive.
Special warnings	• Do not re-freeze thawed skin graft. • Do not irradiate viable skin graft. • Rinse out cryoprotectants before use. • Rinse with an appropriate solution (saline solution or balanced salt solution).

19.3: Glycerol-preserved skin allograft

Tissue/cell product	Glycerol-preserved skin allograft
Definition	Human split-thickness, glycerol-preserved, de-vitalised skin grafts, with epidermis and upper dermis components for the treatment of skin loss.
Established clinical indications	• Temporary biological dressing: – in partial-thickness burns, – on meshed autografts (sandwich technique), – on donor site, – after application of *in vitro* cultured keratinocytes. • Temporary wound coverage after excision in full-thickness burns. • Temporary coverage in toxic epidermolytic necrolysis. • Temporary biological dressing for difficult, non-healing wounds, to protect and preserve the viable granulation tissue from desiccation and necrosis (antalgic and antibacterial effect). • Wound-bed preparation (promoting wound healing).
Critical properties	• Graft thickness ranging 0.2-0.8 mm. • Plain or meshed. • No evidence of microbial growth.
Quality control requirements	• Intact epidermis and upper dermis (normal morphological structure). • Microbiological testing (aerobic and anaerobic bacteria, fungi).
Storage and transport	• The graft is stored in glycerol 85 % solution to keep the tissue preserved before use. • The graft is stored at refrigerator temperature (2-8 °C); storage at room temperature (15-25 °C) during transportation is possible. • Maximum time: storage at 2-8 °C for 5 years.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC must be included in accompanying documentation or on the label: – Size of graft, width and length – Graft thickness – Plain or meshed.
Special warnings	• Rinse out glycerol before use (incubation in a large volume of sterile 0.9 % NaCl solution for 10 min at room temperature). • Not to be used if the storage medium is opaque.

20.1: Cryopreserved femoral artery allograft, antibiotic decontaminated

Tissue/cell product	Cryopreserved femoral artery allograft, antibiotic decontaminated
Definition	Human femoral artery. Decontaminated by incubation with one or more antibiotics, and cryopreserved using slow cooling in the presence of a cryoprotectant.
Established clinical indications	• Replacement of infected prosthetic vascular allografts. • Mycotic abdominal aortic aneurysm. • Chronic ischaemia. • Critical limb ischaemia.
Critical properties	• There should be no visible (or minimal) atheroma or calcification present. • There should be no visible stenosis or dilation present. • Branching arteries should be 2-3 mm in length. • There should be no cuts or significant haematomas in the vessel wall.
Quality control requirements	• Post-decontamination, no viable micro-organisms should be detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms should be detectable on the graft.
Storage and transport	• Grafts should be stored at or below – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers or solid carbon dioxide refrigeration. • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• The grafts should be marked with the Single European Code (SEC). • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age and gender – Key dimensions (proximal and distal annular diameter and length) – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze thawed femoral artery graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

20.2:Cryopreserved heart valve allograft, antibiotic decontaminated

Tissue/cell product	Cryopreserved heart valve allograft, antibiotic decontaminated
Definition	Human heart valve, including the base of the aorta/pulmonary trunk and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled cooling in the presence of a cryoprotectant.
Established clinical indications	• Tetralogy of Fallot. • Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Ventricular septal defect. • Pulmonary stenosis. • Pulmonary atresia. • Aortic stenosis. • Aortic insufficiency. • Absent pulmonary valve syndrome. • Endocarditis. • Ross procedure.
Critical properties	• There should be no visible (or minimal) calcification present in the valve or associated vessel. • Pulmonary valves: there must be a rim of myocardium of at least 2 mm depth surrounding the base of the vessel. • Aortic valves: there must be a rim of myocardium or mitral leaflet of at least 2 mm depth surrounding the base of the vessel. • The associated pulmonary artery should not be cut below the level of the valve leaflet cusps. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decontamination and preservation protocols applied, as demonstrated by a functionality test.
Quality control requirements	• Post-decontamination, no viable micro-organisms should be detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms should be detectable on the graft. • Functionality tests (such as competency).
Storage and transport	• Grafts should be stored at < – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers or solid carbon dioxide refrigeration. • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If graft is shipped in dry ice, it should not be returned to storage at < – 140 °C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• The grafts should be marked with the Single European Code (SEC). • Specific information not coded in the SEC must be included in accompanying documentation: – Donor age and gender – Key dimensions (annular diameter and length/diameter of associated vessels) – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale. • Do not re-freeze thawed heart-valve graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

21.1: Cancellous bone chips

Tissue/cell product	Cancellous bone chips
Definition	Cancellous bone, also referred to as trabecular bone or spongy bone, is the porous mineralised component of bone that has a honeycomb or sponge-like appearance. The bone matrix is organised into a three-dimensional lattice of bony processes (trabeculae) arranged along lines of stress. Cancellous bone chips are sawn or ground to various sizes (generally 1-10 mm diameter/edge length). Cancellous chips provide an osteoconductive matrix for autologous bone remodelling and healing.
Established clinical indications	• Cancellous chips fill bony defects in almost all parts of the skeletal system, including periodontal regions.
Critical properties	• Cancellous chips should only be prepared from bone tissue with suitable morphology and density (contraindications: osteoporosis). The essential native biomechanical properties of the bone must not be significantly altered by the processing protocols. • Processing steps should largely reduce fat and remove blood cells and bone marrow. • Cancellous chips should undergo a process that guarantees absence of any pathogens and viral inactivation. In cases of irradiation, the effect on biomechanical properties of the bone has to be considered. • The bone must not be rendered cytotoxic by the processing protocol.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacterial, fungi). • In cases of lyophilisation (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended.
Storage and transport	• Lyophilised/freeze-dried cancellous chips can be stored and transported at room temperature (15-25 °C). • Freezing and high temperatures (> 30 °C) should be avoided. • Frozen cancellous chips should be stored between − 15 and − 80 °C and transported at or below − 15 °C. • The shelf-life should be justified according to the known range of ambient variables that could affect the critical properties of the graft.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC should be included in accompanying documentation: – Key specifications (e.g. graft dimensions, weight of pack) – Instructions for appropriate rehydration/thawing – Information about potential risks (e.g. transmission of infectious diseases).
Special warnings	• Cancellous chips should be used within 6 hours after rehydration or thawing. • Rehydration time of freeze-dried grafts or thawing time of frozen grafts must be defined by originating tissue establishment and must be performed under sterile conditions. • In cases of lyophilisation (freeze drying): residual moisture or available water.

21.2: Cortical bone struts

Tissue/cell product	Cortical bone struts
Definition	Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons). Cortical strut allografts are diaphyseal segments of bone allograft. Cortical struts are made from full circumferential segments, hemicylinders or flat-planed struts from femur, tibia, humerus or full circumferential segments of fibula by sawing into several sizes. Cortical strut allografts unite to host bone through callus formation, restoring bone stock, and can be used as an onlay biological plate.
Established clinical indications	• Revision arthroplasty and periprosthetic fractures. • Bridging of structural defects in long bones. • Buttress in limb-salvage procedures.
Critical properties	• Cortical struts should only be prepared from bone tissue with suitable morphology and density (contraindications: osteoporosis, osteomalacia). • Processing steps should largely reduce fat and remove blood and bone marrow. The bone must not be rendered cytotoxic by the processing protocol. • Cortical struts should undergo a process that guarantees absence of any pathogens and viral inactivation. In cases of irradiation, the effect on biomechanical properties of cortical bone has to be considered.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacterical, fungi). • In cases of lyophilisation (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended.
Storage and transport	• Lyophilised/freeze-dried cortical struts can be stored and transported at room temperature (15-25 °C). • Freezing and high temperatures (> 30 °C) should be avoided. • Frozen cortical struts should be stored between − 15 and − 80 °C and transported at ≤ − 15 °C. • The shelf-life should be justified according to the known range of ambient variables that could affect the critical properties of the graft.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC should be included in accompanying documentation: – Key specifications (e.g. graft dimensions, weight of pack) – Instructions for appropriate rehydration/thawing – Information about potential risks (e.g. transmission of infectious diseases).
Special warnings	• Cortical struts should be used within 6 hours after rehydration or thawing. • Rehydration time of freeze-dried grafts or thawing time of frozen grafts must be defined by originating tissue establishment and rehydration or thawing must be performed under sterile conditions. • In cases of lyophilisation (freeze drying): residual moisture or available water.

21.3: Patellar tendon allografts

Tissue/cell product	Patellar tendon allografts
Definition	The patellar ligament is the distal portion of the common tendon of the *M. quadriceps femoris*, which continues from the patella to the tibial tuberosity. It is also called the patellar tendon as it is a continuation of the quadriceps tendon.
Established clinical indications	• Reconstruction of the anterior cruciate ligament (ACL). • Extensor mechanism injuries in the knee joint.
Critical properties	• Patellar tendon allografts are procured as a bone-tendon-bone unit, with or without a part of the quadriceps tendon (length 10-20 cm, width 10-40 mm, space between bone blocks 30-80 mm) with a block from the tibial tuberosity (approximately 3.5 cm long and at least 2 cm wide). Tendons 2 cm wide or more are suitable for splitting to provide two patellar tendon allografts. • Patellar tendon allografts should undergo a process that guarantees absence of any pathogens and viral inactivation. These techniques may have a detrimental effect on both the biomechanical and biological properties of the graft and this effect must be considered. • Processing steps should largely reduce fat and remove blood and bone marrow. The bone must not be rendered cytotoxic by the processing protocol.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacterial, fungi). • In cases of lyophilisation (freeze drying) a residual moisture of 5-15 % (w/w) is recommended.
Storage and transport	• Lyophilised/freeze-dried patellar tendon allografts can be stored and transported at room temperature (15-25 °C). • Freezing and high temperatures (> 30 °C) should be avoided. • Frozen patellar tendon allografts should be stored between – 15 and – 80 °C and transported at or below – 15 °C. • The shelf-life should be justified according to the known range of ambient variables that could affect the critical properties of the graft.
Special labelling and accompanying information	• In the EU, the grafts must be labelled with the Single European Code (SEC), as applicable. • Specific information not coded in the SEC should be included in accompanying documentation: – Key specifications (e.g. graft dimensions, weight of pack) – Instructions for appropriate rehydration/thawing – Information about potential risks (e.g. transmission of infectious diseases).
Special warnings	• Patellar tendon allografts should be used within 6 hours after rehydration or thawing. • Rehydration time of freeze-dried grafts or thawing time of frozen grafts must be defined by originating tissue establishment and rehydration or thawing must be performed under sterile conditions. • In cases of lyophilisation (freeze drying): residual moisture or available water.

22.1: Haematopoietic progenitor cells from bone marrow – HPC(M)

Tissue/cell product	Haematopoietic progenitor cells from bone marrow – HPC(M)
Definition	HPCs are found in small numbers in bone marrow. The infused HPC(M) can originate from the recipient (autologous) or from another individual (allogeneic). They can be used as fresh unmanipulated product or can be further processed (e.g. buffy-coat preparation, cell selection, cryopreservation).
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability *a.* for autologous transplantation: – Nucleated cell dose: > 1.0-2.0 × 10^8/kg recipient body weight, – Viable $CD34^+$ cell dose: ≥ 2.0 × 10^6/kg recipient body weight; *b.* for allogeneic transplantation: – Nucleated cell dose: ≥ 2.0-3.5 × 10^8/kg recipient body weight, – Viable $CD34^+$ cell dose: ≥ 2.0-3.50 × 10^6/kg recipient body weight. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient body weight. • In cases of cryopreserved HPC(M), DMSO volume should be less than 1 mL/kg recipient body weight.
Quality control requirements	• Nucleated cell count. • Enumeration of viable $CD34^+$ cells. • Microbiological testing. • ABO Rh blood group for allogeneic products. • Measurement of residual ABO-incompatible red cell volume.
Storage and transport	• Fresh HPC(M) can be stored and transported up to 72 hours at room temperature (15-25 °C) or refrigerated (2-8 °C) as requested by the transplant centre. • Cryopreserved HPC(M) are stored and transported at temperatures equal to or below – 140 °C. • Cryopreserved HPC(M) can be stored for up to 10 years or longer. • Thawed HPC(M) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• In the EU, grafts must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor name (autologous or related donors) or donor ID (unrelated donors) – Recipient name (if permitted), recipient ID (if applicable) – Nucleated cell count and viable $CD34^+$ cell enumeration – ABO Rh blood group – Volume – Identity of the collection facility and/or donor registry – Identity of processing and distribution facility – Instructions for appropriate thawing, if applicable.
Special warnings (if needed)	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing. • If presence of microbial contamination, consider antibiotic treatment in the recipient.

22.2: Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)

Tissue/cell product	Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)
Definition	HPCs are found in umbilical cord blood (UCB). The infused HPC(CB) can originate from the recipient (autologous) or from another individual (allogeneic). UCB units are distributed cryopreserved as whole blood or buffy-coat enriched.
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability *a.* for autologous transplantation: – Nucleated cell dose: ≥ 2.0×10^7/kg body weight (after thawing), – $CD34^+$ cell dose: ≥ 1.2×10^5/kg body weight (after thawing); *b.* for allogeneic transplantation: UCB units 6/6 or 5/6 HLA-matched – Nucleated cell dose: > 2.0×10^7/kg body weight (after thawing), – $CD34^+$ cell dose: > 1.2×10^5/kg body weight (after thawing); UCB units 4/6 HLA-matched – Nucleated cell dose: > 3.0×10^7/kg body weight (after thawing), – $CD34^+$ cell dose: > 1.7×10^5/kg body weight (after thawing). • Absence of microbial contamination (the presence of microbial contamination will not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient body weight.
Quality control requirements	• Total nucleated cell count. • Viable $CD34^+$ cell enumeration. • Viability of $CD45^+$ and $CD34^+$ cells. • Microbiological testing. • ABO Rh blood group and verification HLA typing. • Measurement of residual ABO incompatible red cell volume. • CFU or other validated potency assay.
Storage and transport	• Cryopreserved HPC(CB) are stored and transported at temperatures equal to or below – 150 °C. • Cryopreserved HPC(CB) can be stored for more than 10 years. • Thawed HPC(CB) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• In the EU, grafts must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor name (autologous or related donors) or donor ID (unrelated donors) – Recipient name, recipient ID (if applicable) – Nucleated cell count and viable $CD34^+$ cell enumeration – Result of a potency assay – ABO Rh blood group – Volume – Identity of the collection facility and /or donor registry – Identity of processing and distribution facility – Instructions for appropriate thawing and washing if units have not been red cell reduced prior to cryopreservation – Circular of Information brochure (including: handling instructions for the use of cellular therapy products, with indications, contraindications, side-effects and hazards, dosage and infusion recommendations).
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing.

22.3: Haematopoietic progenitor cells from peripheral blood apheresis – HPC(A)

Tissue/cell product	Haematopoietic progenitor cells from peripheral blood apheresis – HPC(A)
Definition	HPC(A) are procured by apheresis from the mononuclear cell fraction of circulating blood after their mobilisation from the bone marrow. The infused HPC(A) can originate from the recipient (autologous) or from another individual (allogeneic). They can be used as fresh unmanipulated product or further processed (e.g. cell selection, cryopreservation).
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability *a.* for autologous transplantation: – Viable $CD34^+$ cell dose: $\geq 2.0 \times 10^6$/kg recipient body weight; *b.* for allogeneic transplantation: – Target viable $CD34^+$ cell dose: approximately $> 5.0 \times 10^6$/kg recipient body weight, – Minimum viable $CD34^+$ cell dose: $1.5\text{-}3.5 \times 10^6$/kg body weight. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient weight. • In case of cryopreserved HPC(A), DMSO volume should be less than 1 mL/kg recipient body weight.
Quality control requirements	• Nucleated cell count. • Viable $CD34^+$ cell enumeration. • Microbiological testing. • ABO Rh blood group for allogeneic products. • Measurement of residual ABO-incompatible red cell volume.
Storage and transport	• Fresh HPC(A) can be stored and transported up to 72 hours at room temperature (15-25 °C) or refrigerated (2-8 °C) as requested by the transplant centre. • Fresh HPC(A) can be stored up to 72 hours without cryopreservation. • Cryopreserved HPC(A) are stored and transported at temperatures equal or below – 140 °C. • Cryopreserved HPC(A) can be stored for up to 10 years or longer. • Thawed HPC(A) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• In the EU, grafts must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor name (autologous or related donors) or donor ID (unrelated donors) – Recipient name (if permitted), recipient ID (if applicable) – Viable $CD34^+$ cell enumeration – ABO Rh blood group – Volume – Identity of the collection facility and /or donor registry – Identity of processing and distribution facility – Instructions for appropriate thawing (if applicable).
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing. • If presence of microbial contamination, consider antibiotic treatment in the recipient.

22.4: Mononuclear cells from unstimulated peripheral blood apheresis – MNC(A)

Tissue/cell product	Mononuclear cells from unstimulated peripheral blood apheresis – MNC(A)
Definition	Unstimulated mononuclear cells are procured by apheresis from the circulating blood. The procured cells can originate from the recipient (autologous) or from another individual (allogeneic). Unstimulated mononuclear cells can be used as fresh non-manipulated products or further processed (e.g. cryopreservation, cell selection, starting material for ATMPs).
Established clinical indications	• MNC(A) after allogeneic stem cell transplantation from the original HPC donor are used in cases of relapse and mixed chimerism or as relapse prophylaxis to enhance the graft-*versus*-malignancy effect, to promote immune reconstitution and prevent infection complications. • MNC(A) for generation of cellular therapies and ATMPs (e.g. NK-cell therapy, virus-specific T-cells, CAR-T cells).
Critical properties	• Cellularity/viability *a.* After allogeneic transplantation to enhance immunity and graft-*versus*-malignancy effect: – Escalating cell doses of $CD3^+$ cells, depending on the clinical situation and the transplant setting (e.g. in case of relapse from 1.0×10^6/kg to 1.0×10^8/kg body weight), – $CD3^+$ cell dose $> 1.0 \times 10^8$/kg body weight per infusion should be avoided due to increased risk of graft-*versus*-host disease; *b.* As starting material for generation of cellular therapy and ATMPs: – Required cell dose according to the specific protocol. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient).
Quality control requirements	• Nucleated and mononuclear cell count. • Viability. • Viable $CD3^+$ cells enumeration. • Microbiological testing. • ABO Rh blood group for allogeneic products.
Storage and transport	• Fresh MNC(A) can be stored and transported up to 72 hours at room temperature (15-25 °C) or refrigerated (2-8 °C) as requested by the transplant centre. • Fresh MNC(A) can be stored up to 72 hours without cryopreservation. • Cryopreserved MNC(A) are stored and transported at temperatures equal to or below – 140 °C. • Cryopreserved MNC(A) can be stored for up to 10 years or longer. • Thawed MNC(A) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• In the EU, grafts must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC must be included in accompanying documentation: – Donor name (autologous or related donors) or donor ID (unrelated donors) – Recipient name, recipient ID (if applicable) – Total nucleated and mononuclear cell count – Viable $CD3^+$ cell count – ABO Rh blood group (allogeneic products) – Volume – Identity of the collection facility and /or donor registry – Identity of processing and distribution facility. – Instructions for appropriate thawing, if applicable.
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing (if applicable). • If presence of microbial contamination, consider antibiotic treatment in the recipient.

27.1: Vitrified oocytes for non-partner donation

Tissue/cell product	Vitrified oocytes for non-partner donation
Definition	Vitrified human oocytes obtained after controlled ovarian hyperstimulation, to be used for non-partner oocyte donation in *in vitro* oocyte fertilisation. Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised.
Established clinical indications	Women needing a non-partner oocyte donation to achieve a pregnancy. The most common indications are: • Premature ovarian failure, either primary or secondary, including surgical oophorectomy, irreversible gonadal damage after chemotherapy or radiotherapy, Turner syndrome and other chromosomal disorders causing gonadal dysgenesis. • Start of natural menopause, or other age-related infertility. • Carriers of genetic diseases that cannot be treated by pre-implantation genetic diagnosis for monogenic/single gene defects (PGT-M). • Carriers of structural abnormalities that cannot be treated by pre-implantation genetic diagnosis for chromosomal structural rearrangements (PGT-SR). • Carriers of mitochondrial diseases.
Critical properties	• Maturation status of the oocytes is metaphase II (MII). • Absence of giant oocyte size.
Quality control requirements	• Oocytes vitrified 38-40 h post-ovulation induction when possible. • Morphological assessment of oocyte size and maturation status.
Storage and transport	• Temperature below – 140 °C.
Special labelling and accompanying information	• In the EU, oocyte samples originating from non-partner donation and transported to another tissue establishment for human application must be labelled with a full Single European Code (SEC). • Specific information not coded in the SEC must be included in accompanying documentation, for example: – Number of shipped samples – Type of medium used for storage, including batch information – Type of storage device – Number of oocytes per storage device – Instructions for warming. • In cases when the container is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the donor file that may have implications for usage.

27.2: Cryopreserved sperm for non-partner donation

Tissue/cell product	Cryopreserved sperm for non-partner donation
Definition	Cryopreserved human spermatozoa obtained by ejaculation, to be used in non-partner sperm donation for intra-uterine insemination or *in vitro* oocyte fertilisation. Processed by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled cooling rate down to a temperature of approximately – 100 °C and thereafter transferred to liquid nitrogen.
Established clinical indications	Couples or individuals in need of a non-partner sperm donation to achieve a pregnancy, either by intrauterine insemination or by *in vitro* fertilisation of oocytes. The most common indications are: • Azoospermia. • Fertilisation failure. • Absence of a male partner. • Carriers of genetic diseases that cannot be treated by pre-implantation genetic diagnosis for monogenic/single gene defects (PGT-M). • Carriers of structural abnormalities that cannot be treated by pre-implantation genetic diagnosis for chromosomal structural rearrangements (PGT-SR).
Critical properties	• Presence of post-thaw viable and motile sperm.
Quality control requirements	• The number of motile spermatozoa after test thawing must be adequate for the intended use: intra-uterine insemination (IUI), routine *in vitro* fertilisation (IVF) or microinjection (ICSI).
Storage and transport	• Cryopreserved sperm can be shipped in liquid nitrogen or on carbon dioxide ice.
Special labelling and accompanying information	• In the EU, sperm samples originated from non-partner donation and transported to another tissue establishment for human application must be labelled with a full Single European Code (SEC). • Specific information not coded in the SEC must be included in accompanying documentation, for example: – Number of shipped samples – Pre-cryopreservation sperm concentration and motility – Number of motile spermatozoa per device – Type of medium used for storage, including batch information – Type of storage device – Instructions for thawing. • In cases when the container is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered event and reactions in the donor file that may have implications for usage.

Part E. Good Practice Guidelines

Good Practice Guidelines for tissue establishments that follow EU Directives

Introduction

High-quality, safe and efficacious procedures for the donation, procurement, importation, testing, processing, preservation, storage and distribution of human tissues and cells for human application are essential for donors and recipients alike. Tissues and cells are health products of an exceptional nature, and all member states should endeavour to safeguard public health by promoting a high level of safety and quality in these substances when used for human application. This objective should be attained, maintained and continually optimised through the identification and implementation of key quality and safety criteria in relation to all the above-mentioned procedures in tissue establishments.

In the field of blood and blood components, Good Practice Guidelines (GPG) have been included in the *Guide for the preparation, use and quality assurance of blood components* since the 18th edition, published in 2015. In 2016, Directive 2005/62/EC was amended by Directive 2016/1214 to require EU member states to take into account the GPG jointly developed by the Commission and the European Directorate for the Quality of Medicines & Healthcare of the Council of Europe and published by the Council of Europe.

Following this approach, and aiming to promote and assure a high level of quality in the field of human tissues and cells, the European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM) took the decision to incorporate equivalent GPG in the 4th edition of the *Guide to the quality and safety of tissues and cells for human application*.

These guidelines do not introduce new requirements, but rather consolidate the guidance that is already defined in existing legislation and scientific guidelines. The GPG are therefore intended to elaborate on the basic requirements set out in the European Union Tissues and Cells Directives and to detail the key elements which should be defined and controlled within the quality system of tissue establishments that are required to comply with that directive. The GPG incorporate and amplify not only the associated recommendations from the main chapters of the *Guide to the quality and safety of tissues and cells for human application*, but also relevant elements from the detailed principles of Good Manufacturing Practice (GMP) as referred to in Article 47 of EU Directive 2001/83/EC, the results of relevant EU-funded projects and expert opinion consistent with current scientific knowledge.

The GPG should be seen as a complementary document for tissue establishments (and their inspectors or auditors) that describes in detail, and from a practical point of view, the key elements for achieving comprehensive quality management in a tissue establishment.

1. General principles

1.1. Tissue establishments

1.1.1. The term 'tissue establishment' (TE) became widely used in Europe following publication of EU Tissues and Cells Directive 2004/23/EC, which defines it as: 'a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells for human application are undertaken. It may also be responsible for procurement or testing of tissues and cells'.

1.1.2. In the field of medically assisted reproduction (MAR), the term 'TE' refers to the laboratories in MAR centres or clinics, but also to banks of gametes and embryos. These centres or clinics often include clinical units in which the patients are treated. In the context of these guidelines, the term 'TE' will be used to refer to all the banks, units, centres and clinics mentioned above.

1.2. EU tissues and cell legislation

1.2.1. The EU tissues and cells directives created a benchmark for the standards that must be met if carrying out any activity involving tissues and cells for human application, including gametes, embryos and germinal tissue. The directives also require that systems be put in place to ensure that all the tissues and cells used in human applications are traceable from donors to recipients and vice versa.

1.2.2. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 applies to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells intended for human application (including reproductive cells used in MAR procedures). It introduced obligations on EU member states and their authorities to supervise human tissue and cell procurement, to authorise and inspect TEs, to ensure traceability and vigilance and to maintain a publicly accessible register of national TEs. This directive also laid down the rules on donor selection and evaluation and on the quality and safety of tissues and cells (e.g. quality management, tissue and cells reception, processing and storage conditions).

1.2.3. Commission Directive 2006/17/EC established specific technical requirements for each step in the human tissue and cell preparation process, in particular the requirements for the procurement of human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for donors, tissue and/or cell donation, procurement and reception procedures at the TE and requirements for the direct distribution to the recipient of specific tissues and cells. Directive 2006/17/EC was amended in 2012 by Commission Directive 2012/17/EC about certain technical requirements for the testing of human tissues and cells.

1.2.4. Commission Directive 2006/86/EC includes traceability requirements, notification of serious adverse reactions and events (SAREs) as well as certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

1.2.5. In 2015, two new Commission Directives were adopted. Directive 2015/565 amended Directive 2006/86/EC, providing detailed requirements on the coding of human tissues and cells. Directive 2015/566 was an implementing directive on the procedures for verifying equivalent standards of quality and safety of imported tissues and cells.

1.3. Using this Guide

1.3.1. These guidelines are based on a quality management system (QMS) approach. They form the basis of good practice in all TEs and should be used in preparation for both inspection and continuous improvement.

1.3.2. For each topic, the guidelines aim to provide sufficient detail to make TEs aware of the essential matters which should be considered as a minimum in the context of a risk-based analysis which takes full account of the specific protocols and risk-mitigation strategies of each TE that are relevant to the risks associated with the processing, testing and implantation of the types of tissues and cells concerned.

2. Quality management system

2.1. General requirements

2.1.1. Quality management is a wide-ranging concept covering all matters that individually or collectively influence the quality of tissues and cells. It is the sum total of the organised arrangements made with the objective of ensuring that tissues are of the quality required for their intended use. Quality management therefore incorporates good practice.

2.1.2. Each TE must develop and maintain a quality system which facilitates meeting all the relevant minimum requirements identified in the EU tissues and cells directives and which is based on the principles of good practice, incorporating quality risk management and taking into account the relevant elements of EU GMP as in Directive 2001/83/EC.

2.1.3. Quality must be recognised as being the responsibility of all persons involved in the processes of the TE, with management ensuring a systematic approach towards quality and the implementation and maintenance of a quality system.

2.1.4. Attainment of this quality objective is the responsibility of senior management. It requires the participation and commitment both of staff, in many different departments and at all levels in the organisation, and of the organisation's suppliers and distributors. To achieve this quality objective reliably there must be a comprehensive, well-designed and correctly implemented quality system incorporating good practice and quality risk management.

2.1.5. Each actor in the supply chain should establish, document and fully implement a comprehensive and well-designed quality system to deliver quality assurance based on the principles of quality risk management by incorporating good practice and quality control.

2.1.6. The basic concepts of quality management, good practice and quality risk management are interrelated. They are described here in order to emphasise their relationships and their fundamental importance to the processing of tissues and cells.

2.2. Quality system

2.2.1. The quality system encompasses quality management, quality assurance, continuous quality improvement, personnel, premises and equipment, documentation, donation, procurement, testing and processing, storage, release for circulation including distribution, quality control, tissues and cells recall, external and internal auditing, contract management and self-inspection. The design of the system should incorporate appropriate risk-management principles including the use of appropriate tools.

2.2.2. The quality system must ensure that all critical processes are specified in appropriate procedures and/or instructions and are carried out in accordance with the standards and specifications of good practice and comply with appropriate regulations as set out in the chapters in this Guide.

2.2.3. The quality system must be designed to assure the quality and safety of processed tissues and cells, as well as to ensure donor and staff safety and end-user service. This strategy requires the development of clear policies, objectives and responsibilities. It also requires implementation, by means of quality planning, quality control, quality assurance and quality improvement, to ensure the quality and safety of tissues and cells, and to provide end-user satisfaction.

2.2.4. Senior management has the ultimate responsibility of ensuring that an effective quality system is in place and resourced adequately, and that roles and responsibilities are defined, communicated and implemented throughout the organisation. Senior management's leadership and active participation in the quality system is essential. This leadership should ensure the support and commitment to the quality system of staff at all levels and sites within the organisation.

2.2.5. Senior management should establish a quality policy that describes the overall intentions and direction of the TE in relation to quality. They should ensure quality system management and governance of good practice through reviews of the performance of the QMS at regular intervals so as to verify its effectiveness, and they should ensure continuous and systematic improvement of all processes affecting the quality and safety of tissues and cells and the quality system itself and introduce corrective measures if deemed necessary. Senior management is also in charge of regulatory supervision to ensure compliance with all legal and regulatory frameworks.

2.2.6. The quality system must be defined and documented. A quality manual or equivalent document should be established and contain a description of the quality system (including management responsibilities).

2.2.7. All TEs should be supported by an independent quality-assurance programme for fulfilling quality assurance. That programme must be involved in all quality-related matters, and must review and approve all appropriate quality-related documents.

2.2.8. All procedures, premises and equipment that have an influence on the quality and safety of tissues and cells (and tissues and cells components) must be validated or qualified before introduction and must be periodically re-validated or re-qualified, as determined on the basis of quality risk management and change management.

2.2.9. A general policy regarding qualification of facilities and equipment, as well as validation of processes, automated systems and laboratory tests, must be in place. The formal objective of qualification or validation is to ensure compliance with the intended use and regulatory requirements.

2.3. **Change control**

2.3.1. A formal change-control system must be in place to describe the actions to be taken to plan, evaluate and document any planned change which is proposed for the range and/or specifications of procured or processed tissues and cells, the processes, equipment, environment (or site), method of processing or testing or any other change that may affect the reproducibility of a process, the quality and safety of tissues and cells, or the health of donors or recipients/patients.

2.3.2. Change-control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a tissues or cells product of the desired quality, consistent with the approved specifications. Supporting data, e.g. copies of documents, should be reviewed to confirm that the impact of the change has been demonstrated prior to final approval.

2.3.3. The potential impact of a proposed change should be evaluated, and the degree of revalidation or additional testing, qualification and validation needed should be determined, based on the principles of quality risk management.

2.3.4. Changes should be authorised and approved by the Responsible Person(s) or relevant personnel in accordance with the TE's quality system.

2.3.5. After implementation of any change, an evaluation should be undertaken to confirm that the quality objectives were achieved and that there was no unintended deleterious impact.

2.3.6. Where temporary and time-limited changes are implemented, provisions should be in place to ensure and verify that the changes are reversed as appropriate.

2.3.7. All changes should be evaluated for any requirement of notification to, or approval from, a national Health Authority.

2.4. **Deviations**

2.4.1. Procedures must be in place for notifying the Responsible Person in a timely manner of any significant deviations, non-compliance with regulatory commitments (e.g. in submissions or responses to regulatory inspections), tissues and cells defects, or testing errors and related actions (e.g. recalls, regulatory actions). Adequate resources should be made available for the timely resolution of deviations.

2.4.2. The investigation of deviations must include an assessment of component impact, including a review and evaluation of relevant operational documentation and an assessment of deviations from specified procedures. An appropriate level of root cause analysis must be applied during the investigation of deviations. This can be determined using quality risk-management principles. In cases where the true root cause(s) of the issue cannot be determined, consideration should be given to identifying and addressing the most likely root cause(s).

2.4.3. An associated system for the implementation of corrective and preventive actions must be in place. Appropriate corrective and/or preventive actions (CAPAs) should be identified and taken in response to investigations, with a view to ensuring that existing quality problems are identified and corrected, and that recurrence of the problem is prevented. The need to consider a recall of tissues and cells or the need to quarantine materials should also be considered.

2.4.4. The effectiveness of CAPAs should be monitored and assessed, in line with quality risk-management principles.

2.4.5. The systems in place for the management of deviations should be linked as appropriate to the systems in place for the management of SAREs.

2.4.6. Where it is considered that a deviation or associated SARE may have the potential to impact another procurement organisation or TE, or an organisation responsible for human application (ORHA), the details of the deviation should be formally communicated to them so that they may undertake such investigations and actions as they may consider necessary.

2.4.7. Data relating to deviations should be routinely analysed to identify quality problems that may require corrective action or to identify trends that may require preventive action.

2.5. **Process quality review**

2.5.1. Regular process quality reviews should be conducted with the objective of verifying the consistency of the existing preparation process and the appropriateness of current specifications for all starting materials (including tissues and cells) and for processed tissues and cells, as well as highlighting trends and identifying improvements which

may be required. Such a review should normally be conducted annually, taking into account the conclusions of previous reviews; it should be documented and include all tissues and cells which are imported, exported or intended for use in the manufacture of other products. Quality reviews may be grouped by tissue/cell type, where scientifically justified.

2.5.2. A quality review of tissues and cells that are ready for circulation may also be considered as an instrument for surveying the overall quality status of tissues and cells processing, including procurement. It may include:

2.5.2.1. review of starting materials, including tissues and cells;

2.5.2.2. review of critical in-process controls;

2.5.2.3. review of results of quality control and quality monitoring;

2.5.2.4. review of all changes;

2.5.2.5. review of the qualification status of equipment;

2.5.2.6. review of technical agreements and contracts;

2.5.2.7. review of all significant deviations and the CAPAs implemented;

2.5.2.8. review of the findings of internal and external audits and inspections;

2.5.2.9. review of complaints and recalls;

2.5.2.10. review of donor-acceptance criteria;

2.5.2.11. review of donor deferrals;

2.5.2.12. review of look-back cases.

2.5.3. The results of process quality reviews should be evaluated, and an assessment should be made whether any CAPA or any revalidation should be undertaken. Reasons for any such CAPA should be documented. Agreed CAPAs should be completed in a timely and effective manner. There should be management procedures for the ongoing management and review of these actions, and the effectiveness of these procedures should be verified during self-inspection.

2.6. Good practice

2.6.1. Good practice is the part of quality management that ensures that tissues and cells are consistently processed and controlled according to quality standards appropriate to their intended use. Good practice is concerned with donation, procurement, processing, preservation, storage (all hereinafter included in the generic term 'preparation'), import, release for circulation (including distribution) and quality control. The basic requirements are as follows:

2.6.1.1. All processes are defined clearly and reviewed systematically in the light of experience and shown to be capable of consistently delivering tissues and cells of the required quality and complying with their specifications. This strategy includes ensuring that:

2.6.1.1.1. critical steps and significant changes to the process are validated;

2.6.1.1.2. all requirements are provided including:

2.6.1.1.2.1. appropriately qualified and trained personnel;

2.6.1.1.2.2. adequate premises and space;

2.6.1.1.2.3. suitable equipment and services;

2.6.1.1.2.4. correct materials, containers and labels;

2.6.1.1.2.5. approved procedures and instructions;

2.6.1.1.2.6. suitable storage and transport;

2.6.1.1.3. instructions and procedures are written in an instructional form in clear and unambiguous language, and are applicable specifically to the facilities provided;

2.6.1.1.4. operators are trained to carry out procedures correctly;

2.6.1.1.5. records are made, manually and/or by recording instruments, during preparation which demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality of the tissues and cells was as expected;

2.6.1.1.6. any significant deviations are fully recorded and investigated;

2.6.1.1.7. records of preparation processes, storage and release for circulation (including distribution) enable the complete history of the tissues and cells to be traced and these records are retained in a comprehensible and accessible form;

2.6.1.1.8. the release for circulation (including distribution) of the tissues and cells minimises any risk to their quality;

2.6.1.1.9. a system is available to recall any tissues and cells (including those processed using a batch of critical materials that have been distributed or issued);

2.6.1.1.10. complaints about tissues and cells are examined, the causes of quality defects are investigated and appropriate measures are taken in respect of the defective tissues and cells components to prevent reoccurrence.

2.6.1.2. Quality control is the part of good practice that is concerned with sampling, specifications and testing, as well as with the organisation, documentation and release procedures which ensure that materials are not released for use in processing, and tissues and cells are not released for circulation, including distribution, until their quality has been judged to be satisfactory and also that the necessary and relevant tests have been carried out. The basic requirements are:

2.6.1.2.1. adequate facilities, trained and qualified personnel and approved procedures are available for sampling, inspecting and/or testing starting materials (including tissues and cells), packaging materials, intermediate components and finished/ready-for-circulation tissues and cells, and also, if appropriate, for monitoring environmental conditions;

2.6.1.2.2. samples of starting materials (including tissues and cells), packaging materials, intermediate and processed tissues and cells are taken by approved personnel and methods;

2.6.1.2.3. test methods are validated;

2.6.1.2.4. records are made, manually and/or by recording instruments, which demonstrate that all the required sampling, inspecting and testing procedures were actually carried out. Any deviations are recorded and investigated fully;

2.6.1.2.5. the processed tissues and cells comply with the specifications and are correctly labelled;

2.6.1.2.6. records are made of the results of inspection and of the testing of materials and intermediate and processed tissues and cells. to show they have been formally assessed against specifications;

2.6.1.2.7. no tissues and cells are released for circulation, including distribution, that do not comply with the requirements of the relevant authorisations.

2.7. **Quality risk management**

2.7.1. A quality risk-management approach, consisting of a systematic process for the assessment, control, communication and review of risks to quality across the life-cycle of tissues and cells, should be applied. Appropriate statistical tools should be used (where appropriate) in the assessment of ongoing process capability.

2.7.2. It must be ensured that the risks inherent in the use and handling of biological material are identified and minimised, consistent with maintaining adequate quality and safety for the intended purpose of the tissues and cells (Directive 2006/86/EC Annex I A.5). Donor selection, and tissues and cells procurement, processing, storage and distribution activities, should therefore be subjected to a comprehensive risk assessment encompassing all the process steps with respect to the procedures, materials, tests, personnel, premises and equipment involved.

2.7.3. All components of the risk-management process should be linked to the authorised activities of the TE and all elements of the QMS should incorporate the principles of quality risk management.

2.7.4. Any evaluation of the risk to quality must be based on scientific knowledge, experience with the process and, ultimately, protection of the donor and recipient/patient.

2.7.5. The level of effort, formality and documentation of the quality risk-management process should be commensurate with the level of risk.

2.7.6. Risk assessments should be based on an analysis of the risks related to the application of the specific type(s) of tissues and cells and should be undertaken with the primary objective of identifying, where relevant, all those factors which could result in cross-contamination, contamination with adventitious agents, the transmission of disease or infectious agents, the transmission of inherited conditions or mix-ups, or which could render the tissues or cells clinically ineffective or harmful to the recipient/patient.

2.7.7. Such risks could, for example, derive from but are not limited to:

2.7.7.1. the sensitivity of donor screening protocols and tests;

2.7.7.2. procurement procedures;

2.7.7.3. biological properties of the procured tissues and cells;

2.7.7.4. the absence of standardised quality-control tests;

2.7.7.5. the use of potentially infective or known infective materials;

2.7.7.6. processing, storage and transport procedures and environment.

2.7.8. Risk assessments and associated management plans should identify and describe the principal activities of the TE and the circumstances to which the different phases of the plan apply.

2.7.9. Risk-mitigation strategies should be developed on the basis of prospective risk analysis in order to maximise the quality and safety of tissues and cells and to protect recipients/patients, personnel and the process itself, as well as other linked or proximal processes.

2.7.10. Risk management should serve as documentation of the rationale for key safety- or quality-related decisions, such as in the case of actions to be taken in relation to deviations and to determine the eligibility of impacted tissues and cells for clinical use.

2.7.11. All risk-assessment and risk-management plans should include documentation on:

2.7.11.1. the scope of, and circumstances for conducting, the assessment;

2.7.11.2. the individuals assigned to the work programme;

2.7.11.3. identification of the hazards associated with the scope/circumstances;

2.7.11.4. an estimate of their severity and probability of occurrence;

2.7.11.5. the risk analysis, evaluation and control measures for these hazards;

2.7.11.6. the scientific grounds for acceptance / rejection of the decision;

2.7.11.7. a rationale for the acceptability of the residual risk;

2.7.11.8. a statement of acceptance or otherwise of the residual risk.

2.7.12. Risk management should be used to support decision making regarding the specific qualification/validation activities that need to be performed. The associated risk assess-

ment should highlight the critical points in the processes, thus allowing the development of an appropriate validation plan.

2.7.13. Risk-management principles and methodologies should be incorporated into staff training programmes.

2.8. Exceptional release

2.8.1.1. In exceptional circumstances, an ORHA may agree with a TE, or a procurement organisation in the case of direct distribution, that tissues or cells which do not meet defined release criteria can be released for use in a specific recipient. Although directives 2004/23/EC, 2006/17/EC and 2006/86/EC lay down a number of specific requirements for performing a risk assessment when managing specified aspects of the quality and safety of tissues or cells for human use, nonetheless, in exceptional circumstances where any other defined requirements of the directives have not been complied with or cannot be complied with, and where clinical use of the impacted tissues and cells is required due to urgent medical need, the limited availability of alternative therapeutic options and the expected clinical benefit, a comprehensive documented risk assessment must be used to inform the decision of the Responsible Person as to whether the tissues or cells may be released for use.

2.8.1.2. In such circumstances, the physician treating the intended recipient should work with the nominated registered medical practitioner who advises on and oversees the medical activities of the TE, in conducting the risk assessment and a risk–benefit analysis for the intended recipient. All associated discussions and conclusions must be documented and the treating physician must sign their agreement with the exceptional release and their acceptance of any implied risk for the intended recipient.

2.8.1.3. In the case of microbiological contamination of autologous tissues and cells, or tissues and cells received from a specific allogenic donor whereby a repeated procurement cannot be conducted or involves a high degree of risk, the risk assessment and risk–benefit analysis must be based on the nature and extent of the contamination and must specifically consider the risk based on identification of the contaminating micro-organisms and the potential for adequate prophylaxis of the intended recipient.

2.9. Self-inspection, audits and improvements

2.9.1. Self-inspection or audit systems must be in place for all elements of operations to verify compliance with the standards. They must be carried out regularly by trained and competent persons, in an independent way, and according to approved procedures.

2.9.2. All results must be documented, and appropriate CAPAs must be implemented in a timely and effective manner.

3. Management of outsourced activities (contractual arrangements)

3.1. General principles of outsourcing

3.1.1. Outsourced activities that may impact on the quality and safety or efficacy of the tissues and cells must be correctly defined, agreed and controlled in order to avoid misunderstandings which could result in tissues and cells or work of unsatisfactory quality. There must be a written contract covering these activities, tissues and cells or processes to which they are related, and any technical arrangements made in connection with it.

3.1.2. All outsourced arrangements for tissue or cell procurement, processing and testing, including any proposed changes, must be done in accordance with a written contract, with reference to the specification for the tissues or cells concerned.

3.1.3. The responsibilities of each party must be clearly documented to ensure that the principles of good practice are maintained.

3.1.4. The contract giver is the establishment or institution that subcontracts particular work or services to a different institution and is responsible for setting up a contract defining the duties and responsibilities of each party.

3.1.5. The contract acceptor is the establishment or institution that performs particular work or services under a contract for a different institution.

3.2. The contract giver

3.2.1. The contract giver is responsible for assessing the competence of the contract acceptor to successfully carry out the work being outsourced and for ensuring, by means of the contract, that the principles and guidelines of good practice are followed.

3.2.2. The contract giver must provide the contract acceptor with all the information necessary to carry out the contracted operations correctly and in accordance with the specification and any other legal requirements. The contract giver must ensure that the contract acceptor is fully aware of any problems associated with the materials, samples or the contracted processes that might pose a hazard to the premises, equipment, personnel, other materials or other tissues or cells of the contract acceptor.

3.2.3. The contract giver must ensure that all tissues or cells, analytical results and materials delivered by the contract acceptor comply with their specifications and that they have been released under a quality system approved by the Responsible Person or other authorised person.

3.3. The contract acceptor

3.3.1. The contract acceptor must have adequate premises, equipment, knowledge, experience and competent personnel to satisfactorily carry out the work requested by the contract giver.

3.3.2. The contract acceptor must ensure that all products, materials or test results delivered by the contract giver are suitable for their intended purpose.

3.3.3. The contract acceptor must not pass to a third party any of the work entrusted under the contract without the contract giver's prior evaluation and approval of the arrangements. Arrangements made between the contract acceptor and any third party must ensure that the relevant information is made available in the same way as between the original contract giver and contract acceptor.

3.3.4. The contract acceptor must refrain from any activity that may adversely affect the quality of the tissues or cells processed and/or analysed for the contract giver.

3.4. **The contract**

3.4.1. A contract must be drawn up between the contract giver and the contract acceptor that specifies their respective responsibilities relating to the contracted operations. All arrangements for tissues or cells procurement, processing and testing must be in compliance with the requirements of good practice and with regulatory requirements, and must be agreed by both parties.

3.4.2. The contract must specify the procedure, including the necessary evidence to be provided by the contract acceptor, by which the Responsible Person or other authorised person releasing the tissues or cells can ensure that each component has been processed and/or distributed in compliance with the requirements of good practice and regulatory requirements.

3.4.3. The contract must clearly describe who is responsible for purchasing materials, for testing and releasing materials, for undertaking tissues or cells procurement and for processing and testing (including in-process controls). In the case of subcontracted analyses, the contract must state the arrangements for the collection/procurement of samples, and the contract acceptor must agree that they can be subject to inspections by the Health Authorities.

3.4.4. Processing and distribution records, including reference samples if relevant, must be kept by, or be available to, the contract giver. Any records relevant to assessment of the quality of the tissues or cells in the event of complaints or a suspected defect must be accessible and specified in the defect/recall procedures of the contract giver.

The contract must permit the contract giver to audit the facilities of the contract acceptor.

4. Personnel and organisation

4.1. Personnel must be available in sufficient numbers and with the necessary qualifications and experience to carry out the activities related to the procurement, testing, processing, storage and release for circulation (including distribution) of tissues and cells for human application and they must be trained and assessed as competent to perform their tasks.

4.2. Management has the ultimate responsibility to determine and provide adequate and appropriate resources (human, financial, materials, facilities and equipment) to implement and maintain the QMS and continually improve its suitability and effectiveness through participation in management reviews. The responsibilities placed on any one individual should not be so extensive as to present any risk to quality.

4.3. There should be an organisation chart in which the relationships between key personnel are clearly shown in the managerial hierarchy.

4.4. All personnel should have up-to-date job descriptions, which clearly set out their tasks and responsibilities. The correct understanding of responsibilities by individuals should be assessed and recorded.

4.5. Personnel in responsible positions should have adequate authority to carry out their responsibilities. Their duties may be delegated to designated deputies of a satisfactory qualification level. There should be no gaps or unexplained overlaps in the responsibilities of those personnel concerned with the application of good practice.

4.6. Traceability must be ensured to personnel performing critical processing tasks or approving critical results or documents. If such personnel sign paper documents, whether regularly or occasionally, personnel signature lists should be available.

4.7. All personnel must receive initial and continued training appropriate to their specific tasks. Training records must be maintained. Training programmes must be in place and must include the principles of good practice.

4.8. Training should be provided for all personnel whose duties take them into processing areas or into laboratories (including the technical, maintenance and cleaning personnel).

4.9. There should be written policies and procedures to describe the approach to training, including a record of training that has taken place, its contents and an assessment of its effectiveness.

4.10. Personnel must be provided with initial/basic training, updated training as required when procedures change or when scientific knowledge develops, and adequate opportunities for relevant professional development. The training programme must ensure and document that each individual:

4.10.1. has demonstrated competence in the performance of their designated tasks;

4.10.2. has an adequate knowledge and understanding of the scientific/technical processes and principles relevant to their designated tasks;

4.10.3. understands the organisational framework, quality system and health and safety rules of the establishment in which they work; and

4.10.4. is adequately informed of the broader ethical, legal and regulatory context of their work.

4.11. The contents of training programmes must be periodically assessed, and the competence of personnel evaluated regularly.

4.12. Only personnel who are authorised by defined procedures and documented as such may be involved in the procurement, processing, testing and distribution processes, including quality control and quality assurance.

4.13. There must be written safety and hygiene instructions in place, adapted to the activities to be carried out.

4.14. It is the organisation's responsibility to provide instructions on hygiene and health conditions that may be relevant to the quality of tissues and cells (e.g. during procurement) and to ensure that staff report any relevant health problems.

4.15. Steps should be taken to ensure as far as is practicable that no person affected by an infectious disease or having open lesions on the exposed surface of their body is engaged in the processing of tissues and cells. Medical examinations should be carried out when necessary to assure fitness for work and personal health. There should be instructions ensuring that health conditions that can be of relevance to the quality of tissues and cells are reported by personnel.

4.16. Visitors or untrained personnel should, preferably, not be taken into the procurement, processing and laboratory areas. If this is unavoidable, they should be given information in advance, particularly about personal hygiene and the prescribed protective clothing. They should be closely supervised.

4.17. Eating, drinking, chewing or smoking, or the storage of food, drink, smoking materials or personal medication in the processing, testing and storage areas should be prohibited. In general, any unhygienic practice within the processing areas or in any other area where the tissues or cells might be adversely affected should be forbidden.

4.18. There should be a written policy outlining the requirements for wearing protective garments in the different areas. The requirements should be appropriate to the activities to be carried out.

4.19. Personnel must be trained in the gowning requirements appropriate to various area classifications. The competence of personnel working in Grade A/B areas to comply with the gowning requirements must be reassessed at least annually.

4.20. Every person entering the processing areas should wear clean clothing suitable for the processing activity with which they are involved and this clothing should be changed when appropriate. Additional protective garments appropriate to the operations to be carried out (e.g. head, face, hand and/or arm coverings) should be worn when necessary. Jewellery and make-up must be removed before entering a clean room.

4.21. The clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way as to protect the operator and tissues and cells from the risk of contamination.

4.22. The description of clothing required for clean areas is as follows:

- **Grade D**: Hair and, where relevant, beard and moustache should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.
- **Grade C**: Hair and where relevant beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.
- **Grade A/B**: Sterile headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a sterile face mask should be worn to prevent the shedding of droplets and particles. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser-legs should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

4.23. Outdoor clothing should not be brought into changing rooms leading to Grade B and C rooms. For every worker in a Grade A/B area, clean (sterilised) protective garments (including face masks) should be provided every time there is an entry into the clean area; the need to exit and re-enter the clean area for a different processing step/different batch should be determined by the risk of the activity. Gloves should be regularly disinfected during operations. Upon exit from a clean area there should be a visual check of the integrity of the garment.

4.24. Clean area clothing should be cleaned and handled in such a way that it does not gather additional contaminants which can later be shed. When working in a contained area, protective clothing should be discarded before leaving the contained area

4.25. Personnel working in clean areas must be given specific training on aseptic processing, including the basic aspects of microbiology.

4.26. Particular attention must be given to the qualification of the aseptic technique of personnel working in Grade A environments with Grade B backgrounds. Prior to participating in routine aseptic processing operations, personnel should be qualified through participation in successful process-simulation tests. The usual approach is to conduct simulated processes using culture medium in place of, or added to, tissues or cells.

4.27. Microbial monitoring of personnel working in A/B areas should be performed after critical operations and when leaving the A/B area. A system of disqualification of personnel should be established based on the results of the monitoring programme, as well as other parameters that may be relevant. Once disqualified, retraining/requalification is required before the operator can be involved in aseptic operations. It is advised that the retraining/requalification includes participation in a successful process-simulation test.

5. Premises

5.1. General requirements

5.1.1. Premises must be suitable for carrying out the intended procedures in order to prevent errors (e.g. mix-ups, contamination, cross-contamination or improper labelling of tissues and cells).

5.1.2. Environmental conditions such as lighting, temperature, humidity and ventilation should be appropriate and controlled to assure safety and comfort to patients, donors and personnel, and to assure the accurate functioning of equipment during processing and storage.

5.1.3. There must be adequate equipment and materials for the activities performed.

5.1.4. Premises must be secure to prevent the entrance of unauthorised people and should not be used as if by right as a routeway by personnel who do not work there.

5.1.5. Facilities should have an appropriate design to permit ease of maintenance and cleaning. Cleaning and sanitation must be performed on a regular basis and documented. The efficacy of the methods used must be validated and monitored.

5.1.6. A written safety manual and personal protective equipment must be available to minimise the risks to the health of personnel and visitors.

5.1.7. All waste generated by the facilities must be disposed of in accordance with applicable laws and regulations.

5.2. Donor area

5.2.1. There must be a suitable space for the confidential interviews of living donors or the relatives of deceased donors, and for physical examination of the donor.

5.3. Procurement area

5.3.1. The facility must be of adequate size to allow proper operations and ensure donor privacy and anonymity.

5.3.2. Facilities in which tissues or cells are procured must meet appropriate grades of air quality and cleanliness where applicable. The appropriate standard of cleanliness will

depend on the type of tissues or cells being procured, the degree of exposure of the tissues or cells during the procurement process, and the decontamination or sterilisation processes that will subsequently be applied to the tissues or cells during processing.

5.3.3. The procurement facility should be divided into different areas of adequate size to prevent improper labelling and packaging, mix-ups or cross-contamination of tissues or cells.

5.3.4. In cases of tissue/cell donation from living donors (except tissues which are surgical by-products) tissues and cells procurement must be carried out in an area appropriately equipped for the initial treatment of donors experiencing adverse reactions associated with the donation. Access to an intensive care unit and or emergency service must be available, where applicable.

5.4. **Processing area**

5.4.1. The adequacy of the processing and in-process storage areas should permit the orderly and logical positioning of equipment and materials so as to minimise the risk of cross-contamination and to minimise the risk of errors or omission or wrong application of any of the processing or control steps.

5.4.2. Processing of tissues and cells that are exposed to the environment, but are not subject to a subsequent microbial inactivation process, must take place in an environment with specified air quality with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current EU GMP Annex I and Directive 2003/94/EC and with a background environment appropriate for the processing of the tissues and cells concerned, but at least equivalent to GMP Grade D in terms of particles and microbial counts.

5.4.2.1. While Grade D is specified as the minimum background environment, the actual background environment which is utilised must be selected and justified on the basis of an evaluation of the risks associated with the processing, testing and implantation of the types of tissues and cells concerned. Some national requirements may specify Grade C or B backgrounds for certain processes or types of tissue or cell.

5.4.2.2. A less stringent processing environment than Grade A with background D may be acceptable where:

5.4.2.2.1. a validated microbial inactivation or validated terminal sterilisation process is applied, or

5.4.2.2.2. where it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissue or cell, or

5.4.2.2.3. where it is demonstrated that the mode and route of application of the tissue or cell to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with cell and tissue transplantation, or

5.4.2.2.4. where it is not technically possible to carry out the required process in a Grade A environment.

5.4.2.3. The risk assessment for determination of the processing environment must consider potentially significant factors, including :

5.4.2.3.1. tissue or cell contamination during open *versus* closed processing;

5.4.2.3.2. effectiveness of the processing method to remove contaminants;

5.4.2.3.3. suboptimal detection of contaminants due to the sampling method;

5.4.2.3.4. transfer of contaminants at transplantation.

5.4.2.4. The associated guidelines on environmental monitoring, relevant to the determined classification for the processing environment, should be considered at least in the context of a risk-based analysis which takes full account of the specific protocols and risk-mitigation strategies relevant to the risks associated with the processing, testing and implantation of the types of tissues and cells concerned.

5.4.3. Clean rooms and laminar flow cabinets must be classified, re-qualified and monitored in accordance with EN ISO14644 and EU GMP Annex 1.

5.4.4. Critical facility parameters identified to be a risk to the tissues and cells, such as temperature, humidity, air-supply conditions, pressure differentials, particle numbers and microbial contamination, must be checked, monitored and recorded.

5.4.5. Environmental monitoring programs are an important tool by which the effectiveness of contamination-control measures can be assessed. The environmental monitoring program should include an assessment of non-viable and viable contamination and air-pressure differentials.

5.4.6. The monitoring locations should be determined having regard to the risks (e.g. at locations posing the highest risk of contamination) and the results obtained during the qualification of the premises.

5.4.7. The number of samples, volume, frequency of monitoring, alert and action limits should be appropriate, taking into account the risks and the overall control strategy for the establishment. Sampling methods should not pose a risk of contamination to the processing activities.

5.4.8. **Non-viable particulate monitoring**

5.4.8.1. Airborne particle monitoring systems should be established to obtain data for assessing potential contamination risks and to ensure maintenance of the designated environment in the clean room. Environmental monitoring is also expected for isolators and biosafety cabinets.

5.4.8.2. The degree of environmental control of non-viable particulates and the selection of the monitoring system should be adapted to the specific risks of tissues and cells and

of the preparation process/processing (e.g. live organisms). The frequency, sampling volume or duration, alert limits and corrective actions should be established case by case having regard to the risks. It is not necessary for the sample volume to be the same as that used for qualification of the clean room.

5.4.8.3. Appropriate alert and actions limits should be defined. With a view to identifying potential changes that may be detrimental to the process, the alert limits for grades B to D should be lower than those specified as action limits and should be based on the area performance.

5.4.8.4. The monitoring system should ensure that when alert limits are exceeded, the event is rapidly identified (e.g. alarm settings). If action limits are exceeded, appropriate corrective actions should be taken. These should be documented.

5.4.8.5. The maximum permitted particle concentrations in accordance with Annex 1 of EU GMP are shown in the table here:

Grade	Maximum number of particles equal to or greater than 0.5 µm		
	At rest (per m^3)	In operation (per m^3)	ISO classification (at rest/in operation)
A	3 520	3 520	5/5
B	3 520	352 000	5/7
C	352 000	3 520 000	7/8
D	3 520 000	Not defined	8

5.4.8.6. When the risk assessment (see 5.4.2.3) concludes that the most stringent air quality standard is required to achieve an acceptable level of risk, then normally Grade A in full compliance with GMP for aseptic processing (i.e. with a Grade B background, accessed via grades C and D) should be applied. This implies that particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly, except where duly justified (e.g. contaminants in the process that would damage the particle counter, production of particles by a process itself, e.g. bone cutting or grinding, or when this would present a hazard to the tissues or cells). In such cases, monitoring during equipment set-up operations should take place (i.e. prior to exposure of the tissues and cells to the hazard). For this most stringent standard, monitoring should also be performed during simulated operations.

5.4.8.7. For Grade B areas, there should be particle monitoring during critical operations, within the limitation referred to in 5.4.8.6, albeit the monitoring does not need to cover the entire duration of the critical processing. The Grade B area should be monitored at an appropriate frequency and with suitable sample size to permit the detection of changes in levels of contamination.

5.4.8.8. The monitoring strategy for grades C and D should be set having regard to the risks and in particular the nature of the operations conducted.

5.4.8.9. When there are no critical operations ongoing (i.e. at rest), sampling at appropriate intervals should be conducted. While at rest, the heating, ventilating and air-conditioning (HVAC) system should not be interrupted, as this may trigger the need for requalification. In the event of an interruption, a risk assessment should be conducted to determine any actions that may be required, taking account of the activities performed in the affected areas (e.g. additional monitoring).

5.4.8.10. While not required for qualification purposes, the monitoring of the ≥ 5.0 µm particle concentration in Grade A and B areas is required for routine monitoring purposes as it is an important diagnostic tool for early detection of failures. While the occasional indication of ≥ 5.0 µm particle counts may be false counts, consecutive or regular counting of low levels is an indicator of a possible contamination and it should be investigated. Such events may, for example, be indicative of early failure of the HVAC system or filling equipment failure, or it may be diagnostic of poor practices during machine set-up and routine operation.

5.4.9. **Viable particle monitoring**

5.4.9.1. Checks to detect the presence of specific micro-organisms in the clean room (e.g. yeast, moulds) should be performed as appropriate. Viable particle monitoring is also expected for isolators and biosafety cabinets.

5.4.9.2. Where aseptic operations are performed, monitoring should be frequent, using methods such as settle plates, volumetric air and surface sampling (e.g. swabs and contact plates). Rapid microbial monitoring methods should be considered and may be adopted after validation of the premises.

5.4.9.3. Continuous monitoring is required during critical operations where the tissues and cells are exposed to the environment. Surfaces and personnel should be monitored after critical operations. Critical operations should be identified by risk assessment.

5.4.9.4. The following recommended maximum limits apply for microbiological monitoring of clean areas in accordance with Annex 1 of EU GMP:

Grade	Air sample CFU/per m^3	Settle plates (diameter 90 mm) CFU/4 hours*	Contact plates (diameter 55 mm) cfu/plate
A†	< 1	< 1	< 1
B	10	5	5
C	100	50	25
D	200	100	50

* Individual settle plates may be exposed for less than 4 hours. Where settle plates are exposed for less than 4 hours the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 hours.

† It should be noted that for a Grade A the expected result should be 0 cfu recovered; any recovery of 1 cfu or greater should result in an investigation.

5.4.9.5. Appropriate alert and actions limits should be defined. With a view to identifying potential changes that may be detrimental to the process, the alert limits for grades B to

D should be lower than those specified as action limits and should be based on the characteristics of the work area.

5.4.9.6. If micro-organisms are detected in a Grade A area, they should be identified to at least genus level and the impact thereof on tissues and cells quality and on the suitability of the premises for the intended operations should be assessed.

5.4.10. **Air pressure**

5.4.10.1. An essential part of contamination prevention is the adequate separation of areas of operation. To maintain air quality, it is important to achieve a proper airflow from areas of higher cleanliness to adjacent less clean areas. It is fundamental for rooms of higher air cleanliness to have a substantial positive pressure differential relative to adjacent rooms of lower air cleanliness. For clean rooms these pressure cascades should be clearly defined and continuously monitored with appropriate methods (e.g. alarm settings). Adjacent rooms of different grades should have a pressure differential of 10-15 Pa (guidance values).

5.4.10.2. Negative pressure may be required in specific areas for containment reasons (e.g. handling of viral-positive material). In such cases, the negative pressure areas should be surrounded by a positive pressure clean area of appropriate grade.

5.5. **Storage areas**

5.5.1. Storage rooms must be located in a secure area and access must be limited to authorised personnel.

5.5.2. Storage areas should be of appropriate size to allow orderly storage of materials and reagents, and of tissues and cells.

5.5.3. Dedicated areas must be available for storing tissues and cells in quarantine, and/or for storing unqualified materials.

5.5.4. Storage areas for tissues and cells should be maintained within defined temperature limits. Where special storage conditions are required (e.g. temperature, humidity) these must be checked, monitored and recorded.

5.5.5. An alarm system should be in place to alert users in a timely manner to any deviation from the pre-defined storage conditions. Alarm systems placed in storage devices must be continuously active and able to alert personnel on a 24-hour basis.

5.5.6. Provisions must be in place in the event of equipment or power failure.

5.5.7. Oxygen sensors must be appropriately placed and personal protection equipment must be available in areas where liquid nitrogen is present.

5.6. **Ancillary areas**

5.6.1. Suitable facilities for changing clothes and for washing hands should be readily accessible.

5.6.2. Staff rest and refreshment areas should be separate from other rooms.

5.6.3. Archive store and administrative areas should be protected against unauthorised access to ensure that records and documents are maintained in a confidential manner as required by applicable laws and regulations.

6. Equipment and materials

6.1. General requirements

6.1.1. TEs must have equipment and materials appropriate to the activities for which they are authorised.

6.1.2. All critical equipment must be designed, located, qualified, calibrated and maintained to suit its intended purpose and must comply with the general safety requirements of this Guide and the specific requirements relevant to the type(s) of tissues and cells.

6.1.3. Equipment with an appropriate range and precision for measuring, weighing, recording and control should be available, and should be calibrated and checked at defined intervals using appropriate methods.

6.1.4. All critical equipment must be identified and qualified, regularly inspected and preventively maintained in accordance with the manufacturers' instructions. The qualification and maintenance status of each item of equipment must be available.

6.1.5. Where equipment or materials affect critical processing or storage parameters (e.g. temperature, pressure, particle counts, microbial contamination levels), they must be identified and must be the subject of appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times.

6.1.6. A temperature monitoring system should be utilised to document temperatures and to alert staff when temperatures have deviated from acceptable limits. Procedures should be in place for reviewing temperatures. If storage utilises liquid nitrogen, either liquid nitrogen levels or temperature should be monitored and documented at an interval specified in the standard operating procedures (SOPs) and determined by validation.

6.1.7. Procedures for the operation of each piece of critical equipment, detailing the action to be taken in the event of malfunctions or failure, must be available and appropriate records must be kept.

6.1.8. The services that could impact on the tissues/cells quality (i.e. compressed air, heating, ventilating and air conditioning) should be qualified and scheduled in a maintenance programme.

6.1.9. Equipment must be selected to minimise any hazard to donors, personnel or tissues and cells.

6.1.10. All validated processes must use qualified equipment. For any equipment used in validated processes, qualification results must be documented, regular maintenance and calibration must be carried out and documented according to established procedures, and the qualification and maintenance status of each item of equipment must be available.

6.1.11. Records of maintenance activities should be clear and comprehensible and should detail the specific activities performed as part of maintenance.

6.1.12. All modifications, enhancements or additions to qualified systems and equipment must be managed through the change-control procedure of the TE. The effect of each change to the system or equipment, as well as its impact on quality and safety, must be determined to identify the extent of revalidation required.

6.1.13. Instructions for use, maintenance, servicing, cleaning, disinfection and sanitation must be available. These activities must be performed regularly and recorded accordingly.

6.1.14. Repair and maintenance operations should not present any hazard to the donor or staff, or to the quality of the tissues and cells or tissues and cells components.

6.1.15. Equipment should be designed or selected so that it can be thoroughly cleaned and, where necessary, decontaminated. This should be performed according to detailed and written procedures. It should be stored only in a clean and dry condition.

6.1.16. Washing/cleaning solutions and equipment should be chosen and used so that they are not sources of contamination or toxicity.

6.1.17. Equipment should be installed in such a way as to prevent any risk of error or of contamination.

6.1.18. Fixed pipework should be clearly labelled to indicate the contents and, where applicable, the direction of flow.

6.1.19. Distilled, deionised and, where appropriate, other water pipes should be sanitised according to written procedures that detail the action limits for microbiological contamination and the measures to be taken.

6.2. **Calibration and monitoring of equipment**

6.2.1. There should be a calibration plan that defines the principles that decide which equipment needs to be calibrated and the frequency of recalibration.

6.2.2. All equipment with a critical measuring function must be calibrated against a traceable standard if available. Adequate records of such tests should be maintained, including the values obtained prior to any adjustment. Calibration reports should include the range of uncertainty of the calibration method and its traceability to a national standard. The

report and/or calibration certificate must be reviewed and signed to show acceptance of the document. Any failed calibrations require investigation of the potential impact.

6.2.3. Observation of trends and analyses of calibration and monitoring results should be a continuous process. Intervals of calibration and monitoring should be determined for each item of equipment to achieve and maintain a desired level of accuracy and quality. The calibration and monitoring procedure should be based on a recognised international standard. The calibration status of all equipment that requires calibration should be readily available.

6.2.4. To ensure appropriate performance of a system or equipment, a monitoring plan should be developed and implemented. The plan should take into account the criticality of the system or equipment, and should specify monitoring, user-notification and problem-resolution mechanisms. When appropriate, equipment should be subject to continuous monitoring linked to an alarm system. If an unusual event is observed, personnel should follow the standard response described in the monitoring plan. The standard response should involve the notification of affected personnel and, where appropriate, initiation of a resolution response to the problem and risk assessment of the affected tissues and cells. Depending on the severity of the problem and the criticality of the system or equipment, a back-up plan should be in place and be implemented to keep the process or system operating.

6.2.5. Assessment of the performance of equipment should occur in the following situations:

6.2.5.1. upon commissioning of new equipment, which must include the design, installation, and operational and performance qualifications;

6.2.5.2. after any relocation, repairs or adjustments that might potentially alter the functioning of equipment;

6.2.5.3. after any major repair or modification, when the equipment should be checked and validated before its release;

6.2.5.4. if ever a doubt arises that the equipment is not functioning appropriately.

6.2.6. The ability of a supplier to maintain its activities relating to a system or equipment must be requalified on a regular basis; notably to anticipate weaknesses in services or to manage changes in the system, equipment or supplier. The periodicity and detail of the requalification process should be linked to the level of risk of using the system or equipment and should be planned for each supplier.

6.2.7. Defective equipment should be labelled clearly as such and, if possible, removed from processing areas.

6.3. **Data-processing systems**

6.3.1. If computerised systems are used, software, hardware and back-up procedures should be validated/qualified before use, be checked regularly to ensure reliability and be main-

tained in a validated/qualified state. Hardware and software must be protected against unauthorised use or unauthorised changes. The back-up procedure must prevent loss of or damage to data at expected and unexpected downtimes or function failures.

6.3.2. Systems must be properly maintained at all times. Documented maintenance plans must be developed and implemented.

6.3.3. Changes in computerised systems must be validated; applicable documentation must be revised, and relevant personnel trained appropriately, before any critical change is introduced into routine use. Computerised systems must be maintained in a validated /qualified state. This must include user testing to demonstrate that the system is correctly performing all specified functions, both at initial installation and after any system modifications.

6.3.4. There must be a hierarchy of permitted user access to enter, amend, read or print data. Methods of preventing unauthorised entry must be in place, such as personal identity codes or passwords that are changed regularly.

6.3.5. All necessary measures must be taken to ensure protection of data. These measures must ensure that safeguards against unauthorised additions, deletions or modifications of data and transfer of information are in place, to resolve data discrepancies and to prevent unauthorised disclosure of such information.

6.3.6. Computer systems designed to control decisions related to inventories and release of tissues and cells should prevent the release of all tissues and cells considered not acceptable for release. Preventing release of any tissues and cells from a future donation from a deferred donor should be possible.

6.4. Equipment and materials for procurement and processing and storage

6.4.1. Critical equipment and materials should meet documented requirements and specifications and, when applicable, Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC and Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. In this context, 'critical' means those items of equipment and materials that come in contact with the tissues or cells or influence the critical quality/safety attributes of the tissues and cells directly (e.g. an additive) or indirectly (e.g. donor testing kits).

6.4.2. Procurement, processing and storage equipment must be managed in accordance with the standards and specifications laid down in the directives and with due regard to relevant national and international regulation, standards and guidelines covering the sterilisation of medicines and medical devices. Qualified, sterile instruments and procurement devices must be used for tissue and cell procurement. The appropriate equip-

ment and instruments should be used, in order to guarantee the quality of the specific recovered tissue.

6.4.3. Wherever possible, only Conformité Européenne (CE) marked medical devices should be used, and all relevant staff must have received appropriate training on the use of such devices.

6.4.3.1. Devices manufactured within a health institution, and lacking CE marking, may be used within the institution only if they comply with the requirements in Regulation (EU) No. 2017/746, Article 5, 5.

6.4.4. Sterile instruments and devices must be used for tissue and cell procurement. Instruments or devices must be of good quality, validated or specifically certified and regularly maintained for the procurement of tissues and cells.

6.4.5. When reusable instruments must be used, a validated cleaning and sterilisation procedure for removal of infectious agents should be in place.

6.4.6. Materials and parts of equipment that come into contact with tissues and cells must not be reactive, additive or absorptive to such an extent that they affect the quality of the tissues and cells and thus present any hazard.

6.4.7. A written SOP must be in place to regulate the specific materials that come into contact with tissues and cells during processing, the addition of therapeutic products to tissues and cells, the choice of those media and products, their characteristics, their source and control and the associated requirements for asepsis and labelling. A procedure to select the materials must be in place.

6.4.8. A controlled list should be constructed of all materials that come into contact with the tissues or cells or that influence the quality or safety of the tissues or cells. Detailed specifications for such critical reagents and consumables must be documented.

6.4.9. Only materials from qualified suppliers that meet the documented specifications should be used.

6.4.10 All changes to equipment and materials should be managed in accordance with the principles of change control.

6.4.11. Documented systems for purchasing equipment and materials should be available. These should identify the specific requirements for establishing and reviewing contracts for the supply of both equipment and materials.

6.4.12. The contracting process should include:

6.4.12.1. checks prior to awarding the contract to help ensure that suppliers meet the organisation's needs;

6.4.12.2. appropriate checks on received goods to confirm that they meet specifications;

6.4.12.3. the requirement for manufacturers to provide a certificate of analysis for critical material;

6.4.12.4. checks to ensure that goods in use continue to meet specifications;

6.4.12.5. regular contact with suppliers to help understand and resolve problems;

6.4.12.6. performance of periodic risk-based audits.

6.4.13. Specifications for the material in contact with tissues and cell should include, if applicable:

6.4.13.1. a description of the materials, including the designated name and the internal code reference; the reference, if any, to a pharmacopoeia monograph; the approved suppliers and, if possible, the original producer of the products; a specimen of printed materials;

6.4.13.2. directions for sampling and testing or reference to procedures;

6.4.13.3. qualitative and quantitative requirements with acceptance limits;

6.4.13.4. storage conditions and precautions;

6.4.13.5. the maximum period of storage before re-examination.

6.4.14. When using processing media and/or added therapeutic products, their source, lot number and expiration date must be recorded in the relevant processing documentation.

6.4.15. Materials used during the processing of tissues and cells should be verified as being:

6.4.15.1. free of viral contamination (certificate should be available);

6.4.15.2. free of transmissible spongiform encephalopathy (TSE) contamination (certificate should be available);

6.4.15.3. produced under GMP conditions when available;

6.4.15.4. suitable for human use when available;

6.4.15.5. of defined identity, purity, sterility and quantification of endotoxins;

6.4.15.6. free of human/animal origin reagents if possible;

6.4.15.7. free of antibiotics whenever possible;

6.4.15.8. with quantified final residues of reagents whenever possible;

6.4.15.9. with risk assessment of potential residues in the final tissues and cells.

6.4.16. The specifications of the materials used to perform any evaluation of the donor should be described and these materials should not have any negative impact on the maintenance of the donors and reliability of the evaluation results.

6.4.17. Only materials from approved suppliers that meet their documented requirements and specifications must be used. Batch acceptance testing or checking of each delivery of materials should be carried out, and documented, before release for use in tissue or cell procurement or processing. Critical materials must be released by a person qualified to perform this task.

6.4.18. Manufacturers of sterile materials should provide a certificate of release for each batch. The TE should define acceptance criteria for such certificates in writing, and should include at least the name of the material, manufacturer, compliance with relevant requirements (e.g. pharmacopoeias or regulations for medical devices) and confirmation that the materials are sterile and pyrogen-free as appropriate.

6.4.19. All incoming materials should be checked to ensure that the consignment corresponds to the order.

6.4.20. The status of materials (quarantined, released, rejected) should be indicated clearly.

6.4.21. Materials must be stored under the conditions established by the manufacturer and in an orderly manner that permits segregation by status, batch and lot as well as stock rotation.

6.4.22. Storage and use of materials should follow the 'first-in first-out' principle (i.e. the material that entered storage first should be used first) taking into account the expiry date of materials.

7. Qualification and validation

7.1. General principles

7.1.1. TEs should establish a system to document evidence that provides a high degree of assurance that a specific process, piece of equipment or environment will consistently produce processed tissues and cells meeting pre-determined specifications and quality attributes.

7.1.2. All critical equipment and technical devices must be identified and qualified.

7.1.3. All critical processing procedures must be validated and must not render the tissues and cells clinically ineffective or be harmful to the recipient.

7.1.4. TEs should identify what validation work is needed to prove control of the critical aspects of their particular processes.

7.1.5. Any significant changes to the facilities, equipment or processes that might affect the quality of the tissues and cells should be qualified/validated, and authorised by a Health Authority if applicable.

7.1.6. A risk-assessment approach should be used to determine the scope and extent of validation/qualification. Such risk assessment should take into account all the equipment (e.g. autoclave, incubator, freeze dryer), facilities (e.g. clean rooms, laminar flow module), electronic systems (e.g. clean rooms environmental monitoring system, tissues processing system) and processes (e.g. musculoskeletal processing, skin processing, clean

rooms disinfection, tissue transport, analytical methods) which may affect the quality of processed tissues and cells.

7.1.7. The results from the risk-assessment study of the scope of validation/qualification activities within a TE should be covered in a Validation Master Plan.

7.2. **Documentation**

7.2.1. As a minimum, the Validation Master Plan should consist of:

7.2.1.1. description of the TE;

7.2.1.2. list of equipment, facilities, electronic systems and processes that need to be qualified or validated;

7.2.1.3. current state of validation/qualification of each element within the scope of the Plan;

7.2.1.4. validation/qualification programme;

7.2.1.5. validation/qualification activities and who is responsible;

7.2.1.6. procedures related to validation/qualification activities;

7.2.1.7. criteria for requalification or revalidation.

7.2.2. The activities of qualification or validation should be described in a protocol containing at least:

7.2.2.1. the objective(s);

7.2.2.2. scope of the activities;

7.2.2.3. who is responsible;

7.2.2.4. any related documents;

7.2.2.5. the stages of qualification or validation;

7.2.2.6. the acceptance criteria.

7.2.3. A validation/qualification report should be issued reflecting the results of the activities containing at least:

7.2.3.1. the objective(s);

7.2.3.2. scope of the activity;

7.2.3.3. who was responsible;

7.2.3.4. any related documents;

7.2.3.5. any deviations from the protocol;

7.2.3.6. detailed results;

7.2.3.7. conclusions.

7.3. Facility, system and equipment qualification

7.3.1. Qualification for new facilities, systems and equipment

7.3.1.1. The qualification of new facilities, systems or equipment begins with Design Qualification (DQ) and progresses successively through Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ).

7.3.1.1.1. DQ is the documented verification that the proposed design of the facilities, equipment or system is suitable for the intended purpose. During DQ the compliance of the design with good practice should be demonstrated and documented.

7.3.1.1.2. IQ is the documented verification that the equipment or system, as installed or modified, complies with the approved design, the manufacturer's recommendations and/or user requirements. IQ should be performed on all critical facilities, systems and equipment. The IQ protocol should include, but need not be limited to, the following:

7.3.1.1.2.1. verification that all facilities and equipment comply with the requirements of the purchase order;

7.3.1.1.2.2. verification of CE-approval where required;

7.3.1.1.2.3. verification that the location and environmental conditions of the equipment/installation are correct according to the manufacturer's recommendations and internal specifications;

7.3.1.1.2.4. verification that items are installed in accordance with internal specifications and identified correctly with the manufacturer;

7.3.1.1.2.5. verification of serial numbers of all items/parts;

7.3.1.1.2.6. verification that all parts of the equipment are free from defects;

7.3.1.1.2.7. verification that the connections of electricity, water, steam, pressure, vacuum, etc. are functional and that their operating ranges are appropriate to the proper functioning of the installation;

7.3.1.1.2.8. identification of the items that require calibration, with checks of appropriate calibration certificates and documentation of the programme and procedure for periodic calibration;

7.3.1.1.2.9. verification of the existence of instructions for performing preventive maintenance.

7.3.1.1.3. OQ is the documented verification that the equipment or system, as installed or modified, performs as intended throughout the anticipated operating ranges. OQ should follow successful completion of IQ. The OQ protocol should include, but need not be limited to, the following:

7.3.1.1.3.1. tests that have been developed from knowledge of processes, systems and equipment;

7.3.1.1.3.2. tests to include a condition or a set of conditions encompassing upper and lower operating limits, sometimes referred to as 'worst case' conditions;

7.3.1.1.3.3. identification of critical operating variables, tests performed, alarms, security devices and acceptance criteria;

7.3.1.1.3.4. verification that the operation of various items of equipment/installation that are connected to the mains and put into operation is correct.

7.3.1.1.4. The completion of a successful OQ should allow the finalisation of calibration, operating and cleaning procedures, operator training and preventive maintenance requirements. It should permit a formal 'release' of the facilities, systems and equipment.

7.3.1.1.5. PQ is the documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly, based on the approved process method and specifications. PQ should follow successful completion of IQ and OQ. Although PQ is described as a separate activity, it may in some cases be appropriate to perform it in conjunction with OQ, or concurrently with processing activities. The PQ protocol should include, but need not be limited to, the following:

7.3.1.1.5.1. tests, using materials, qualified substitutes or simulated tissues and cells, that have been developed from knowledge of the process and the facilities, systems or equipment;

7.3.1.1.5.2. tests that include a condition or set of conditions encompassing upper and lower operating limits;

7.3.1.1.5.3. a process description or reference to protocol development;

7.3.1.1.5.4. a list of equipment involved;

7.3.1.1.5.5. the critical parameters and operating ranges;

7.3.1.1.5.6. a description of the tests to be performed, or control variables, sample taking, time and reference method sampling and analytical methods;

7.3.1.1.5.7. the acceptance criteria.

7.3.2. **Qualification of established (in-use) facilities, systems and equipment**

7.3.2.1. Evidence should be available to support and verify the operating parameters and limits for the critical variables of the operating equipment.

7.3.2.2. The procedures and records for calibration, cleaning, preventive maintenance, operating and operator training of the facilities, systems and/or equipment in use should be documented.

7.3.3. **Qualification of clean rooms**

7.3.3.1. Clean rooms and laminar flow cabinets must be classified, requalified and monitored in accordance with the requirements of EU GMP Annex 1 and the relevant requirements of ISO. The associated tests to be carried out for clean rooms should include at least:

7.3.3.1.1. air change (renewal) rate per hour within one room checked against specification;

7.3.3.1.2. airflow-visualisation test;

7.3.3.1.3. absolute filters integrity – the grade of sealing of the filters and the absence of leaks in the filter material checked;

7.3.3.1.4. total count of airborne particles (viable or not) be checked according to specifications;

7.3.3.1.5. temperature and relative humidity recorded during the test and checked according to specifications;

7.3.3.1.6. pressure differential between areas checked according to specifications;

7.3.3.1.7. recovery test (normally tested for Grade A and B clean rooms), i.e. check the time required for a clean room to recover the specified classification after an out-of-specification period;

7.3.3.1.8. laminar flow velocities in laminar flow areas;

7.3.3.1.9. HVAC system operations and alarms;

7.3.3.1.10. electricity back-up systems.

7.3.3.2. All these tests should be performed at least in an 'at rest' situation. The particle counting test should also be performed in an 'in operation' situation.

7.3.4. **Qualification of laminar flow hoods**

7.3.4.1. The tests to be carried out for laminar flow hoods should include:

7.3.4.1.1. speed and uniformity of the air, i.e. check that the average speed meets the specified acceptance criteria and that there is uniformity;

7.3.4.1.2. absolute filters integrity – check the grade of sealing of the filters and the absence of leaks in the filter material;

7.3.4.1.3. particle counting – check the total count of airborne particles (viable or not) according to specifications;

7.3.4.1.4. electronic test – check all the operating controls (light, ultra-violet light, fan) and alarms;

7.3.4.1.5. airflow visualisation (for biological safety cabinets), where the test objective is to study the behaviour of air inside and outside the cabin with the help of a smoke generator.

7.3.4.2. All these tests should be performed at least in an 'at rest' situation; the particle-counting test should also be performed in an 'in operation' situation.

7.4. **Process validation**

7.4.1. Facilities, systems and equipment to be used should be qualified before use, and analytical testing methods should be validated.

7.4.2. Processes that have been in use for some time should also be validated.

7.4.3. Staff taking part in the validation work should have been appropriately trained.

7.4.4. All facilities, systems, equipment and processes should be periodically evaluated to verify that they are still operating in a valid manner.

7.4.5. **Prospective validation**

7.4.5.1. Process validation should normally be completed prior to the distribution of any tissue or cell; this is called prospective validation.

7.4.5.2. Prospective validation should include, but need not be limited to, the following:

7.4.5.2.1. short description of the process;

7.4.5.2.2. summary of the critical processing steps to be investigated;

7.4.5.2.3. list of the equipment/facilities to be used (including measuring, monitoring and/or recording equipment) together with its calibration status;

7.4.5.2.4. specifications for release of the processed tissues and cells;

7.4.5.2.5. list of analytical methods, as appropriate;

7.4.5.2.6. proposed in-process controls with acceptance criteria;

7.4.5.2.7. additional testing to be carried out, with acceptance criteria and analytical validation, as appropriate;

7.4.5.2.8. sampling plan;

7.4.5.2.9. methods for recording and evaluating results;

7.4.5.2.10. teams/departments and persons responsible;

7.4.5.2.11. proposed timetable.

7.4.5.3. Using this defined process (including specified components) of prospective validation, a series of batches of the final tissues or cells may be produced under routine conditions.

7.4.5.4. The number of process runs carried out and observations made should be sufficient to allow the normal extent of variation and trends to be established and to provide sufficient data for evaluation. Three consecutive batches/runs all within the finally agreed parameters are generally considered to constitute validation of the process.

7.4.5.5. Batches, where applicable, made for process validation should be the same size as the routine scale batches.

7.4.6. **Concurrent validation**

7.4.6.1. In exceptional circumstances it may be acceptable not to complete a validation programme before routine processing starts and instead to validate a process during routine processing (concurrent validation). The decision to carry out concurrent validation must be justified, documented and approved by authorised personnel.

7.4.6.2. Documentation requirements for concurrent validation are the same as specified for prospective validation.

7.4.7. **Retrospective validation**

7.4.7.1. Retrospective validation is only acceptable for well-established processes. Validation of such processes should be based on historical data. The steps involved require preparation of a specific protocol and reporting of the results of the data review, leading to a conclusion and a recommendation.

7.4.7.2. Retrospective validation is not appropriate where there have been recent changes in relation to starting materials, the tissues or cells, operating procedures or equipment.

7.4.7.3. The source of data for this validation should include, but need not be limited to:

7.4.7.3.1. batch processing and packaging records;

7.4.7.3.2. process-control charts;

7.4.7.3.3. maintenance log books;

7.4.7.3.4. records of personnel changes;

7.4.7.3.5. process-capability studies;

7.4.7.3.6. processed tissues and cells data;

7.4.7.3.7. storage-stability results.

7.4.7.4. Batches selected for retrospective validation should be representative of all batches made during the review period, including any batches that failed to meet specifications, and should be sufficient in number to demonstrate process consistency.

7.4.7.5. Additional testing of retained samples may be needed to obtain the necessary amount or type of data to retrospectively validate the process.

7.4.7.6. Retrospective validation generally requires that data from ten to thirty consecutive batches be examined to assess process consistency, but fewer batches may be examined if this can be justified; the justification must be documented.

7.4.8. **Process validation for aseptic processing in Grade A areas with Grade B background**

7.4.8.1. The validation of aseptic processing should include a process-simulation test, which is the performance of the manufacturing process using a sterile microbiological growth medium and/or placebo (e.g. culture media of cells which are demonstrated to support the growth of bacteria) to test whether the defined procedures are adequate to prevent contamination during processing. Results and conclusions should be recorded. The process-simulation test should follow as closely as possible the routine preparation process and it should be conducted in the same locations where the processing occurs. The process simulation should focus on all operations carried out by personnel

involving open process steps. All potential interventions and challenges to the process should be considered.

7.4.8.2. An appropriate simulated model using alternative materials may be acceptable provided that this is duly justified.

7.4.8.3. Alternative approaches may also be developed for steps that take a long time. The simulation of reduced times for certain activities (e.g. centrifugation, incubation) should be justified having regard to the risks. In some cases, it may also be acceptable to split the process into key stages which are simulated separately, provided that all transitions between stages are also evaluated. When a closed system is used for the processing of tissues and cells, the process simulation should focus on the steps related to the connections to the closed system.

7.4.8.4. In cases of processing of various types of tissues and cells, consideration can be given to the matrix and/or bracketing approach. Under a bracketing approach, only samples at the extremes of certain design factors would undergo a full process simulation. This approach can be accepted if the handling of different tissues and cells is similar (same equipment and processing steps). Under a matrix approach, it may be possible to combine process simulations for different tissues and cells sharing similar processing steps, provided that the worst case is covered by the matrix approach. The use of bracketing and matrixing together should be duly justified.

7.4.8.5. Filled containers should be inverted to ensure that the medium/placebo touches all parts of the container/closure and should then be incubated. The selection of the incubation duration and temperature should be justified and appropriate for the process being simulated and the selected medium/placebo.

7.4.8.6. All contaminants from the filled containers should be identified. The results should be assessed, in particular in relation to the overall quality of the processed tissues and cells and the suitability of the preparation process. The target should be zero growth. Any growth detected should be investigated. If the growth detected is indicative of potential systemic failure, the potential impact on tissues and cells processed since the last successful simulation test of this type should be assessed, and adequate corrective and preventive actions should be taken.

7.4.8.7. Process-simulation tests intended to support initial validation should be performed with three consecutive satisfactory simulation tests per preparation process.

7.4.8.8. Process simulation (one run) should be repeated periodically to provide ongoing assurance of the ability of the process and the staff to ensuring aseptic manufacturing. The frequency should be determined on the basis of a risk assessment but should generally not be less than once every six months (for each preparation process).

7.4.8.9. In the case of infrequent processing (i.e. if the interval between the processing activities is more than six months), it is acceptable that the process-simulation test is done

just before the next scheduled processing activity, provided that the results of the process-simulation test are available prior to the start of processing. Nevertheless, in cases of long periods of inactivity (i.e. over one year), the validation prior to restart of processing should be done with three runs.

7.4.8.10. When considering the frequency of the simulation test, the establishment is required to consider also the relevance of the simulation test for the training of personnel and their ability to operate in an aseptic environment.

7.4.8.11. A process simulation should also be conducted in cases where there is any significant change to the process (e.g. modification of HVAC system or equipment). In such cases, three runs are required.

7.5. **Cleaning and disinfection validation**

7.5.1. Cleaning and disinfection validation should be performed in order to confirm the effectiveness of a cleaning or disinfection procedure.

7.5.2. Cleaning or disinfection intervals and methods should be determined.

7.5.3. The rationale for selecting the limits for carry-over of tissues and cells residues, cleaning agents and microbial contamination should be logically based on the materials involved. The limits should be achievable and verifiable.

7.5.4. Residues of tissues and cells or cleansing agents should be checked based on risk assessment. Validated analytical methods having sensitivity to detect residues or contaminants should be used. The detection limit for each analytical method should be sufficiently sensitive to detect the established acceptable level of the residue or contaminant.

7.5.5. Normally only cleaning or disinfection procedures for tissues and cells contact surfaces of the equipment need to be validated. Consideration should be given to non-contact parts.

7.5.6. The intervals between use and cleaning/disinfection and between cleaning/disinfection and reuse should be validated.

7.5.7. For cleaning and disinfection procedures for tissues and cells and processes which are similar, it is considered acceptable to select a representative range of similar tissues and cells and processes. A single validation study utilising a 'worst case' approach can be carried out which takes account of the critical issues.

7.5.8. Typically, three consecutive applications of the cleaning or disinfection procedure should be performed and shown to be successful in order to prove that the method is validated.

7.5.9. 'Test until clean' is not considered an appropriate alternative to cleaning validation.

7.5.10. Products which simulate the physicochemical properties of the substances to be removed may exceptionally be used instead of the substances themselves, where such substances are either toxic or hazardous.

7.6. Revalidation

7.6.1. Revalidation should be performed when there is a change, in any equipment, facilities or process, that is considered significant because it affects the quality of the tissues and cells. These changes should be approved through a change-control procedure.

7.6.2. When the tissues and cells quality review confirms that the system or process is consistently producing material meeting its specifications, there is no need for revalidation.

8. Donation

8.1. General requirements

8.1.1. All necessary measures should be taken to ensure that any promotion and publicity activities in support of the donation of human tissues and cells comply with guidelines or legislative provisions laid down by the member states and the associated restrictions or prohibitions on advertising the need for, or availability of, human tissues and cells with a view to offering or seeking financial gain or comparable advantage.

8.1.2. The donation of tissues and cells must be voluntary and unpaid. No financial gain, or inducement, or any other compensation should be given to the living donor or the deceased donor's family. In the case of unrelated living donors, an allowance to cover any costs incurred should be acceptable if justifiable and transparent. Member states must define the conditions under which compensation may be granted.

8.1.3. Any extra medical costs related to the donation process of tissues or cells (e.g. serological or bacteriological testing) must not be charged to the donor or to a deceased donor's family. These costs must be met by the TE.

8.1.4. The activities related to tissue and cell procurement must be carried out in such a way as to ensure that donor evaluation and selection is carried out in accordance with the requirements referred to in Article 28(d) and (e) of Directive 2004/23/EC.

8.1.5. In the case of an autologous donation, the suitability criteria must be established in accordance with the requirements referred to in Article 28(d) of Directive 2004/23/EC.

8.1.6. The results of the donor evaluation and testing procedures must be documented, and any major anomalies must be reported in accordance with the requirements referred to in the Annex of Directive 2004/23/EC.

8.1.7. TEs must take all necessary measures to ensure that tissue and cell procurement, packaging and transportation comply with the requirements referred to in Article 28(b), (e)

and (f) of Directive 2004/23/EC. The tests required for donors must be carried out by a laboratory accredited, designated, authorised or licensed by the Health Authority or authorities.

8.1.8. TEs must implement a system for the identification of human tissues and cells, in order to ensure the traceability of all human tissues and cells.

8.1.9. The procurement of human tissues or cells must be authorised only after all mandatory consent or authorisation requirements in force in the member state concerned have been met.

8.1.10. TEs must, in keeping with the national legislation, take all necessary measures to ensure that donors, their relatives or any persons granting authorisation on behalf of the donors are provided with all appropriate information as referred to in the Annex of Directive 2004/23/EC.

8.1.11. Before the procurement of tissues and cells proceeds, an authorised person must confirm and record:

8.1.11.1. that consent for the procurement has been obtained in accordance with Article 13 of Directive 2004/23/EC;

8.1.11.2. how and by whom the donor has been reliably identified.

8.2. **Donor recruitment/referral**

8.2.1. Procedures for recruiting living donors in an ethical manner, for ensuring their safety and well-being and for the identification and referral of all potential deceased donors should be implemented and maintained in accordance with the applicable legislation.

8.2.2. Professionals involved in the recruitment of living donors, and in the identification and referral of deceased donors must be trained and appropriately qualified.

8.2.3. In the case of both living and deceased donors, screening must be performed to exclude any contraindications to donation.

8.2.4. A coding system must be in place, with unique codes physically attached and recorded in documents and/or electronic data, to guarantee traceability and biovigilance at all stages from donor screening until tissue and cell application (e.g. the donor is identified with a wrist band and/or different labels attached to the body). A donation code must be applied to all tissues procured. The donation code must be traceable to the donor code.

8.2.5. The coding system must be designed to relate all transplants of a certain donor to a unique donor number in order to guarantee traceability and biovigilance from donor screening until tissue transplantation.

8.2.6. A potential donor should receive a donor identification number/donor code before any further procedures are started. All documentation and/or electronic data that are

collected from the donor should be traceable to this number. All body materials (e.g. blood, tissue, fluid) that are procured from this donor must be given a donation number, traceable to the donor identity number/donor code.

8.2.7. The method of verifying the donor's identity should be described in an identification procedure. This procedure should be followed before starting the procurement and should enable the identity of the donor to be established beyond any doubt. The verification should be performed based on at least two independent factors like date of birth and name, or name and hospital patient number.

8.2.8. The source of the donor's identity should be documented. For living donors this should include officially recognised means of identification such as identity cards, passports, etc. For deceased donors, the presence of toe tags, wrist bands or other confirmation of the deceased's identity should be noted. At least two independent forms of identification, such as name, date of birth, address or hospital number, must be used to verify a deceased donor's identity.

8.2.9. **Living donors**

8.2.9.1. Recruitment of donors must be voluntary and unpaid, and informed consent must be obtained in advance.

8.2.9.2. Recruitment of non-partner donors in MAR, whether performed by public health system or by private clinics (where allowed by national legislation), must be authorised by the Health Authority, and the donation of reproductive material must strictly follow the same principles of voluntary and unpaid donation.

8.2.9.3. Recruitment of persons not able to consent should never be done through public registries. In addition, in some countries, specific regulations restrict donation in these circumstances (e.g. some countries do not allow procurement of peripheral blood progenitor cells from minors and/or administration of growth factors).

8.2.10. **Deceased donors**

8.2.10.1. Identification and referral of deceased tissue donors must be in compliance with the national deceased donation programme.

8.2.10.2. A system must be established between the procurement organisation and the corresponding TE to ensure that any deceased individual who is a potential donor can be detected within an adequate period of time to allow an effective donation.

8.3. **Donor consent**

8.3.1. Procedures or protocols for expressing consent to donation, depending on the type of donor, the specific circumstances and the different legal systems for consent, must be implemented and maintained.

8.3.2. There should be an authorised person who confirms and records that consent for the procurement has been obtained in accordance with both Article 13 of Directive 2004/23/EC and the legislation in place in the member state.

8.3.3. Consent should be recorded and/or documented in the donor/patient's record.

8.3.3.1. Informed consent must be obtained for living donors. The informed consent must include an explanation, in understandable terms, of all the reasonable risk and potential harm, both for the donor and recipient, as well as all the tests to be performed.

8.3.3.2. Informed consent must be obtained from all donors for the use of their tissue and/or cell for specific purposes and for serological testing.

8.3.3.3. The person in charge of the donation process must ensure that the donor has been properly informed of at least those aspects relating to the donation and procurement process outlined in point 8.3.3.5. This information must be given prior to the procurement.

8.3.3.4. The information must be given by a trained and appropriately qualified person able to transmit it in an appropriate and clear manner, using terms that are easily understood by the donor.

8.3.3.5. The information must cover: the purpose and nature of the procurement, its consequences and risks; analytical tests, if they are performed; the recording and protection of donor data and medical confidentiality; the therapeutic purpose and potential benefits of the donation; and information on the applicable safeguards intended to protect the donor.

8.3.3.6. The donor must be informed that he/she has the right to receive the confirmed results of the analytical tests, clearly explained.

8.3.3.7. Information must be given on the necessity for obtaining the applicable mandatory consent, certification and authorisation in order that the tissue and/or cell procurement can be carried out.

8.3.4. Tissue or cell procurement must not be carried out on a person who does not have the capacity to consent, other than as specified in point 8.3.4.1.

8.3.4.1. In the case of a donor who is a minor or a donor with no legal capacity, the consent must be obtained from parents or the legal representative, provided the following conditions are met:

8.3.4.1.1. there is no compatible donor available who has the capacity to consent;

8.3.4.1.2. the recipient is a brother or sister of the donor;

8.3.4.1.3. the donation has the potential to be life-saving for the recipient;

8.3.4.1.4. the authorisation of the donor's representative, or of an authority or a person or body provided for by law, has been given specifically and in writing and with the approval of the competent body;

8.3.4.1.5. the potential donor concerned does not object.

8.3.5. In the case of discarded tissue or surgical by-product, the donor must be made aware that he or she can express any intention as to how he or she desires such surgical residues to be dealt with, or else the surgical residues must be handled by the healthcare institution as it deems fit.

8.3.6. All information must be given and all necessary consents and authorisations must be obtained in accordance with the legislation in force in member states.

8.3.6.1. The confirmed results of the donor's evaluation must be communicated and clearly explained to the relevant persons in accordance with the legislation in member states.

8.3.6.2. In cases where a legal process applies, judicial consent must be obtained according to local regulations before starting any procurement activities. When judicial authorisation is needed due to an unknown cause of death, the transplant co-ordinator should be the person responsible to ask consent from the judge on call and in charge of the investigation. Procurement should only be carried out if it does not affect the judicial autopsy.

8.3.7. The request for donation must be explained in understandable terms by a healthcare professional familiar with the donation process.

8.3.7.1. The discussion about consent should be conducted in a suitable environment. The person who requests the consent of the donor should have received specific training for this purpose.

8.3.7.2. The use of tissue from a deceased donor must be in accordance with relevant national and international legislation.

8.4. **Donor selection and evaluation**

8.4.1. Procedures for donor selection and evaluation should be implemented and maintained. They should take place before each procurement and comply with the requirements referred to above.

8.4.2. Selection criteria for donors should be based on an analysis of the risks related to the application of the specific cells/tissues. Indicators of these risks must be identified by review of the medical and behavioural history, biological testing, physical examination (for living donors) or *post mortem* examination (for deceased donors) and any other appropriate investigation.

8.4.3. The TE or procurement organisation must ensure that donors comply with the selection criteria set out in the annexes to Directive 2006/17/EC:

8.4.3.1. Annex I for donors of tissues and cells, except donors of reproductive cells;

8.4.3.2. Annex III for donors of reproductive cells.

8.4.4. The TE or procurement organisation must ensure that:

8.4.4.1. donors of tissues and cells, except donors of reproductive cells, undergo the biological tests set out in point 1 of Annex II of Directive 2006/17/EC;

8.4.4.2. the tests referred to in point 8.4.4.1. are carried out in compliance with the general requirements set out in point 2 of Annex II of Directive 2006/17/EC;

8.4.4.3. donors of reproductive cells undergo the biological tests set out in points 1, 2 and 3 of Annex III of Directive 2006/17/EC;

8.4.4.4. the tests referred to in point 8.4.4.3 above are carried out in compliance with the general requirements set out in point 4 of Annex III of Directive 2006/17/EC.

8.4.5. In the case of living donors, the health professional responsible for obtaining the health and social history must ensure that the donor has:

8.4.5.1. understood the information provided;

8.4.5.2. had an opportunity to ask questions and been provided with satisfactory responses;

8.4.5.3. confirmed that all the information provided is true to the best of his/her knowledge.

8.4.6. An authorised person must collect and record the donor's relevant medical and behavioural information according to the requirements described in section 1.4 of Annex IV of Directive 2006/17/EC.

8.4.7. In order to acquire the appropriate information, different relevant sources must be used, including at least an interview with the donor, for living donors, and the following when appropriate:

8.4.7.1. the medical records of the donor;

8.4.7.2. an interview with a person who knew the donor well, for deceased donors;

8.4.7.3. an interview with the treating physician;

8.4.7.4. an interview with the general practitioner;

8.4.7.5. the autopsy report, for deceased donors.

8.4.8. In the case of a deceased donor, and in the case of a living donor when justified, a physical examination of the body must be performed to detect any signs that may be sufficient in themselves to exclude the donor or which must be assessed in the light of the donor's medical and personal history.

8.4.9. The complete donor records must be reviewed and assessed for the donor's suitability and signed by a qualified health professional.

8.4.10. All donor data must be recorded and kept for 30 years after the use of the donated tissue and cells or after their utilisation. Data must be protected from unauthorised viewing.

8.4.11. The donor selection and evaluation process must be performed by trained and appropriately qualified personnel in accordance with SOPs, and described in detail in records. A set of authorised SOPs should define responsibilities and describe how procedures should be carried out and by whom.

8.4.12. The following list of actions for donor selection and evaluation must be conducted and verified in accordance with point 8.4.11:

8.4.12.1. donor identification;

8.4.12.2. donor/donor family consent details;

8.4.12.3. donor's medical history (including genetic disease, a family history of disease, exclusion criteria, additional exclusion criteria for deceased child donors);

8.4.12.4. donor's social history (including personal, travel, behavioural, risk assessment);

8.4.12.5. donor's physical examination (including exclusion signs);

8.4.12.6. donor's psychological examination for living haematopoietic progenitor cell (HPC) donors and medically assisted reproduction donors;

8.4.12.7. blood sample procurement;

8.4.12.8. evaluation of test results for markers of transmissible disease;

8.4.12.9. final decision making about eligibility.

8.4.13. In the case of living donors, a face-to-face interview must be conducted, during which a standardised questionnaire should be completed. The interviews should be done, documented and signed by a trained and appropriately qualified person. For deceased donors, alternative sources of information should be used.

8.4.14. **Living donors**

8.4.14.1. *Autologous living donor*

8.4.14.1.1. If the removed tissues and cells are to be stored or cultured, the same minimum set of biological testing requirements must apply as for an allogeneic living donor.

8.4.14.1.2. Positive test results will not necessarily prevent the tissues or cells or any product derived from them being stored, processed and reimplanted, if appropriate isolated storage facilities are available to ensure no risk of cross-contamination with other grafts and/or no risk of contamination with adventitious agents and/or mix-ups.

8.4.14.2. *Allogeneic living donor (except donors of reproductive cells)*

8.4.14.2.1. Allogeneic living donors must be selected on the basis of their health and medical history, an interview by a qualified and trained healthcare professional with the donor, and clinical investigations and tests in compliance with point 8.4.14.2.3. This assessment must include relevant factors that may assist in identifying and screening out persons

whose donation could present a health risk to others, such as the possibility of transmitting diseases or health risks to themselves.

8.4.14.2.2. For any donation, the procurement process must not interfere with or compromise the health or care of the donor. In the case of cord blood or amniotic membrane donation, this applies to both mother and baby.

8.4.14.2.3. Selection criteria for allogeneic living donors must be established and documented by the TE (and the transplanting clinician in the case of direct distribution to the recipient), based on the specific tissue or cells to be donated, together with the donor's physical status and medical and behavioural history and the results of clinical investigations and laboratory tests establishing the donor's state of health.

8.4.14.2.4. The same exclusion criteria must be applied as for deceased donors with the exception of factors related to an unknown cause of death. Depending on the tissue or cell to be donated, other specific exclusion criteria may need to be added, such as:

8.4.14.2.4.1. in the case of HPC, the potential for transmission of inherited conditions.

8.4.14.3. *Donor of reproductive cells*

8.4.14.3.1. Donor-selection criteria and laboratory testing do not need to be applied in the case of partner donation of reproductive cells for direct use.

8.4.14.3.2. Reproductive cells that are processed and/or stored and reproductive cells that will result in the cryopreservation of embryos must meet the following criteria:

8.4.14.3.2.1. the clinician responsible for the donor must determine and document, based on the patient's medical history and therapeutic indications, the justification for the donation and its safety for the recipient and any child(ren) that might result;

8.4.14.3.2.2. biological tests must be carried out to assess the risk of cross-contamination in compliance with the general requirements set out in point 2 of Annex III of Directive 2006/17/EC;

8.4.14.3.2.3. positive results will not necessarily prevent partner donation in accordance with national rules.

8.4.14.3.3. In the case of donations of reproductive cells other than by partners, donor-selection criteria and laboratory testing must be applied in accordance with Annex III, point 3 of Directive 2006/17/EC.

8.4.14.3.3.1. Donors must be selected on the basis of their age, health and medical history, provided on a questionnaire, and through a personal interview performed by a qualified and trained healthcare professional.

8.4.14.3.3.2. This assessment must include relevant factors that may assist in identifying and screening out persons whose donation could present a health risk to others, such as the possibility of transmitting diseases (such as sexually transmitted infections), or

health risks to themselves (e.g. superovulation, sedation or the risks associated with the egg-procurement procedure or the psychological consequences of being a donor).

8.4.14.3.3.3. Complete information on the associated risk and on the measures undertaken for its mitigation must be communicated and clearly explained to the recipient.

8.4.15. **Deceased donor**

8.4.15.1. National and local requirements for confirmation of death must be complied with before tissue procurement begins.

8.4.15.2. In the case of deceased donors, the cause, time and circumstances of death must be verified and recorded. The sources of information used for deceased donors must be reviewed. Transferring information from donation records to a new document should be carried out by trained and appropriately qualified staff from the TE.

8.4.15.3. Donor age criteria should be established, documented and recorded.

8.4.15.4. The person responsible for confirming the donor's identity must do this by means of at least three factors, such as age, race, date of birth or medical history number.

8.4.15.5. *General criteria for exclusion*

8.4.15.5.1. Cause of death unknown, unless autopsy provides information on the cause of death after procurement and none of the general criteria for exclusion set out in the present section applies.

8.4.15.5.2. History of a disease of unknown aetiology.

8.4.15.5.3. Presence, or previous history, of malignant disease, except for primary basal cell carcinoma, carcinoma *in situ* of the uterine cervix, and some primary tumours of the central nervous system that have to be evaluated according to scientific evidence and must be considered as part of the risk assessment in decision making. Donors with malignant diseases can be evaluated and considered for cornea donation, except for those with retinoblastoma, haematological neoplasm and malignant tumours of the anterior segment of the eye. Malignant melanoma with known metastatic disease also excludes use of ocular tissue, including avascular cornea. Any vascularised ocular tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and should be evaluated as discussed above.

8.4.15.5.4. Risk of transmission of diseases caused by prions. This risk applies, for example, to:

8.4.15.5.4.1. people diagnosed with Creutzfeldt–Jakob disease, variant Creutzfeldt–Jacob disease, or other prion disease, or having a family history of non-iatrogenic Creutzfeldt–Jakob disease;

8.4.15.5.4.2. people with a history of rapid progressive dementia or degenerative neurological disease, including those of unknown origin;

8.4.15.5.4.3. recipients of hormones derived from the human pituitary gland (such as growth hormones) and recipients of grafts of cornea, sclera and *dura mater*, and persons that have undergone undocumented neurosurgery (where *dura mater* may have been used).

8.4.15.5.5. Systemic infection which is not controlled at the time of donation, including bacterial diseases, systemic viral, fungal or parasitic infections, or significant local infection in the tissues and cells to be donated. Donors with bacterial septicaemia may be evaluated and considered for eye donation but only where the corneas are to be stored by organ culture to allow detection of any bacterial contamination of the tissue.

8.4.15.5.6. History, clinical evidence or laboratory evidence of HIV, acute or chronic hepatitis B (HBV), except in the case of persons with a proven immune status, hepatitis C (HCV) and human T-lymphotrophic virus (HTLV) I/II, including evidence of transmission risk or evidence of risk factors for these infections.

8.4.15.5.7. History of chronic, systemic autoimmune disease that could have a detrimental effect on the quality of the tissue to be retrieved.

8.4.15.5.8. Indications that test results of donor blood samples will be invalid due to:

8.4.15.5.8.1. the occurrence of haemodilution, according to the specifications in Annex II, section 2 of Directive 2006/17/EC, where a pre-transfusion sample is not available;

8.4.15.5.8.2. treatment with immunosuppressive agents;

8.4.15.5.9. Evidence of any other risk factors for transmissible diseases on the basis of a risk assessment, taking into consideration donor travel and exposure history and local infectious disease prevalence;

8.4.15.5.10. Presence on the donor's body of physical signs implying a risk of transmissible disease(s) as described in Annex IV, point 1.2.3 of Directive 2006/17/EC;

8.4.15.5.11. Ingestion of, or exposure to, a substance (such as cyanide, lead, mercury, gold) that may be transmitted to recipients in a dose that could endanger their health;

8.4.15.5.12. Recent history of vaccination with a live attenuated virus where a risk of transmission is considered to exist;

8.4.15.5.13. History of xenotransplantation that involves the transplantation, implantation or infusion into a human recipient of live xenogeneic cells, tissues or organs or of human bodily fluids, cells, tissues or organs that have had *ex vivo* contact with live xenogeneic materials (unless justified on the basis of a documented risk assessment).

8.4.15.6. *Additional exclusion criteria for deceased child donors*

8.4.15.6.1. Any children born from mothers with HIV infection or that meet any of the general exclusion criteria must be excluded as donors until the risk of transmission of infection can be definitely ruled out.

8.4.15.6.1.1. Children aged less than 18 months born from mothers with HIV, HBV, HCV or HTLV infection, or at risk of such infection, and who have been breastfed by their mothers during the previous 12 months, cannot be considered as donors regardless of the results of the analytical tests.

8.4.15.6.1.2. Children of mothers with HIV, HBV, HCV or HTLV infection, or at risk of such infection, and who have not been breastfed by their mothers during the previous 12 months and for whom analytical tests, physical examinations and reviews of medical records do not provide evidence of HIV, HBV, HCV or HTLV infection, can be accepted as donors.

8.4.16. Procedures should be in place to ensure that abnormal findings arising from the donor-selection and evaluation process are properly reviewed by a qualified health professional and that appropriate action is taken. The reason for rejection of a donor should be recorded.

9. Donor testing

9.1. Testing of donations for infectious markers and agents is a key factor in ensuring that the risk of disease transmission is minimised and that tissues and cells are suitable for their intended purpose.

9.2. Each donation must be tested in conformity with the requirements laid down in EU directives, especially in Annex II and III Directive 2006/17 EC.

9.3. The tests must be carried out by a qualified laboratory, authorised as a testing centre by the Health Authority, using CE-marked testing kits where appropriate. The type of test used must be validated for the purpose in accordance with current scientific knowledge.

9.4. Additional donor testing for other markers or agents may be required, taking into account the epidemiological profile in any given region or country.

9.5. The tests must be carried out on the donor's serum or plasma; they must not be performed on other fluids or secretions unless specifically justified clinically using a validated test for such a fluid.

9.6. TEs may accept tissues and cells from donors with haemodilution of more than 50 % only if the testing procedures used are validated for such diluted plasma or if a pre-transfusion sample is available.

9.7. **Donation samples**

9.7.1. **Deceased donors**

9.7.1.1. In the case of a deceased donor, blood samples must have been obtained just prior to death or, if not possible, as soon as possible after death (within 24 hours after death).

9.7.2. **Living donors**

9.7.2.1. In the case of HPC donors, blood samples must be taken for testing within 30 days prior to donation.

9.7.2.2. In the case of non-HPC and non-reproductive cell living donors:

9.7.2.2.1. where tissues and cells are going to be stored for less than 180 days, blood samples must be obtained at the time of donation or, if not possible, within 7 days after donation;

9.7.2.2.2. where tissues and cells are going to be stored for 180 day or longer, blood samples can be taken up to 30 days prior to and 7 days after donation, and repeat sampling and testing is required after an interval of 180 days.

9.7.2.2.3. Repeat blood sampling and testing are not required after an interval of 180 days if:

9.7.2.2.3.1. the blood sample, taken at the time of procurement or within 7 days after procurement, is additionally tested by the nucleic acid amplification technique (NAT) for HIV, HBV and HCV;

9.7.2.2.3.2. the processing includes an inactivation step that has been validated for HIV, HBV and HCV.

9.7.2.3. In the case of neonatal donors, tests can be carried out on the donor's mother to avoid medically unnecessary procedures upon the infant.

9.7.2.4. In the case of reproductive non-partner donors:

9.7.2.4.1. all serum samples must be obtained at the time of donation;

9.7.2.4.2. sperm donations must be quarantined for ≥ 180 days after the last procurement, after which repeat testing is required, but quarantine is not necessary if at each donation serology testing is combined with NAT for HIV, HBV and HCV;

9.7.2.4.3. the same testing approach must be used for oocyte donors, allowing for the safe use of cryopreserved oocytes (after quarantine and re-testing after 180 days) or fresh oocytes (if NAT is done at the time of donation); oocyte donation may be considered as starting at the first day of stimulation, and the sample for testing may be taken at that time.

9.7.2.5 In the case of reproductive partner donors:

9.7.2.5.1 blood samples must be taken before the first donation and this must be done ≤ 3 months before the first donation. For further partner donations, additional blood samples must be obtained according to national legislation, but ≤ 24 months from the previous sampling.

9.8. The procedure used for the labelling of laboratory samples with donation numbers must be designed to avoid any risk of identification error and mix-up.

9.9. Upon receipt of samples at the laboratory, positive identification of the samples received against those expected should be carried out.

9.10. Laboratory personnel must be thoroughly instructed, trained and competent to operate the test system.

9.11. Each step of the handling and processing of samples should be described, as should the conditions of pre-analytical treatment of specimens (e.g. centrifugation), storage and transportation (duration, temperature, type of container, storage after testing).

9.12. There must be data confirming the suitability of any laboratory reagents used in testing of donor samples.

9.13. All laboratory testing procedures must be validated before use.

9.14. Screening algorithms should be defined precisely in writing (by SOPs) for dealing with initially reactive specimens and for resolving discrepancies in results after re-testing.

9.15. If additional NAT testing is performed, a thoroughly validated system of labelling/identification of samples should be in place.

9.16. There must be a reliable process in place for transcribing, collating and interpreting results.

9.17. There must be clearly defined procedures to resolve discrepant results. Appropriate confirmatory testing must take place. In the case of confirmed positive results, appropriate donor management must take place, including the provision of information to the donor and follow-up procedures.

9.18. The quality of the laboratory testing must be assessed regularly by participation in a formal system of proficiency testing, such as an external quality-assurance programme.

10. Procurement

10.1. General requirements

10.1.1. Procurement activities must be authorised by the appropriate Health Authority.

10.1.2. The procurement of human tissues or cells must be authorised only after all mandatory consent or authorisation requirements in force in the member state concerned have been met.

10.1.3. Procurement of human tissues or cells must take place only after donor consent/authorisation requirements have been satisfied.

10.2. Procurement procedures

10.2.1. Procedures must be authorised and appropriate for the type of donor and the type of tissue or cells procured and must be standardised. The procurement procedures must

be appropriate for the type of donor and the type of tissue/cells donated. There must be procedures in place to protect the safety of the living donor.

10.2.2. The procurement procedures must protect those properties of the tissue/cells that are required for their ultimate clinical use, and at the same time minimise the risk of microbiological contamination during the process, particularly when tissues and cells cannot subsequently be sterilised.

10.2.3. Policies and procedures must be in place to minimise the risk of tissue or cell contamination by staff who might be infected with transmissible diseases.

10.2.4. The sequence in which the various tissues are procured must be well defined to assure the quality of each type of tissue.

10.2.5. If deceased donation occurs after organ donation, sterility should be ensured throughout the whole procedure, including during organ procurement.

10.2.6. Selection of the use of suboptimal conditions must be supported by written justification and be authorised by the relevant Health Authority.

10.2.7. Sample cultures of the tissues or cells procured should be taken and an appropriately validated culture method must be used.

10.2.8. Time limits for procurement should be validated by quality assessments and by tests for microbiological contamination.

10.2.9. Once the tissue is procured and until it arrives at the TE, critical variables related to maintaining the quality of the tissues or cells (e.g. temperature, sterile packaging) must be controlled and recorded.

10.2.10. Once the tissues and cells have been retrieved from a deceased donor body, the donor body must be reconstructed so that it is as similar as possible to its original anatomical appearance.

10.2.11. Where appropriate, the staff and equipment necessary for body reconstruction of deceased donors must be provided. Such reconstruction must be completed effectively.

10.3. **Procurement report**

10.3.1. The organisation performing the procurement must produce a procurement report, which is passed on to the TE. This report must contain at least:

10.3.1.1. the identification, name and address of the TE to receive the cells/tissues;

10.3.1.2. donor identification data (including how and by whom the donor was identified);

10.3.1.3. description and identification of procured tissues and cells (including samples for testing);

10.3.1.4. identification of the person who is responsible for the procurement session, including signature;

10.3.1.5. date, time (where relevant, start and end) and location of procurement, the procedure (SOP) used and any incidents that occurred; also, where relevant, environmental conditions at the procurement facility (description of the physical area where procurement took place);

10.3.1.6. for deceased donors, conditions under which the body is kept, i.e. refrigerated (or not), time of start and end of refrigeration;

10.3.1.7. ID/batch numbers of reagents and transport solutions used.

10.3.2. The report must also contain the date and time of death where possible.

10.3.3. Where sperm is procured at home, the procurement report must state this and must contain only the following:

10.3.3.1. the name and address of the TE to receive the cells/tissues;

10.3.3.2. the donor identification.

10.3.4. The date and time of procurement may be included, where possible.

10.4. Following procurement, all recovered tissues and cells must be packaged and labelled as described in section 12: Packaging, coding and labelling.

11. Processing

11.1. General requirements

11.1.1. Each tissue and cell preparation process must be authorised by the Health Authority after evaluation of the donor-selection criteria and procurement procedures, the protocols for each step of the process, the quality-management criteria and the final quantitative and qualitative criteria for the cells and tissues. This evaluation must comply at least with the requirements set out in Directive 2006/86 Annex II.

11.1.2. TEs are responsible for the determining the suitability of the received tissues/cells for processing and for the quality and safety assessment of the processed tissue/cell products before distribution. Decisions on suitability should be made by a person who is appropriately qualified.

11.2. Reception of the tissues/cells at the TE

11.2.1. Each TE must ensure that the tissue and cells received are quarantined until they, along with the associated documentation, have been inspected or otherwise verified as conforming to requirements. The review of relevant donor/procurement information and thus acceptance of the donation needs to be carried out by specified/authorised persons.

11.2.2. Tissue and cells must be held in quarantine until such time as the requirements relating to donor testing and information have been met in accordance with Directive 2004/23/EC Article 15. Received tissues and cells awaiting final test results, or subject to additional testing or confirmatory medical assessment, must be held in quarantine until such test results or medical data become available.

11.2.3. When the procured tissues/cells arrive at the TE, there must be documented verification that the consignment, including the transport conditions, packaging, labelling and associated documentation and samples, meet the requirements of Annex IV of Directive 2006/17/EC and the specifications of the receiving establishment.

11.2.4. Each TE must have a documented policy and specifications against which each consignment of tissues and cells, including samples, can be verified. These must include the technical requirements and other criteria considered by the TE to be essential for the maintenance of acceptable quality. The TE must have documented procedures for the management and segregation of non-conforming consignments, or those with incomplete test results, to ensure that there is no risk of contamination of other tissues and cells being processed, preserved or stored.

11.2.5. TEs must ensure that all donations of human tissues and cells are subjected to tests in accordance with the requirements referred to Directive 2004/23/EC Article 28(e) and that the selection and acceptance of tissues and cells comply with the requirements referred to in Directive 2004/23/EC Article 28(f).

11.2.6. TEs must ensure that human tissue and cells and associated documentation comply with the requirements referred to in Directive 2004/23/EC Article 28(f).

11.2.7. TEs must verify and record the fact that the packaging of human tissue and cells received complies with the requirements referred to in Article 28(f). All tissues and cells that do not comply with those provisions must be discarded.

11.2.8. The procurement report and shipping record (if the donation was transported by a third party) should be cross-checked with the contents of the package.

11.2.9. The acceptance or rejection of received tissues or cells must be documented.

11.2.10. The data that must be registered at the TE (except for donors of reproductive cells intended for partner donation) include:

11.2.10.1. consent/authorisation, including the purpose(s) for which the tissues and cells may be used (i.e. therapeutic or research, or both therapeutic use and research) and any specific instructions for disposal if the tissue or cells are not used for the purpose for which consent was obtained;

11.2.10.2. all required records relating to the procurement and the taking of the donor history, as described in the donor documentation section;

11.2.10.3. results of physical examination, of laboratory tests and of other tests (such as the autopsy report, if used in accordance with Directive 2006/17/EC Annex IV point 1.2.2.);

11.2.10.4. for allogeneic donors, a properly documented review of the complete donor evaluation against the selection criteria by an authorised, trained and qualified person;

11.2.10.5. in the case of cell cultures intended for autologous use, documentation of the possibility of medicinal allergies (such as to antibiotics) of the recipient.

11.2.11. In the case of reproductive cells intended for partner donation, the data to be registered at the TE include:

11.2.11.1. consent, including the purpose(s) for which the tissues and cells may be used (such as reproductive only and/or for research) and any specific instructions for disposal if the tissue or cells are not used for the purpose for which consent was obtained;

11.2.11.2. partner identification;

11.2.11.3. place of procurement;

11.2.11.4. tissues and cells obtained and relevant characteristics.

11.3. **Processing**

11.3.1. TEs must include in their SOPs all processes that affect quality and safety and must ensure that they are carried out under controlled conditions. TEs must ensure that the equipment used, the working environment, the process design, validation and control conditions are all in compliance with the requirements referred to in Directive 2004/23/EC Article 28 (h).

11.3.2. Any modifications to the processes used in the preparation of tissues and cells must also meet the criteria laid down in paragraph 11.3.1 above. Any substantial modification to the processes and parameters in reference to tissue and cell processing should be reported to the national Health Authority and, if required, authorised by that authority before processing begins.

11.3.3. The procedures must be documented in SOPs, which must conform to the validated method and to the standards laid down in Directive 2006/86/ Annex I(E), points 1 to 4.

11.3.4. TEs must ensure that all processes are conducted in accordance with the approved SOPs.

11.3.5. The critical processing procedures must be validated and must not render the tissues or cells clinically ineffective or harmful to the recipient. This validation may be based on studies performed by the establishment itself, or on data from published studies or, for well-established processing procedures, by retrospective evaluation of the clinical results for tissues supplied by the establishment. Processing methods should be designed to ensure the safety and biological functionality of processed tissues and cells.

11.3.6. If validation is based on retrospective evaluation of the clinical results for tissues or cells supplied by the establishment, data should be collected and analysed that include the number of tissues or cells implanted following processing by the method under consideration, and the time period (start and end dates/times) during which these implantations occurred.

11.3.7. It should be demonstrated that the validated process can be carried out consistently and effectively in the TE environment by the staff available.

11.3.8. If physicochemical methods are to be applied, these procedures must be adapted to the type of tissue or cell and should be validated.

11.3.9. The processing procedures must undergo regular critical evaluation to ensure that they continue to achieve the intended results.

11.3.10. Before implementing any significant change in processing, the modified process must be validated and documented.

11.3.11. Tissues or cells from different donors should not be pooled during processing unless this is the only way in which clinical efficacy can be achieved on an individual patient basis. Traceability must be fully ensured if there is pooling of different tissues and cells from two or more donors during processing.

11.3.12. Pooled tissues or cells should be treated as a single batch while also ensuring that the original donations are fully traceable.

11.3.13. In the case of pooling during the process, a risk evaluation should be in place to preclude cross-contamination.

11.3.14. If the process includes a sterilisation or viral-inactivation step, process-specific validation studies should be completed to demonstrate the log reduction achieved by the process.

11.3.15. Where a microbial inactivation procedure is applied to the tissue or cells, it must be specified, documented and validated.

11.3.16. In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during processing or storage, with tissues or cells from another donor, unless they are intentionally pooled.

11.3.17. A separate set of clean, sterile instruments should be used for each donor.

11.3.18. The procedures used to prevent or reduce contamination during processing should be validated depending on the type of tissue and how it is processed.

11.3.19. The use of antibiotics during procurement, processing and preservation must be recorded, and the end user should be aware of the use of antibiotics.

11.3.20. Maximum times from procurement until processing and storage must be defined. For deceased donors, maximum times from circulatory arrest (cardiac arrest or, for organ donors, cross-clamp time) to procurement must also be defined.

11.3.21. Procurement, processing and storage times must be documented in the records for tissues and cells.

11.3.22. TEs must ensure that human tissues and cells are correctly identified at all times. Each delivery of tissues or cells must be assigned a unique identifying code, in accordance with Directive 2006/86/EC Art. 10, 10a, 10b.

11.3.23. Each processed tissue or cell product should have a unique identifying code, i.e. the Single European Code (SEC), that is also recorded in the processing records.

11.3.24. The acceptance, rejection or disposal of tissues and cells, including those to be discarded, must be properly documented.

11.4. **Storage**

11.4.1. Maximum storage time and storage conditions must be defined and validated for each type of tissue and cell.

11.4.2. There must be a system in place to separate and distinguish tissues and cells prior to release/in quarantine from those that are released and from those that are rejected, in order to prevent mix-ups and cross-contamination.

11.4.3. A documented risk assessment approved by the Responsible Person must be undertaken to determine the fate of all stored tissues and cells following the introduction of any new donor selection or testing criterion or any significantly modified processing step that enhances safety or quality.

11.5. **Disposal**

11.5.1. TEs must include in their SOPs special provisions for the handling of tissues and cells to be discarded, in order to prevent the contamination of other tissues or cells, the processing environment or personnel. These procedures must comply with national regulations.

11.5.2. Disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and for the human body.

11.5.3. There should be documentation to show that the conditions for disposal of HPC, as defined prior to procurement, have been met, including (where applicable) the option to transfer the cells to another facility if the designated recipient is still alive after the agreed storage period.

12. Packaging, coding and labelling

12.1. General principles

12.1.1. Labelling and packaging operations should be designed to prevent any cross-contamination or mix-ups. Simultaneous operations should be avoided or adequate measures should be taken to ensure that no cross-contamination or mix-ups occur.

12.1.2. Facilities where packaging or labelling operations have taken place should be inspected and documented before starting any other operation so as to guarantee that all the previous materials have been removed.

12.1.3. There should be written procedures describing the receipt, identification, quarantine, sampling, examination and/or testing, release and handling of packaging and labelling materials.

12.1.4. Records should be maintained for each shipment of labels and packaging materials showing receipt, examination or testing, and whether accepted or rejected.

12.1.5. All packaging and labelling materials should be stored and managed in a safe manner in order to avoid any cross-contamination or mix-up, which could result in incorrectly identified/packaged tissues/cells.

12.2. Packaging and labelling

12.2.1. Containers should provide adequate protection against any deterioration or contamination of the tissues/cells that may occur during storage and transportation, and should resist the processing techniques used (e.g. sterilisation).

12.2.2. Containers should be clean and sanitised to ensure that they are suitable for their intended use. These containers should not alter the quality, safety and efficacy of the tissues/cells.

12.2.3. Labels should be designed to adhere firmly to the container under all storage and transport conditions and the processing techniques used.

12.2.4. Type of label to be used, as well as the labelling methodology, should be defined and established in written procedures.

12.2.5. Labels applied to containers, equipment or premises should be clear, unambiguous and in the agreed format of the TE.

12.2.6. All excess labels should be destroyed or maintained in a secure manner, when necessary, to prevent cross-contamination or mix-ups.

12.2.7. Obsolete labels should be destroyed according to written procedures.

12.2.8. Printed labels should be carefully examined to ensure that the information on the label conforms to the corresponding tissue/cells. The results of this examination should be documented.

12.2.9. A printed label, representative of those used, should be included in the processing records.

12.2.10. A unique donation code must be allocated to the donated tissues and cells, during procurement or at the end of the recovery process, to ensure proper identification of all donated material. The registration system must ensure traceability to the donor identity/donor code and must comply with the coding requirements of Directive 2006/86/EC.

12.2.11. Following procurement, all recovered tissues and cells must be packaged in a manner which minimises the risk of contamination and must be stored at temperatures that preserve the required characteristics and biological function of the cells/tissues. The packaging must also prevent contamination of those persons responsible for packaging and transportation of the tissues and cells.

12.2.12. The packaged cells/tissues must be shipped in a container which is suitable for the transport of biological materials and which maintains the safety and quality of the contained tissues or cells.

12.2.13. Any accompanying tissue or blood samples for testing must be accurately labelled to ensure identification with the donor, and must include a record of the time when and the place where the specimen was taken.

12.2.14. **Primary packaging and labelling operation after procurement**

12.2.14.1. Every package containing tissues and cells must be labelled at the time of procurement. The primary tissue/cell container must indicate the donation identification or code and the type(s) of tissue and cell.

12.2.14.2. Where the size of the package permits, the following information must also be provided:

12.2.14.2.1. date (and time where possible) of donation;

12.2.14.2.2. details of any blood transfusion before the procurement, and the haemodilution risk;

12.2.14.2.3. hazard warnings;

12.2.14.2.4. nature of any additives/transport medium (if used);

12.2.14.2.5. in the case of autologous donations, the label must state 'for autologous use only';

12.2.14.2.6. in the case of directed donations, the label must identify the intended recipient;

12.2.14.2.7. If any of the information above cannot be included on the primary package label, it must be provided on a separate sheet accompanying the primary package.

12.2.15. **Secondary packaging and labelling operation after procurement**

12.2.15.1. When tissues/cells are shipped by an intermediary, every shipping container must be labelled at least with the following:

12.2.15.1.1. **TISSUES AND CELLS** and **HANDLE WITH CARE;**

12.2.15.1.2. the identification of the establishment from which the package is being transported (address and phone number) and a contact person in the event of problems;

12.2.15.1.3. the identification of the TE of destination (address and phone number) and the person to be contacted to take delivery of the container;

12.2.15.1.4. the date and time of the start of transportation;

12.2.15.1.5. specifications concerning conditions of transport relevant to the quality and safety of the tissues and cells;

12.2.15.1.6. in the case of living tissues and cells, the indication **DO NOT IRRADIATE;**

12.2.15.1.7. when tissues and cells are known to be positive for a relevant infectious disease marker, the indication **BIOLOGICAL HAZARD;**

12.2.15.1.8. in the case of autologous donors, the indication **FOR AUTOLOGOUS USE ONLY;**

12.2.15.1.9. specifications concerning storage conditions (such as **DO NOT FREEZE**).

12.2.16. **Final labelling for distribution**

12.2.16.1. The primary tissue/cell container must provide the following:

12.2.16.1.1. type of tissues and cells, identification number or (SEC) code of the tissue/cells, and lot or batch number where applicable;

12.2.16.1.2. identification of the TE from which the tissues/cells are sent;

12.2.16.1.3. expiry date of the tissues/cells;

12.2.16.1.4. in the case of an autologous donation, this information has to be specified (**FOR AUTOLOGOUS USE ONLY**) and the donor/recipient has to be identified;

12.2.16.1.5. in the case of directed donations, the label must identify the intended recipient;

12.2.16.1.6. when tissues and cells are known to be positive for a relevant infectious disease marker, they must be marked as **BIOLOGICAL HAZARD.**

12.2.16.2. If any of the information under points 12.2.16.1.4 and 12.2.16.1.5 above cannot be included on the primary container label, it must be provided on a separate sheet accompanying the primary container. This sheet must be packaged with the primary container in a manner that ensures that they remain together.

12.2.16.3. The following information must be provided either on the label or in accompanying documentation:

12.2.16.3.1. description (definition) and, if relevant, dimensions of the tissues;

12.2.16.3.2. morphology and functional data where relevant;

12.2.16.3.3. date of distribution of the tissue/cells;

12.2.16.3.4. biological investigations carried out on the donor and results;

12.2.16.3.5. storage recommendations;

12.2.16.3.6. instructions for opening the container or package, and any required manipulation/ reconstitution;

12.2.16.3.7. expiry dates after opening/manipulation;

12.2.16.3.8. instructions for reporting serious adverse reactions and/or events;

12.2.16.3.9. presence of potential harmful residues (e.g. antibiotics, ethylene oxide).

12.2.17. **External labelling of the shipping container**

12.2.17.1. For transport, the primary container must be placed in a shipping container that must be labelled with at least the following information:

12.2.17.1.1. identification of the originating TE, including an address and phone number;

12.2.17.1.2. identification of the destination organisation responsible for human application (ORHA), including address and phone number;

12.2.17.1.3. a statement that the package contains human tissue/cells and the warning **HANDLE WITH CARE;**

12.2.17.1.4. where living cells are required for the function of the graft, such as stem cells, gametes and embryos, the warning **DO NOT IRRADIATE** must be added;

12.2.17.1.5. recommended transport conditions (e.g. keep cool, in upright position);

12.2.17.1.6. safety instructions/method of cooling (when applicable).

12.3. **Coding**

12.3.1. The TE must have effective and accurate systems to uniquely identify and label cells/ tissues received and distributed. An identifying code (SEC) must be allocated by the TE to all procured tissues and cells, including tissues and cells imported from countries outside the EU, to ensure proper identification of the donor and the traceability of all donated material and to provide information on the main characteristics and properties of the tissues and cells.

12.3.2. The code must incorporate at least:

12.3.2.1. donation identification;

12.3.2.2. unique ID number;

12.3.2.3. identification of the TE;

12.3.2.4. tissues and cells identification;

12.3.2.5. tissues and cells code (basic nomenclature);

12.3.2.6. split number (if applicable);

12.3.2.7. expiry date.

13. Quality control (including microbiological control)

13.1. General principles

13.1.1. A quality-control system must be in place to ensure that tissues and cells are not released for use until their quality has been assessed as satisfactory. Activities such as verification steps, sampling and testing should be carried out to assess that the tissues and cells, and also materials, equipment and processes, comply with established acceptance criteria.

13.1.1.1. All records which are critical to the safety and quality of the tissues and cells must be protected from unauthorised amendment and kept so as to ensure readability and access throughout their specified retention period, after expiry date, clinical use or disposal.

13.1.1.2. Samples for quality control should be representative of the tissues and cells from which they are taken and should be procured and recorded in accordance with written procedures that describe the method of sampling, including the amount of sample to be taken, precautions to be observed and storage conditions.

13.1.1.3. At all stages of quality-control testing, sampling containers must be labelled with relevant information on their identity and date of sampling.

13.1.2. There must be a person responsible for quality control who supervises all quality-control procedures who ensures that the premises and equipment where quality-control operations are carried out are appropriate and maintained under suitable conditions and who also ensures that all personnel working under his/her responsibility are adequately trained.

13.1.3. Written procedures (SOPs) must be in place that govern quality control at key stages during processing. The SOPs should include at least:

13.1.3.1. the test method;

13.1.3.2. the sample size and sampling plan;

13.1.3.3. the accepted criteria.

13.1.4. Sampling and testing methods must be validated to show the representativeness of the sample and the suitability of the selected methods.

13.1.4.1. The performance of the testing procedures should be regularly assessed.

13.1.5. Records related to quality-control testing should be part of the documentation of tissues and cells. Data to be recorded and maintained should include:

13.1.5.1. name, manufacturer and batch number of the material or products used;

13.1.5.2. reference to the relevant specifications and testing procedures, and to the equipment used;

13.1.5.3. test results, including observations and calculations, and reference to any certificates of analysis;

13.1.5.4. date of testing;

13.1.5.5. identification of the persons who performed the testing;

13.1.5.6. identification of the person who verified the testing;

13.1.5.7. statement of approval or rejection of the test results;

13.1.6. Minimum acceptance criteria should be based on a defined specification for each type of tissue and cell.

13.1.7. Non-conforming tissues and cells must be identified and separated from conforming tissues and cells. The fate of non-conforming tissues and cells must be decided by the Responsible Person in charge of the TE, according to written procedures.

13.2. **Microbiological control**

13.2.1. The microbiological safety of tissues and cells is based on donor selection and minimisation of initial contamination, with control and monitoring of contamination during the entire procurement process. All facilities that procure, process or store tissues and cells should have access to the services of a microbiology laboratory with a fully implemented QMS and to the advice of a suitably qualified expert microbiologist.

13.2.2. Microbiological control should be carried out at least on representative samples of the tissues and cells before final release, and on representative samples of tissues and cells at the time of procurement.

13.2.2.1. In exceptional cases, if sampling of the finished tissues and cells product is not feasible, the storage medium or rinsing or washing solutions can be tested as surrogates.

13.2.2.2. In cases where the nature of the procured tissues and cells does not allow sampling of the starting material, an alternative sampling approach, such as liquids in contact with the starting material, may be followed.

13.2.2.3. Where applicable, a sample of the tissues/cells storage, transport or rinsing solution should be tested.

13.2.2.4. In-process testing should be performed at relevant steps of the preparation process, according to a risk assessment that has to take into consideration the nature of the tissues

and cells, their origin and procurement, the critical steps during processing and their intended application.

13.2.2.5. For cells such as HPC, in which a closed system is used for processing and where no further steps are conducted, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point is applicable.

13.2.2.6. For processed tissues and cells sterilised in their final container by a validated sterilisation process, if the release is intended to rely on process data only and not on final product testing for sterility, then validated procedures for all critical processing steps and a fully validated sterilisation method must be applied.

13.2.2.7. For tissue and cells obtained from processing that includes decontamination, such as treatment with antibiotics and anti-fungal agents, methods for finished tissue and cell testing must be evaluated carefully to assess possible inhibition of microbial growth due to decontaminating agents or their residues.

13.2.2.8. Microbiological testing methods for the detection of bacteria and fungi should follow the procedures outlined in the *European Pharmacopoeia* (*Ph. Eur.*), according to the method employed and the type of tissue or cell analysed. Chapter 2.6.27 of *Ph. Eur.* deals specifically with cell-based preparations or products where the examination for sterility cannot be applied according to Chapter 2.6.1 of *Ph. Eur.*, while General Monograph 5.1.6 gives indications on implementation and the use of alternative microbiological methods. Microbiological examination of non-sterile tissues and cells should be performed according to indications given in Chapters 2.6.12 and 2.6.13 of *Ph. Eur.*

13.2.2.9. Whatever the applied method, its suitability must be shown with respect to specificity, sensitivity and robustness.

13.2.2.10. If release of the tissues and cells is necessary before the end of the officially verified/required incubation period, a 'negative-to-date' reading of the results may be carried out. In this case, intermediate results of the final testing in combination with final results of in-process controls should be used for tissues and cells release.

13.2.2.11. If micro-organisms are detected after tissues and cells release, pre-defined measures such as identification and antibiotic sensitivity of the species must be carried out, and information must be provided to clinicians caring for the patient.

13.2.3. Depending on the type of preparation process, it may be necessary to complement the microbial test concept by additional tests for specific infectious agents such as mycoplasma (*Ph. Eur.* 2.6.7).

13.2.3.1. Testing should be conducted at those manufacturing steps at which mycoplasma contamination would most likely be detected, such as after pooling or procurement but before washing.

13.2.3.2. As mycoplasmas are cell-associated micro-organisms that may locate within the cell, testing should always include the cellular matrix, if possible.

13.2.4. Depending on the intended application of the tissues and cells, and the estimated impact of endotoxins on the recipient, routine testing for endotoxins may be required. If deemed necessary, it should be carried out according to *Ph. Eur.* 2.6.14.

13.2.4.1. In the case of tissues and cells obtained from culture, an endotoxin test should be carried out on them before release to the patient.

13.2.4.2. In any procedure in which animal-derived products are used, endotoxin testing should be performed.

13.2.4.3. Because raw materials are potential sources of endotoxins, raw materials certified by their manufacturers to be free of endotoxins should be employed in culture of tissues and cells.

13.2.4.4. For certain cells that must be administered immediately and that cannot be cryopreserved without damaging the viability and quality of the cells, a rapid method for endotoxin testing may be employed.

13.2.5. Each batch of the microbiological culture medium and plates to be used must be tested for its growth-promoting capacities by using a 'growth-promotion test' in accordance with *Ph. Eur.*

13.2.6. The methods employed for sterility testing must be validated in the presence of the intended sample material (method suitability test). The method suitability test must be carried out using the bacterial and fungal species indicated in the chapters applicable from *Ph. Eur.*

13.2.7. Source material that demonstrates contamination must be rejected unless the preparation undergoes decontamination and/or terminal sterilisation, and the detected quantity and quality of micro-organisms can be reliably inactivated or removed by the intended procedure, or if it is justified by exceptional clinical circumstances.

14. Distribution, import/export and recall

14.1. Release

14.1.1. The distribution process, meaning transportation and delivery of cells or tissues intended for human application or for use in further manufacture, must be validated.

14.1.2. Prior to distribution a comprehensive record review must ensure that all elements of procurement, processing and storage have met the established quality criteria, including the identity of the tissues and cells.

14.1.3. Packaged tissues or cells should be examined visually for appropriate labelling and container integrity.

14.1.4. Tissues and cells should not be distributed without an order from a physician or other authorised health professional.

14.1.5. In cases of incomplete eligibility of the donor, the tissues and cells must be released only for documented urgent medical need and after a risk assessment has been performed.

14.1.6. Distribution for clinical application must be restricted to authorised ORHAs or to authorised health professionals and must comply with all applicable national laws and regulations. In cases where tissues or cells require additional procedures such as thawing to be undertaken by the ORHA, the associated specific instructions must be provided to the ORHA by the TE.

14.2. **Transport**

14.2.1. Equipment used to ensure the maintenance of critical transport or shipment must be qualified and validated.

14.2.2. The capacity of the transport container to maintain the required environmental conditions, and the length of time that these conditions can be maintained, should both be determined by validation and documented.

14.2.3. The container/package must be secure, and shipment conditions such as temperature and time limit must be defined to ensure maintenance of the required properties of the tissues and/or cells.

14.2.4. Data loggers or temperature indicators must be used when it is mandatory to monitor temperature during transport or shipment of tissues or cells requiring a continuous temperature-controlled environment.

14.2.5. The mode of transport or shipment must comply with the applicable laws and regulations on transportation of biological substances.

14.2.6. An alternative plan of transport or shipping should be available in case of emergency situations, to prevent possible clinical complications for the recipient.

14.2.7. Couriers should be appropriately trained and should be able to contact the receiving facility on a 24-hour basis in cases of delay during transit.

14.2.8. Viable tissues and cells, including stem cells, gametes and embryos, must not be exposed to irradiation. Appropriate arrangements in accordance with relevant national legislation should be in place to ensure that such tissues and cells are not exposed to irradiation during transport, including at security screenings and border crossings.

14.3. **Agreements**

14.3.1. Written agreements for the shipment of tissues and cells must be in place between the shipping company and the TE.

14.3.2. A service-level agreement between the exporting TE and importing TE must clearly define the relevant roles and responsibilities, including procedures for transport, packaging and required environmental conditions.

14.3.3. The agreement should specify how tissues and cells will be identified, and a unique identifying code must allow unambiguous identification and traceability.

14.4. **Export**

14.4.1. Exported tissues and cells must be procured, handled, stored, transported, used and disposed of in accordance with the consent that has been given by the donor.

14.4.2. Tissues and cells should be exported only to countries that have proper controls on the use of donated material and only for the purposes for which they can lawfully be used in the country of destination.

14.5. **Import**

14.5.1. TEs must be authorised for the import of tissues and cells from non-EU countries by their respective Health Authorities.

14.5.2. The importing TE should assess and document that the exporting TE has applied the fundamental ethical principles of consent, non-remunerated donation, anonymity and respect for public health.

14.5.3. The importing TE must evaluate the general quality and safety systems at the exporting establishment, along with its licences and accreditations and the donor blood testing.

14.5.4. The importing TE must require that any changes to authorisation status be immediately communicated by the exporting facility.

14.5.5. The agreements between an importing TE and suppliers in other non-EU countries should include provisions for the performance of audits at the exporting facility.

14.5.6. Acceptance at the TE should include a documented procedure to verify compliance with the written agreement in place with the exporter.

14.5.7. Containers should be examined for any evidence of tampering or damage during transport.

14.5.8. Tissues and cells should be stored in quarantine in an appropriate secure location under defined conditions until they, along with the accompanying documentation, have been verified as conforming to requirements.

14.5.9. The importing TE must identify and code the imported tissues and cells with the appropriate SEC.

14.6. **Records**

14.6.1. The courier must provide records of pick-up and delivery to the TE to ensure complete traceability of the tissues and cells.

14.6.2. Documentation obtained from the exporting TE must be archived for the time period required by national regulations (e.g. 30 years in EU member states).

14.7. **Recall**

14.7.1. The TE must have personnel authorised to assess the need for recall and to initiate and co-ordinate the necessary actions.

14.7.2. A recall procedure must be in place that includes a description of the responsibilities, actions to be taken within pre-defined periods of time and requirements for notification to the Health Authorities.

14.7.3. Recall actions must be taken within pre-defined periods of time and must include tracing all relevant tissues or cells and, where applicable, must include trace-back. The purpose of the investigation is to identify any donor who might have contributed to causing the adverse reaction and to retrieve available tissues or cells from that donor, as well as to notify consignees and recipients of components procured from the same donor in the event that they might have been put at risk.

14.7.4. The progress of the recall process should be recorded and a final report issued, including reconciliation of the delivered and recovered quantities of the tissues or cells.

14.7.5. The effectiveness of the arrangements for recalls should be regularly evaluated.

15. Documentation

15.1. **General principles**

15.1.1. Good documentation constitutes an essential part of the quality system and is key to operating in compliance with the requirements of good practice, which includes these guidelines. Various types of documents and media used must be defined fully in the QMS of the organisation.

15.1.2. Documentation may exist in various forms: paper-based, electronic or photographic. The main objective of the system of documentation used must be to establish, control, monitor and record all activities that directly or indirectly impact on all aspects of the quality and safety of tissues and cells as well as any derived medicinal products. The QMS must include sufficient instructional detail to facilitate common understanding of the requirements. It must also provide for adequate recording of the various processes and evaluation of any observations, so that ongoing application of the requirements may be demonstrated.

15.1.3. There are two primary types of documentation used to manage and record good practice compliance: instructions (directions, requirements) and records/reports. Appropriate practices should be applied to each type of document. Suitable controls must be

implemented to ensure the accuracy, integrity, availability and legibility of documents. Instruction documents must be free from errors and available in writing. The term 'written' means recorded or documented on media from which data may be rendered in a readable form for humans.

15.2. **Required good practice documentation**

15.2.1. Documentation must include at least the following items:

15.2.1.1. a quality manual;

15.2.1.2. specifications for materials and reagents;

15.2.1.3. approved SOPs for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;

15.2.1.4. identification and analysis of risks and a risk-mitigation plan;

15.2.1.5. records of the performance of operations, including processing records;

15.2.1.6. records of deviations, complaints and audits;

15.2.1.7. training and competency records of personnel;

15.2.1.8. qualitative and quantitative specifications for tissues and cells;

15.2.1.9. key quality indicators for tissues and cells.

15.2.2. **Instructions (directions or requirements)**

15.2.2.1. Specifications, based on policies and risk assessments, describe in detail the requirements to which the tissues and cells or other materials used or obtained during processing and distribution must conform.

15.2.2.2. Testing instructions detail all the starting materials, equipment and computerised systems (if any) to be used and specify all sampling and testing instructions. If applied, in-process controls must be specified, together with their acceptance criteria.

15.2.2.3. SOPs give directions for performing certain operations.

15.2.2.4. SOPs provide explicit instructions for performing certain discrete operations, and may also record the outcome (e.g. qualification and validation protocols).

15.2.2.5. Technical agreements are agreed between contract givers and acceptors for outsourced activities.

15.2.3. **Records/reports**

15.2.3.1. Records provide evidence of various actions taken to demonstrate compliance with instructions, e.g. activities, events, investigations and a history of all tissues and cells, including their distribution. Records include the raw data that are used to generate other records. For electronic records, regulated users should define which data are to be used

as raw data. All data on which quality decisions are based should be defined as 'raw data'.

15.2.3.2. Certificates of analysis provide a summary of testing results on samples of reagents, products or materials, together with the evaluation of compliance with a stated specification.

15.2.3.3. Reports document the carrying out of particular exercises, projects or investigations, together with the results, conclusions and recommendations.

15.3. **Generation and control of documentation**

15.3.1. Each type of document should be defined and its requirements should be followed, regardless of document forms or media. Complex systems must be understood, well documented and validated, and adequate controls must be in place. Many documents (instructions and/or records) may exist in hybrid forms (i.e. some elements are electronic and others are paper-based). Relationships and control measures for master documents, official copies, data handling and records must be stated for both hybrid and homogeneous systems.

15.3.2. A document-control system, defined in a written procedure, must be established for the review, revision history and archiving of documents, including SOPs. Appropriate controls for electronic documents, such as templates, forms and master documents, must be implemented. Appropriate controls must be in place to ensure the integrity of the record throughout the retention period.

15.3.3. Documents should be designed, prepared, reviewed and distributed with care. Reproduction of working documents from master documents should not allow errors to be introduced through the reproduction process.

15.3.4. There must be a document-control procedure in place to ensure that only current versions are in use.

15.3.5. Documents containing instructions must be approved, signed and dated by appropriate and authorised persons. This may also be undertaken electronically. Documents should have unambiguous content and be uniquely identifiable. The effective date must be defined.

15.3.6. Documents containing instructions should be laid out in an orderly fashion and be easy to check. The style and language of documents should fit with their intended use. SOPs, Work Instructions and Methods should be written in an imperative mandatory style.

15.3.7. Documents within the QMS must be regularly reviewed and kept up to date. A periodic review process should be established to ensure that the documentation for any given process, system or equipment is complete, current and accurate.

15.3.8. All changes to documents must be reviewed, dated, approved, documented and implemented promptly by authorised personnel.

15.3.9. Instructional documents should not be handwritten; however, where documents require the entry of data, sufficient space should be provided for such entries.

15.4. **Good documentation practices**

15.4.1. Records must be legible and may be handwritten, transferred to another medium such as microfilm, or documented in a computerised system.

15.4.2. Records should be made or completed at the time each action is taken and in such a way that all significant activities concerning the coding, donor eligibility, procurement, processing, preservation, storage, transport and distribution or disposal, including aspects relating to quality control and quality assurance of tissues and cells, are traceable.

15.4.3. For every critical activity, the materials, equipment and personnel involved must be identified and documented.

15.4.4. The record system must ensure continuous documentation of the procedures performed from the donor to the recipient. That is, each significant step must be recorded in a manner that permits tissue and cells or procedure to be traced, in either direction, from the first step to final use/disposal.

15.4.5. Any alteration made to the entry on a document must be signed and dated; the alteration must permit reading of the original information. Where appropriate, the reason for the alteration should be recorded. In the case of electronic records, there must be an audit trail, so that it is traceable as to what data has been altered, when it was altered and who altered it.

15.4.6. Access to records (registers and data) must be restricted to persons authorised by the Responsible Person, and to the Health Authority for the purpose of inspection and control measures.

15.4.7. Data protection and confidentiality measures must be in place, in accordance with Article 14 of Directive 2004/23/EC.

15.5. **Retention of documents**

15.5.1. It should be clearly defined which record is related to each activity and where this record is located. Secure controls must be in place to ensure the integrity of the record throughout the retention period. These controls must be validated if appropriate.

15.5.2. Records encompassing identification, donor tests and clinical evaluation of the donor must be retained and must include at least the following details:

15.5.2.1. identification;

15.5.2.2. age;

15.5.2.3. sex;

15.5.2.4. medical and behavioural history;

15.5.2.5. relevant clinical data, laboratory test results and results of any other tests;

15.5.2.6. outcome of physical examination, results of autopsy (if carried out) or preliminary verbal report for deceased donors;

15.5.2.7. completed haemodilution algorithm (where applicable);

15.5.2.8. consent/authorisation forms;

15.5.2.9. for HPC donors, report of donor's suitability for intended recipient and, if donor is unrelated, relevant donor data to confirm suitability.

15.5.3. Donor-testing records must be accessible at the laboratory and must include date and time of sampling, date and time of sample receipt at the testing facility, record of test kits used to test donor sample and the results of donor testing, including repeat testing.

15.5.4. Records of procurement of tissues and cells must be retained. A procurement report should be available that includes:

15.5.4.1. identification of procurement organisation, and person responsible for procurement, including signature;

15.5.4.2. identification of TE receiving the tissue/cells;

15.5.4.3. donor identification data (including how and by whom the donor was identified);

15.5.4.4. donation unique number;

15.5.4.5. date, time and place of donation, and SOP used for procurement;

15.5.4.6. type of donation;

15.5.4.7. description of the procurement area, including environmental conditions;

15.5.4.8. storage conditions for deceased donors (including whether refrigeration was applied, and time of start and end of refrigeration);

15.5.4.9. details of materials, reagents and transport solutions;

15.5.4.10. any incidents during procurement.

15.5.5. Records of the processing of tissues and cells must be retained. A processing report should be available that at least includes the details of:

15.5.5.1. tissues and cells received, and evaluation of their suitability;

15.5.5.2. SOP used to process the tissues and cells;

15.5.5.3. equipment used during processing;

15.5.5.4. materials used during processing;

15.5.5.5. sterilisation or decontamination;

15.5.5.6. cryopreservation and freezing protocols;

15.5.5.7. environmental monitoring;

15.5.5.8. tissues and cells testing, including microbiological testing;

15.5.5.9. any incidents that occurred during processing.

15.5.6. Records of the storage and distribution of tissues and cells must be retained, and reports should be available that detail:

15.5.6.1. storage location (and transfer record if location is changed);

15.5.6.2. date placed in storage and date removed from storage;

15.5.6.3. storage temperature;

15.5.6.4. any incidents that occurred during storage;

15.5.6.5. name of party responsible for distribution;

15.5.6.6. identification of establishment, courier or individual who transported tissues/cells at any stage between procurement and end use;

15.5.6.7. packaging;

15.5.6.8. time and date of distribution and delivery;

15.5.6.9. identification of receiving establishment, clinician or ORHA;

15.5.6.10. any incidents that occurred during distribution.

15.5.7. Records of the clinical application of tissues and cells should be retained by the ORHA and should include:

15.5.7.1. identification of supplier TE;

15.5.7.2. identification of clinician or ORHA;

15.5.7.3. type(s) of tissues and/or cells;

15.5.7.4. tissues and cells identification;

15.5.7.5. identification of the recipient;

15.5.7.6. date of clinical application;

15.5.7.7. any incidents that occurred during clinical applications;

15.5.7.8. any adverse reactions in the recipient;

15.5.7.9. health outcomes of children born following MAR.

15.5.8. Specific retention requirements for certain documentation apply.

15.5.8.1. Records must be retained for a period complying with local, national or EU requirements, as appropriate.

15.5.8.2. Traceability data (that allow tracing from donor to recipient and vice versa) must be retained for a minimum of 30 years.

15.5.8.3. All records (including raw data) that are critical to the safety and quality of the tissues and cells must be kept for at least 10 years after the expiry date, clinical use or disposal.

15.5.8.4. Quality system documentation and associated records should be retained for a minimum of 10 years.

15.5.8.5. For other types of documentation, the retention period should be defined on the basis of the business activity that the documentation supports. These retention periods should be specified.

16. Traceability

16.1. General principles

16.1.1. A robust system must be established and maintained to trace a specific tissue/cell during any step from donor/donation to recipient or child conceived as a result of MAR treatment, and vice versa. In the EU, tissues and cells are traceable from procurement to human application or disposal and vice versa through documentation and the use of the SEC (Directive 2006/86/EC as amended by Commission Directive EU 2015/565). Tissues and cells used for advanced therapy medicinal products must be traceable under this directive at least until transferred to the Advanced Therapy Medicinal Product manufacturer.

16.1.2. All relevant data relating to products and materials coming into contact with tissues and cells must also be traceable.

16.1.3. Each organisation holding tissues or cells must have effective and accurate procedures to uniquely identify and label cells/tissues collected, received, processed, distributed/disposed or used for human application. The application of an SEC does not preclude the additional application of other codes in accordance with member states' national requirements.

16.1.4. For accurate transcription of critical identification information, electronic transfer should be used. If manual transcription is used, double checking of data should be implemented.

16.1.5. Responsibility for traceability among the different organisations involved in procurement, processing, distribution and human application of cells/tissues must be clearly defined. Responsibility should be defined in a written technical and legal agreement.

16.1.6. Traceability data must be kept for the long term after clinical use to allow adequate biovigilance and follow-up. In the EU, information related to traceability, as described in

Annex VI of the Directive 2006/86/EC, must be retained for at least 30 years after application or cell/tissue expiry date. Data that are critical to the safety and quality of cells/tissues should be maintained for at least 10 years.

16.1.7. Traceability data must be stored securely in an appropriate archive. In the case of change of storage location, a link between the previous location and the new location must be established.

16.1.8. Audits of traceability from donor to recipient and vice versa must be included in the quality-management plan.

17. Biovigilance

17.1. General principles

17.1.1. TEs must have documented procedures in place for the reporting of serious adverse reactions and events (SAREs) as defined in Directive 2004/23/EC.

17.1.2. There should be systems in place to ensure that adverse events, adverse reactions and near misses are documented, carefully investigated and, where necessary, followed up by the implementation of corrective actions to prevent recurrence.

17.1.3. Systems must be in place to assure the follow-up of tissue recipients and children conceived after MAR treatment.

17.1.4. There should be procedures in place for reporting SAREs in a timely manner to the Responsible Person for the TE and to the Health Authorities. Adequate resources must be made available for their immediate investigation and resolution, and for the implementation of any corrective and preventive actions.

17.1.5. There should be a co-ordinator, who has responsibility for vigilance and surveillance specified in their job description.

17.1.6. Vigilance programmes should include an activity of scanning for new risks that have not been recognised previously. New risks may be related to donors, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing.

17.1.7. Newly emerging infectious diseases, for which targeted testing can be carried out or which might imply the need to exclude certain donors, represent an example of one type of new risk as described in 17.1.6.

17.1.8. Co-ordination between various systems of vigilance (e.g. blood transfusion or haemovigilance, organ transplantation, medical device vigilance, pharmacovigilance) should be in place at the local level (TE) and at the Health Authority level.

17.1.9. Effective communication of the results of vigilance systems is fundamental to ensuring that the benefits of these programmes are realised in practice. Regular feedback to healthcare professionals is critical to support continued notification of adverse reactions and events.

17.1.10. TEs and clinicians should promote a culture that encourages reporting in a non-punitive context for the benefit of patients and donors. It should be accepted that mistakes do happen and that the human application of tissues and cells is not risk-free.

17.1.11. Programmes of training and awareness should be organised to encourage reporting. The message should be promoted that the reporting and dissemination of vigilance and surveillance information can result in positive improvements for donors and patients.

17.2. **Adverse reactions**

17.2.1. Adverse reactions must be detected, reported, investigated and assessed in terms of severity, imputability, probability of recurrence, frequency of recurrence and consequences.

17.2.2. Efficient systems for rapid quarantine and recall of unsafe tissues or cells must be in place, along with procedures for look-back where donors or recipients are found to have been exposed to a risk.

17.2.3. Important outcomes from each adverse reaction should be disseminated appropriately.

17.2.4. The following are examples of reportable adverse reactions [with abbreviated descriptions in square brackets]:

17.2.4.1. suspected harm in living donor related to procurement [donor harm];

17.2.4.2. unexpected primary infections possibly transferred from donor to recipient (e.g. viral, bacterial, parasitic, fungal, prion) [infection from donor]

17.2.4.3. suspected transmitted infection (viral bacterial, parasitic, fungal, prion) possibly due to contamination or cross-contamination by an infectious agent in the procured tissues, cells or associated materials, between procurement and their clinical application [infection from infected/contaminated tissues and cells];

17.2.4.4. unexpected hypersensitive reactions, including allergy, anaphylactoid reactions or anaphylaxis [hypersensitivity];

17.2.4.5. malignant disease possibly transferred by the tissues or cells (donor-derived, process-associated or other) [malignancy];

17.2.4.6. unexpected delayed or absent engraftment or graft failure (including mechanical failure) [failure];

17.2.4.7. toxic effects on tissues and cells or associated materials [toxicity];

17.2.4.8. unexplained immunological reactions due to tissue or cell mismatch [mismatch];

17.2.4.9. aborted procedure involving unnecessary exposure to risk (e.g. wrong tissue supplied, discovered after patient is anaesthetised and the surgical procedure has begun) [undue risk];

17.2.4.10. suspected transmission of genetic disease by transplantation or gamete/embryo donation [genetic abnormality];

17.2.4.11. suspected transmission of other (non-infectious) illness [other transmission];

17.2.4.12. transfusion-associated circulatory overload in haematopoietic progenitor cell transplantation [volume overload];

17.2.4.13. neurological reaction [insult];

17.2.4.14. severe febrile reaction [fever];

17.2.4.15. other [other].

17.2.5. The TE is responsible for providing clinical-user entities, procurement organisations and critical third parties with clear instructions, forms and guidance on how to notify adverse reactions in accordance with national and local requirements.

17.2.6. If serious adverse reactions (SARs) are detected in relation to tissues or cells that have entered international distribution channels, appropriate international collaboration should ensure that all those involved (clinicians, TEs and Health Authorities) in each of the countries concerned are informed and participate, if necessary, in the investigation and follow-up.

17.3. **Adverse events**

17.3.1. Adverse events can occur at any moment from donor selection to clinical application. For effective detection of adverse events, all relevant parties must be aware of their responsibilities for identifying errors or unexpected results. This includes all staff at TEs and procurement organisations, those working in organisations such as testing laboratories that provide 'third party' services to TEs, and clinical users who may also detect errors at the point of clinical use. In EU Directive 2006/86/EC, the definition of a serious adverse event (SAE) includes those incidents often referred to as 'near misses', i.e. where an error or fault is detected and corrected without causing harm.

17.3.2. Deviations from the requirements of the quality system should be documented and investigated as part of the internal QMS. On occasions, however, a deviation may be of such importance that it should be considered an SAE and reported through the vigilance system.

17.3.3. According to instructions from the European Commission and EU member states for annual vigilance reporting, deviations from SOPs in TEs (or other adverse events) that have implications for the quality and safety of tissues and cells should result in SAE reporting to the Health Authority if one or more of the following criteria apply:

17.3.3.1. inappropriate tissues or cells have been distributed for clinical use, even if not used;

17.3.3.2. aborted procedure involving unnecessary exposure to risk e.g. wrong tissue supplied, discovered after patient had been anaesthetised and the surgical procedure had begun [undue risk];

17.3.3.3. the event could have implications for other patients or donors because of shared practices, services, supplies or donors;

17.3.3.4. the event has resulted in a mix-up of gametes or embryos;

17.3.3.5. the event has resulted in a loss of traceability of tissues or cells;

17.3.3.6. the event has resulted in a loss of any irreplaceable autologous tissues or cells or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;

17.3.3.7. the event has resulted in the loss of a significant quantity of allogeneic tissues or cells.

Appendices

Appendix 1. **General reference documents used**

The experts who developed the chapters in this Guide incorporated principles and specific text from many regulatory, professional and scientific publications. The following are the principal reference documents used.

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Appendix 2. **Acronyms and other abbreviations**

3D	three-dimensional
AATB	American Association of Tissue Banks
AAV	*adeno*-associated virus
Ab	antibodies
ACI	autologous chondrocyte implantation
AFC	antral follicle count
AIDS	acquired immunodeficiency syndrome
ALK	anterior lamellar keratoplasty
AMH	anti-Müllerian hormone
Anti-CMV	antibody to *Cytomegalovirus*
Anti-EBV	antibody to Epstein–Barr virus
Anti-HBc	antibody to hepatitis B core antigen
Anti-HCV	antibody to hepatitis C virus
Anti-HIV-1	antibody to HIV-1
Anti-HIV-2	antibody to HIV-2
ARE	adverse reactions and events
ART	assisted reproductive technology
ARTHIQS	Assisted Reproductive Technologies and Haematopoietic stem cells for transplantation Improvements for Quality and Safety throughout Europe [joint action]
ATMP	advanced therapy medicinal products
aW	available water
BET	bacterial endotoxin test
BFU-E	burst-forming units erythroblast
BM	bone marrow
BMDW	Bone Marrow Donors Worldwide [organisation: now part of WMDA]
BMP	bone morphogenetic proteins
BMSC	bone marrow stromal cells
BSS	balanced salt solution
CAPA	corrective and preventive action
CAR	chimeric antigen receptor
CAT	Committee for Advanced Therapies
CBC	complete blood count
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* infection
CD-P-TO	European Committee (Partial Agreement) on Organ Transplantation of the Council of Europe
CE (marked)	Conformité Européenne
CEA	cultured epithelial autografts
CFU	colony-forming units
CFU-GM	colony-forming units-granulocyte/monocyte
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CJD	Creutzfeldt–Jakob disease
CLET	cultivated limbal epithelial transplantation
CMV	*Cytomegalovirus*
CNS	central nervous system
CNT	Centro Nazionale Trapianti (Italy)
COC	cumulus enclosed oocyte
COD	cause of death
COHS	controlled ovarian hyperstimulation
COMET	cultivated oral mucosal epithelial transplantation
DALK	deep anterior lamellar keratoplasty
DBD	deceased by brain death
DBM	demineralised bone matrix
DBO	Department of Biological Standardisation, OMCL Network & HealthCare (at the European Directorate for the Quality of Medicines & HealthCare)
DC	dendritic cells

DCD	deceased by circulatory death
DH-BIO	Committee on Bioethics of the Council of Europe
DLI	donor lymphocyte infusions
DM	Diabetes mellitus
DMEK	Descemet membrane endothelial keratoplasty
DMSO	dimethyl sulfoxide
DQ	design qualification
DSAEK	Descemet stripping automated endothelial keratoplasty
DVT	deep-vein thrombosis
EATCB	European Association of Tissue and Cell Banks
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein–Barr virus
EC	European Commission
ECCTR	European Cornea and Cell Transplant Registry
ECDC	European Centre for Disease Prevention and Control
ECM	extracellular matrix
ECVAM	European Centre for the Validation of Alternative Methods
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	ethylenediamine tetra-acetic acid
EEBA	European Eye Bank Association
EGF	endothelial growth factor
EGTA	ethylene glycol tetra-acetic acid
EIA	enzyme immunoassay
EK	endothelial keratoplasty
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMDIS	European Marrow Donor Information System
EQSTB	European Union project 'European Quality System for Tissue Banking'
ESBL	extended-spectrum beta lactamases
ESC	embryonic stem cell
ESHRE	European Society for Human Reproduction and Embryology
EU	European Union *not* endotoxin units (*see* IU)
EUROCET	European Registry for Organs, Tissues and Cells
Eurocode IBLS	Eurocode International Blood Labelling Systems
EuroGTP	Euro Good Tissue Practices [EU project]
EuroGTP-II	Good [Tissue] Practices for demonstrating safety and quality through recipient follow-up [EU project]
EUSTITE	European Standards and Training in the Inspection of Tissue Establishments [EU project]
EUTC	European Code for Tissues and Cells
FACS	fluorescence-activated cell sorting
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration (USA)
FED	Fuchs endothelial dystrophy
FIPS	fingerprints
FMEA	failure mode and effects analysis
FMECA	failure mode, effects and criticality analysis
FMT	faecal microbiota transplantation
FNHTR	febrile non-haemolytic reactions
FOS	fastidious organism supplement
FSH	follicle-stimulating hormone
GAG	glycosaminoglycans
G-CSF	granulocyte-colony stimulating factor
GEMM	granulocyte-erythrocyte-macrophage-megakaryocyte
GF	growth factors
GM	granulocytes and macrophages
GM-CSF	granulocyte macrophage-colony stimulating factor
GMP	Good manufacturing practice [EU document]
GPA	glycerol-preserved allografts
GTP	good tissue practice
GV	germinal vesicle
GvHD	graft-*versus*-host disease
GvT	graft-*versus*-tumour
HACCP	hazard analysis and critical control points
hAM	human amniotic membrane
HAV	hepatitis A virus
HBc	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HEPA	high-efficiency particulate air
HES	hydroxyethyl starch
HHV	human *Herpes* virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMG	human menopausal gonadotrophin
HPC	haematopoietic progenitor cells
HPV	human *papilloma* virus
HRV	human *rotavirus*
HSC	haematopoietic stem cells
HSG	hysterosalpingography
HSV	*Herpes simplex* virus
HTLV	human T-lymphotrophic virus
HVAC	heating, ventilating, and air conditioning
HyCoSy	hysterosalpingo-contrast sonography

IATA	International Air Transport Association
ICCBBA	International Council for Commonality in Blood Banking Automation
ICMART	International Committee Monitoring Assisted Reproductive Technologies
ICSI	intracytoplasmic sperm injection
ICU	intensive care unit
IDM	infectious disease marker
IEC	Independent Ethics Committee
IFN	interferon
Ig	immunoglobulin
IL	interleukin
iPSC	induced pluripotent stem cell
IQ	installation qualification
ISCT	International Society for Cellular Therapy
ISN	International Society for Nephrology
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineering
ISSCR	International Society for Stem Cell Research
IT	information technology
IU	international unit (*for endotoxins*)
IUI	intra-uterine insemination
IVF	*in vitro* fertilisation
IVM	*in vitro* maturation
JACIE	Joint Accreditation Committee–ISCT & EBMT
KIR	killer immunoglobulin-like receptors
KLAL	keratolimbal allograft
KPI	key performance indicator
LAL	limulus amoebocyte lysate
LH	luteinising hormone
LSC	limbal stem cells
MCM	metastatic cutaneous melanoma
MESA	microsurgical epididymal sperm aspiration
MII	metaphase II
MNC	mononuclear cells
MPHO	medical products of human origin
MRA	marrow re-populating ability
MRSA	methicillin-resistant *Staphylococcus aureus*
MSC	mesenchymal stromal (stem) cells
MTT	tetrazolium salt assay
NAC	nipple–areola complex
NAT	nucleic acid amplification technique/ nucleic acid test
NEC	necrotising enterocolitis
NICE	National Institute of Health and Clinical Excellence
NK	natural killer
NRT	neutral red test
NtPSC	nuclear-transfer pluripotent stem cells
OA	osteoarthritis
OECD	Organisation for Economic Co-operation and Development
OHSS	ovarian hyperstimulation syndrome
ONT	Organización Nacional de Trasplantes (Spain)
OQ	operational qualification
ORHA	organisation responsible for human application
Parvo-B19	*Parvovirus* B19
PBK	pseudophakic bullous keratopathy
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
PESA	percutaneous epididymal sperm aspiration
PGD	pre-implantation genetic diagnosis, *see* PGT
PGS	pre-implantation genetic screening, *see* PGT
PGT	pre-implantation genetic testing (formerly known as PGD and PGS, *see above*)
PGT-A	pre-implantation genetic screening for aneuploidy screening
PGT-M	pre-implantation genetic diagnosis for monogenic/single gene defects
PGT-SR	pre-implantation genetic diagnosis for chromosomal structural rearrangements
Ph. Eur.	*European Pharmacopoeia*, 9th edn, Strasbourg: Council of Europe 2016
PIC/S	Pharmaceutical Inspectorate Co-operation Scheme
PK	penetrating keratoplasty
PN	ProNucleus
PO	procurement organisation
POI	premature ovarian insufficiency
POSEIDON	Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe [EU project]
PQ	performance qualification
PRF	platelet-rich fibrin
PRIVILEGED	Privacy in Law, Ethics and Genetic Data [EU project]
PROH	1,3-propanedial
PRP	platelet-rich plasma
PVP	PolyVinylPyrrolidone
QC	quality control
QM	quality manager
QMS	quality management system
QRM	quality risk management
RABS	Restricted Access Barrier System
RANTES	regulated on activation, normal T-cell expressed and secreted

RATC	rapid alerts for tissues and cells
RBC	red blood cell
RCT	randomised control trial
RhD	Rhesus D antigen
rhG-CSF	recombinant granulocyte-colony stimulating factor
RP	Responsible Person
RPN	risk priority number
SAE	serious adverse event
SAL	sterility assurance level
SAR	serious adverse reaction
SARE	severe adverse reactions and events
SDS	sodium dodecyl sulfate
SEC	Single European Code
SIG	special interest group
SoHO V&S	Vigilance and Surveillance of Substances of Human Origin
SOP	standard operating procedure
SP-CTO	Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation
S(P)EAR	serious (product) events and reactions
SSC	spermatogonial stem cell
SVF	stromal vascular fraction
T1DM	Type-1 diabetes mellitus
TAMC	total aerobic microbial count
TBV	total blood volume
TCR	T-cell receptor
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TGF	tumour growth factor/transforming growth factor
TNC	total nucleated cells
TNF	tumour necrosis factor
TPV	total plasma volume
TRALI	transfusion-related acute lung injury
TSB	tryptic soy broth
TSE	transmissible spongiform encephalopathy
TTS	The Transplantation Society
TYMC	total combined yeasts/moulds count
UCB	umbilical cord blood
UPS	uninterrupted power supply
V&S	vigilance and surveillance
vCJD	Variant Creutzfeldt–Jakob disease
VEGF	vascular endothelial growth factor
VISTART	Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation [joint action]
VMP	validation master plan
VOC	volatile organic compound
VRE	Vancomycin-resistant *enterococci*
WHO	World Health Organization
WMDA	World Marrow Donor Association
WNV	West Nile virus

Appendix 3. **Glossary**

Acceptance criteria	Requirements needed to meet the relevant quality and safety standards in order to ensure an acceptable final product for human application.
Adipose tissue	Loose connective tissue, composed of adipocytes and stromal vascular fraction, which serves as energy storage and endocrine organ.
Advanced therapy medicinal product	A medicinal product that can be a gene therapy medicinal product, a somatic cell therapy medicinal product, a tissue-engineered product or a combined advanced therapy medicinal product (which is a medicinal product incorporating cells and medical devices or actively implantable medical devices).
Adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells. *See also:* Serious adverse event.
Adverse reaction	Any unintended response, including a communicable disease, in the donor or the recipient that is associated with the procurement or human application of tissues and cells. *See also:* Serious adverse reaction.
Agarose gel electrophoresis	Diagnostic tool to visualise DNA fragments.
Alamar blue	Indicator of cell viability based on resazurin oxidation-reduction.
Allogeneic	Refers to tissues and cells removed from one individual and applied to another of the same species.
Allograft	Tissues or cells transplanted between two genetically different individuals of the same species. The term is synonymous with 'homograft'.
AM	Amniotic membrane.
Ambient temperature	The temperature of the surrounding environment. In temperature-controlled facilities, ambient temperature is usually 17-21 °C for thermal comfort. Referred to as 'room temperature' in this Guide.
Amniotic membrane	The innermost layer of the placental membrane; it surrounds the foetus during pregnancy.
Angiogenesis	Physiological process by which new blood vessels form from pre-existing vessels.
Antibiogram	*See:* Resistogram.
Apheresis	A medical technique in which peripheral blood of a donor or patient is passed through an apparatus that separates one particular constituent and returns the remaining constituents to the donor or patient.
Aseptic techniques	Procedures designed to prevent contamination from micro-organisms and spread of infection.
Assisted reproductive technology	All interventions that include *in vitro* handling of both human oocytes and sperm, or of embryos, for the purpose of reproduction. This includes, but is not limited to, *in vitro* fertilisation (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, gamete and embryo cryopreservation, semen, oocyte or embryo donation and gestational carrier cycles. Thus, ART does not, and ART-only registries do not, include assisted insemination using sperm from a woman's partner or a sperm donor. *See also:* Medically assisted reproduction.

Audit	Periodic, independent and documented examination and verification of activities, records, processes and other elements of a quality system to determine their conformity with specific internal or external requirements. They may be conducted by professional peers, internal quality system auditors or auditors from certification or accreditation bodies.
Autologous	Refers to tissues or cells removed from and applied in the same individual.
Azoospermia	Absence of spermatozoa in the ejaculate.
Bacteraemia	The presence of viable bacteria in the circulating blood.
Banking	Processing, preservation, storage and distribution of tissues and cells for human application or other purposes, including research and training.
Barcode	An optical machine-readable representation of data relating to the object to which it is attached.
Batch	A defined quantity of starting material, packaging material or product processed in one process (or series of processes) so that it can be considered to be homogeneous.
Bioactivity	The effect of a substance upon a living organism.
Biobank	A collection of biological material and the associated data and information stored for research purposes. Also known as a bio-repository.
Bioburden	Total number of viable microorganisms or total microbial count present, on or in tissues or cells or in the environment, usually measured before the application of a decontamination or sterilisation process.
Biochemical cue	Chemical signal that occurs in a biological organism and causes a biological response.
Biocompatibility	Property of a material being compatible with living tissue. Biocompatible materials do not produce a toxic or immunological response when exposed to the body or body fluids.
Biodegradability	Disintegration of materials by biological processes.
Biomechanical cue	Mechanical signal that occurs in a biological organism and causes a biological response.
Bionics	Biologically inspired engineering is the application of biological methods and systems found in nature to the study and design of engineering systems and modern technology.
Biophysical cue	Physical signal that occurs in a biological organism and causes a biological response.
Bioprinting	Combination of cells, growth factors and biomaterials using layer-by-layer deposition to fabricate biomedical parts that maximally imitate natural tissue characteristics, including structures that are later used in medical and tissue engineering fields.
Bisbenzimidine	Organic compound used as a fluorescent stain for DNA in molecular biology applications.
Blastocyst	An embryo, around 5-6 days after fertilisation, with an inner cell mass, outer layer of trophectoderm and a fluid-filled blastocoele cavity.
Blastomere	A cell in a cleavage stage embryo.
Blood groups	ABO or ABo. Both forms are widely used, but this Guide uses O. The O is from German *ohne* ('without') and means the same as o ('zero'): these are red blood cells without A or B antigens on the cell surface.
Bone	The hard, rigid, mineralised form of connective tissue constituting most of the skeleton of vertebrates and composed primarily of calcium salts. There are two types of osseous tissue that form bones: cortical bone (the compact bone of the shaft of a bone that surrounds the marrow cavity) and cancellous or trabecular bone (typically occurs at the ends of long bones, proximal to joints and within the interior of vertebrae). Cancellous bone is highly vascular and frequently contains bone marrow.
Bone marrow	Tissue at the centre of large bones. It is the place where new blood cells are produced. Bone marrow contains two types of stem cell: haematopoietic (which can produce blood cells) and stromal (which can produce fat, cartilage and bone).
Cell	The smallest transplantable and functional unit of life.
Cell culture	Growth of cells in a nutrient medium *in vitro*.
Cell migration	Movement of cells in particular directions, often in response to specific external signals, including chemical signals and mechanical signals.
Circulation	Transfer of tissues or cells from a tissue establishment to another operator for further processing.
Cytotoxicity	Quality of being toxic to cells.
Clean area, clean environment, cleanroom	An area with defined environmental control of particulate and microbial contamination, and constructed and used in such a way as to reduce the introduction, generation and retention of contaminants within the area.
Cleavage stage embryo	Embryo, beginning with the 2-cell stage and up to, but not including, the morula stage.

Clinical evaluation	Clinical follow-up studies for monitoring predefined clinical outcome indicators to evaluate quality, safety and effectiveness/efficacy of tissue or cell product for a defined number of patients.
Coding	A system for unique identification of tissues and cells for human application, comprising a donation identifier and product identifier for the specific type of tissue or cell.
Collagen	Main structural protein.
Colonisation	The natural, biological presence or spread of micro-organisms.
Compatibility testing	Testing for the presence or absence of recipient antibodies to HLA and to blood group antigens present on the tissues or cells for transplantation.
Competent authority	*See:* Health Authority.
Computerised system	A system including the input of data, electronic processing and the output of information, to be used either for reporting or for automatic control.
Consent to donation	Lawful permission or authorisation for removal of human cells, tissues and organs for transplantation. *See also:* Opt-in donation; Opt-out donation.
Contained laboratory, contained area	According to EU GMP, an area constructed and operated in such a manner (and equipped with appropriate air handling and filtration) as to prevent contamination of the external environment by biological agents from within the area.
Contamination	Accidental inclusion or growth of harmful micro-organisms, such as bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and by-products. Contamination is different from colonisation, which is the natural, biological presence of micro-organisms.
Controlled ovarian stimulation	Pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes.
Cord blood	Blood collected from placental vessels and umbilical cord blood vessels after the umbilical cord is clamped and/or severed as a source of haematopoietic progenitor cells.
Cord blood bank	A specific type of tissue establishment in which haematopoietic progenitor cells collected from placental and umbilical cord blood vessels are processed, cryopreserved and stored. It may also be responsible for collection, testing or distribution.
Cornea	The transparent anterior part of the outer fibrous coat of the eye. A collagenous tissue bounded by an outer stratified epithelium and an inner monolayer of endothelial cells. The major refractive component of the eye.
Critical	Potentially having an effect on the quality and/or safety of (or having contact with) tissues and cells.
Cross-contamination	Unintentional transfer of micro-organisms and/or other material from one donation or processing batch to another.
Cryopreservation	Preservation and storage of viable tissues and cells (including gametes and embryos) to preserve viability, either by slow freezing or by vitrification.
Cryoprotectant	A chemical compound that is used to protect cells and tissues against freezing injury.
Cumulus cell	The multi-layered mass of granulosa cells surrounding the oocyte.
DAPI	4',6-diamidino-2-phenylindole, a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA.
Deceased donor	A person declared to be dead according to established medical criteria and from whom cells, tissues or organs have been procured for the purpose of human application. *See also:* Donor after brain death; Donor after circulatory death.
Decontamination	The process of removing or neutralising contaminants.
De-epidermisation	Process by which epidermis is removed from skin.
Delivery rate	Number of deliveries of neonates expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles. It includes deliveries that resulted in the birth of one or more live and/or stillborn babies.
Denudation	The removal or stripping of the cumulus cells from the oocyte.
Design qualification	The first step in the qualification of new equipment or facilities.
Deviation	Departure from an approved instruction/protocol or established standard.
Differentiation	Process by which a less specialised cell becomes a more specialised cell type.
Direct use	Any procedure in which tissues and cells are donated and used without banking or storage.
Discontinuous gradient centrifugation	Sperm-preparation technique based on sedimentation of sperm at different rates depending on density.
Disinfection	A process that reduces the number of viable micro-organisms, but does not necessarily destroy all microbial forms, such as spores and viruses.
Disposal (of tissues/cells)	The act or means of discarding tissues and/or cells.
Distribution	Transportation and delivery of cells or tissues intended for human application.

Donor	An individual, living or deceased, who is a source of tissues or cells for human application and for other purposes including research.
Donor after brain death	A donor who is declared dead based on the irreversible loss of neurological functions. Also known as deceased heart-beating donor.
Donor after circulatory death	A donor who is declared dead based on circulatory criteria. Also known as deceased non-heart-beating donor.
Donor evaluation	The procedure for determining the suitability of an individual, living or deceased, as a donor of cells or tissues.
Donor selection	*See:* Donor evaluation.
Double embryo transfer	Transfer of two embryos.
Effectiveness	Presence of functionality proven by *in vitro* analytics (e.g. potency assays) depending on the mode of action of the tissue or cell product.
Efficacy	Presence of desired clinical effects/ patient outcome depending on the mode of action of the tissue or cell product.
Elastin	Highly elastic protein in connective tissue that allows many tissues in the body to resume their shape after stretching or contracting.
Electron beam irradiation	Use of beta irradiation, usually of high energy under elevated temperatures and nitrogen atmosphere, for sterilisation or cross-linking of polymers.
Embryo	The result of continued development of the zygote to 8 completed weeks after fertilisation, equivalent to 10 weeks of gestational age.
Embryo biopsy	The removal of cells (blastomeres or trophectoderm cells) from the embryo for the purpose of genetic analysis.
Embryo donation	Transfer of an embryo resulting from gametes (spermatozoa and oocytes) that did not originate from the recipient and her partner.
Embryo transfer	Procedure in which one or more embryos are placed in the uterus or Fallopian tube.
Emerging disease	A disease that has recently appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range.
End user	A healthcare practitioner who undertakes human application procedures.
Endotoxins	Large molecules consisting of a lipid and a polysaccharide, which are found in the outer membrane of Gram-negative bacteria.
Error	A mistake or failure to carry out a planned action as intended, or application of an incorrect plan that may or may not cause harm to patients.
Escharectomy	Surgical procedure based on removal of necrotic skin tissue from a full-thickness burn.
Ethylene oxide	Organic compound and toxic gas, being a surface disinfectant widely used in hospitals and the medical equipment industry for sterilisation.
Exceptional release	The distribution for clinical use of a unit of tissues and/or cells that does not fully comply with the defined safety and quality criteria for release. The release is justified by a specific clinical need in which the benefit outweighs the risk associated with the non-compliance. *See also:* Negative-to-date release.
Expert	Individual with the appropriate qualifications and experience to provide technical advice to a health authority inspector.
Expiry date	The date after which tissues or cells are no longer suitable for use. Also known as 'expiration date'.
Export	Act of transporting a tissue or cell intended for human application to another country where it is to be processed further or used directly. In the EU, 'export' refers to transport to a third country (i.e. outside the EU).
Facility	A physical building or part of a building.
Fallopian tube	A long duct in the female abdomen that transports the oocytes that have been released from the ovary to the uterus.
Fascia	A layer of fibrous connective tissue that surrounds muscles, groups of muscles, blood vessels and nerves; it binds some structures together while permitting others to slide smoothly over each other.
Fertilisation	Entry of the oocyte by a spermatozoon followed by combination of their genetic material, resulting in the formation of a zygote.
Fertility	The capacity to establish a clinical pregnancy.
Fertility preservation	Cryopreservation of reproductive tissues or cells to preserve reproductive capacity.
Fibronectin	High-molecular glycoprotein of the ECM that plays a major role in cell adhesion, growth, migration and differentiation.
Final product	Any tissue or cell preparation intended to be transplanted or administered after the final release step.
Follow-up	Subsequent evaluation of the health of a patient, living donor or recipient, for the purpose of monitoring the results of the donation or human application, maintaining care and initiating post-donation or post-application interventions.
Freeze drying	*See:* Lyophilisation.

Freezing	As used in this guide, it means storage of tissues at high sub-zero temperatures, with or without cryo-protectant.
Full-thickness grafts (FTSG)	Graft composed of epidermis and full-thickness dermis (with adnexal structure).
Fungaemia	The presence of fungi in the circulating blood.
Gamma irradiation	Penetrating electromagnetic radiation arising from the radioactive decay of atomic nuclei. It is used for medical equipment sterilisation.
Glycosaminoglycans (GAG)	Long unbranched polysaccharides that are highly polar and thus attract water; useful as a lubricant or shock absorber.
Good laboratory practice	Set of principles that provides a framework within which studies are planned, carried out, monitored, recorded, reported and archived by laboratories conducting testing of all kinds.
Good Manufacturing Practice	An EU standard applied internationally for the safe manufacture of medicinal products. Although the processing of tissues and cells is not normally regulated under medicinal manufacturing legislation, many of the principles of GMP can be applied usefully to tissues and cells for human application.
Good practice	A method or technique that has consistently shown results superior to those achieved by other means and which is currently used as a benchmark.
Graft	Part of the human body that is transplanted in the same or another person to replace a damaged part or to compensate for a defect.
Grafting	*See:* Transplantation.
Haematopoietic progenitor cells	Primitive haematopoietic cells capable of self-renewal as well as maturation into any of the haematopoietic lineages, including committed and lineage-restricted progenitor cells, unless otherwise specified and regardless of tissue source. Also known as 'haematopoietic stem cells'.
Haematoxylin-Eosin	Histology staining used in medical diagnosis.
Haemodilution	In reference to blood samples from a donor, a decrease in the concentration of the donor's plasma proteins, circulating antigens or antibodies and any infectious agent present, resulting from the transfusion of blood or blood components and/or infusion of fluids, e.g., colloid(s) and/or crystalloid(s). Also known as 'plasma dilution'.
Haemolysis	Damage to red cells resulting in the release of haemoglobin into serum/plasma.
Health Authority	In the context of this Guide, the body which has been delegated with the responsibility for ensuring that tissue and cell donation, banking and human application are appropriately promoted, regulated and monitored in the interests of donor and patient safety and public transparency on a national or regional basis by their government. Other terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it.
Heart valve	One of the four structures within the heart that prevent the backflow of blood by opening and closing with each heartbeat. They include two semilunar valves (aortic and pulmonary), the mitral (or bicuspid) valve and the tricuspid valve. They permit blood flow in only one direction.
Homograft	*See:* Allograft.
Human application	Use of tissues or cells on or in a human recipient.
Human error	A mistake made by a person rather than being caused by a poorly designed process or by the malfunctioning of a machine such as a computer.
Human tissues and cells for human application	Material containing or consisting of human tissues and/or cells intended for implantation, transplantation, infusion or transfer into or onto a human recipient.
Hybrid scaffold	Scaffold obtained using different types of materials.
Hydroxiproline quantification	Hydroxiproline is a non-essential amino acid (proline derivative) which results from collagen acid hydrolysis.
Identification of tissues and cells	The labelling of tissues and cells to uniquely designate their origin, use or destination. *See also:* Labelling.
Immune-privileged niche	A certain site of the human body able to tolerate the introduction of antigens without eliciting an inflammatory immune response.
Implantation (in the context of assisted reproductive technologies)	Attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) that starts 5-7 days after fertilisation. *See also:* Transplantation.
Import	In this context, the act of bringing tissues or cells into one country from another for the purpose of human application or further processing.
Importing tissue establishment	A tissue bank or a unit of a hospital or another body established within the EU which is a party to a contractual agreement with a third-country supplier for the import into the EU of tissues and cells coming from a third country and intended for human application.

Imputability	Assessment of the probability that a reaction in a donor or recipient may be attributable to the process of donation or clinical application or to an aspect of the safety or quality of the tissues or cells applied.
In-process control	Checks undertaken during processing to monitor and, if necessary, to adjust the process to ensure that a product conforms to its specification. Control of the environment or equipment may also be regarded as a part of in-process control.
In vitro fertilisation	Assisted reproductive technology procedure that involves extracorporeal fertilisation. It includes conventional *in vitro* insemination and ICSI, for which *see* Intracytoplasmic sperm injection.
In vitro maturation	Refers to the maturation of immature oocytes after procurement from follicles that may or may not have been exposed to exogenous gonadotrophins before retrieval. Also, the *in vitro* process of maturation from immature dendritic cells (DC) to mature DC.
Incident	A generic term for an adverse reaction or adverse event.
Incident reporting (adverse event reporting, serious/critical incident reporting)	A system in a healthcare organisation for collecting, reporting and documenting adverse occurrences that affect patients and are inconsistent with planned care (e.g. medication errors, equipment failures, violations).
Informed consent	A person's voluntary agreement, based upon adequate knowledge and understanding of relevant information, to donate, to participate in research or to undergo a diagnostic, therapeutic or preventive procedure.
Inner cell mass	A group of cells in the blastocyst that give rise to the embryonic structures, the foetus, the yolk sac, the allantois and the amnion.
Inspection	On-site assessment of compliance with local/national regulations on tissues and cells, carried out by officials of the relevant Health Authority.
Installation qualification	The second step in the qualification of new equipment or facilities.
Intracytoplasmic sperm injection	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
Intra-uterine insemination	Procedure in which processed sperm cells are transferred transcervically into the uterine cavity.
Keratoplasty	Corneal transplantation.
Key performance indicator	A quantifiable measure or a set of quantifiable measures used to trace performance over time.
Labelling	Includes steps taken to identify packaged material by attaching the appropriate information to the container or package so it is clearly visible on or through the immediate carton, receptacle or packaging. *See also:* Identification of tissues and cells.
Laminine	High-molecular-weight protein of the extracellular matrix with important roles in cell differentiation, migration and adhesion.
Laparoscopy	A surgical procedure in which a small incision is made through which a viewing tube (laparoscope) is inserted.
Limbal stem cells	The population of stem cells residing in the basal epithelium of the limbus, giving rise to the corneal epithelium.
Limbal tissue	Tissue bridging the junction between the cornea and sclera. Site of the limbal stem cells that renew the corneal epithelium. Limbal stem cell deficiency causes ocular surface disease.
Limbus	The area bridging the junction between the cornea and sclera.
Live birth rate	Delivery of one or more infants with any signs of life expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles.
Living donor	A living person from whom cells or tissues have been removed for the purpose of human application.
Lyophilisation	A controlled freezing and dehydration process through the sublimation of water under vacuum from ice directly to vapour to a residual water content of < 5 %. Typically used to preserve a non-viable perishable material or to make the material more convenient for transport. Also known as freeze drying.
Malignancy	Presence of cancerous cells or tumours with a tendency to metastasise, potentially resulting in death.
Manipulation	Preparation of retrieved tissues or cells to make them suitable for human application. In the context of processing of haematopoietic progenitor cells, this is a laboratory procedure that selectively removes, enriches, expands or functionally alters the cells.
Masson trichrome	Staining protocol used in histology for distinguishing cells from the surrounding connective tissue.

Medically assisted reproduction (MAR)	Reproduction brought about through various interventions, procedures, surgeries and technologies to treat different forms of fertility impairment and infertility. These include ovulation induction, ovarian stimulation, ovulation triggering, all ART procedures, uterine transplantation and intra-uterine, intracervical and intravaginal insemination with semen of husband/partner or donor. *See also:* Assisted reproductive technology (ART).
Medicinal product	Any substance or combination of substances presented as having properties for treating or preventing disease in human beings, or which may be used in or administered to human beings with a view to either making a medical diagnosis, or restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action.
Meiotic spindle	Spindle apparatus composed of microtubules that support and segregate chromosomes during meiotic division.
Metaphase II oocyte	Mature oocyte at the metaphase of the second meiotic division.
MHC II antigen	Class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells and B cells.
Micromanipulation in ART	Technology that allows micro-operative procedures to be done on the spermatozoon, oocyte, zygote or pre-implantation embryo.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is a colourimetric assay for assessing cell metabolic activity.
Musculoskeletal	Tissues that are part of the skeleton and muscular system, including muscles, bones, cartilage, tendons and ligaments, which function in the support and movement of the body.
Negative-to-date release	The release of tissues or cells for human application before completion of testing for bacterial or fungal cultures. The cultures are negative at the time of release.
Next of kin	A person's closest living blood relative or relatives.
Non-compliance	Failure to comply with accepted standards, requirements, rules or laws.
Non-partner donation	Donation of reproductive cells between a man and a woman who do not have an intimate physical relationship; also called 'third-party donation'.
Novelty	Any new tissue and cell preparation or change that could significantly affect the quality and/or safety of tissues and cells and/or the safety of recipients.
Oligozoospermia	Total concentration of spermatozoa $< 15 \times 10^6$/mL.
One-off import	Import of any specific type of tissue or cell that is for the personal use of an intended recipient or recipients known to both the importing tissue establishment and the third-country supplier before the importation occurs. Such an import of any specific type of tissue or cell must not normally occur more than once for any given recipient. Imports from the same third-country supplier taking place on a regular or repeated basis must not be considered to be 'one-off imports' (Directive 2015/566/EC).
Oocyte	The female gamete (egg).
Oocyte cumulus complex	Oocyte surrounded by the granulosa and corona radiate cells.
Oocyte sharing	Refers to a female partner who enters ART treatment and decides to donate a specified number of her retrieved oocytes. Also known as egg sharing.
Operational qualification	Third step in the qualification of new equipment or facilities.
Opt-in donation	System for determining voluntary consent to donate where consent has been given by an individual during their lifetime or by an individual's family after their death. Also known as explicit or express consent.
Opt-out donation	System for determining voluntary consent to donate where donation may proceed unless an individual has expressed an objection during their lifetime. Also known as presumed or deemed consent.
Organ	Differentiated and vital part of the human body, formed by different tissues, that maintains its structure, vascularisation and capacity to develop physiological functions with a significant level of autonomy.
Organ culture	Culture of the whole or parts of an organ in medium *in vitro* to preserve cell–cell and cell–matrix interactions and to maintain structure and function.
Organisation responsible for human application	A healthcare establishment or unit of a hospital or another body that carries out human application of human tissues or cells.
Ovarian hyperstimulation syndrome	An exaggerated systemic response to ovarian stimulation characterised by a wide spectrum of clinical and laboratory manifestations. It is classified as 'mild', 'moderate' or 'severe' according to the degree of abdominal distension, ovarian enlargement and respiratory, haemodynamic and metabolic complications.

Package insert	A document included in the packaging of a distributed tissue or cell product that includes important information for the end users on handling, storage, traceability and adverse outcome reporting and, in some cases, on the product's properties or characteristics.
Packaging	Packaging, including primary and secondary packaging, aims to protect tissues and cells and to present them to the operator (initial or in-process packaging) or to the clinical user (final packaging) in a suitable manner. *See also:* Primary packaging; Secondary packaging.
Packaging material	Any material employed in the packaging of tissues or cells, excluding any outer packaging used for transportation or shipment. Packaging materials are referred to as 'primary' or 'secondary' according to whether or not they are intended to be in direct contact with the product.
Paracetic acid	Organic compound used for the disinfection of medical supplies to prevent biofilm formation.
Partner donation	Donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship.
Percutaneous epididymal sperm aspiration	Sperm aspiration by percutaneous puncture of the epididymis by a fine-needle technique.
Performance qualification	The fourth step in the qualification of new equipment or facilities.
Pericardium	A double-walled sac that contains the heart and the roots of the great vessels.
PicoGreen assay	Selective dsDNA quantification of as little as 25 pg/mL.
Placenta	An organ that connects the developing foetus to the uterine wall to allow nutrient uptake, waste elimination and gas exchange via the mother's blood supply.
Plasma dilution	*See:* Haemodilution.
Polar body	A haploid cell with very little cytoplasm that is formed and is separated from the oocyte during meiosis and that contains a nucleus produced in the first or second meiotic division.
Pooling	Physical contact or mixing in a single container, of tissues or cells from more than one procurement from the same donor, or from two or more donors.
Posthumous donation	The donation of tissue or cells after the donor's death with prior written consent of the donor.
Pre-implantation genetic testing	A test performed to analyse the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities. These include: PGT for aneuploidies (PGT-A); PGT for monogenic/single gene defects (PGT-M); and PGT for chromosomal structural rearrangements (PGT-SR).
Preservation	Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of tissues or cells.
Primary packaging	Any material employed in the packaging of tissues and cells that is intended to be in direct contact with the graft, excluding any outer packaging used for transportation or shipment.
Procedures	Description of all the tasks, operations and processes to be carried out, the precautions to be taken and measures to be applied to ensure the quality and safety of tissues and cells from procurement through processing, testing and storage to human application.
Processing	All operations involved in the preparation, manipulation, preservation, storage and packaging of tissues or cells intended for human application.
Procurement	A process by which tissues or cells are made available for banking or human application. This process includes donor identification, evaluation, obtaining consent for donation, donor maintenance and retrieval of tissues, cells or organs.
Procurement organisation	A healthcare establishment or a unit of a hospital or another body that undertakes the procurement of human tissues or cells.
Proliferation	Rapid reproduction of a cell.
Pronucleus	The nucleus of the sperm or the oocytes during the process of fertilisation, after the sperm has entered the oocytes but before they fuse.
Prophase I oocyte	Immature oocyte at the prophase of the first meiotic division.
Propidium Iodide	Fluorescent intercalating agent used to evaluate cell viability or DNA content in cell cycle analysis.
Pyrogenic	Producing or produced by heat or fever.
Pyrogens	Substance, typically produced by a bacterium, which produces fever when introduced or released into the blood.

Qualification	According to EU GMP, the action of proving that any equipment works correctly and actually leads to the expected results. More generally, qualification is applied to the inputs to a process, i.e. equipment, facilities, materials and software (and their suppliers), as well as to operators and the relevant written procedures.
Quality	Fulfilment of a specific set of standards, characteristics and requirements.
Quality assurance	The actions planned and performed to provide confidence that all systems and elements that influence the quality of the product are working as expected, both individually and collectively.
Quality control	The part of quality management focused on fulfilling quality requirements. In terms of preparation, it concerns sampling specifications and testing; for an organisation, it relates to documentation and release procedures, which together ensure that the necessary and relevant tests have actually been carried out and that materials have not been released for use until their quality has been judged to be satisfactory.
Quality improvement	The actions planned and performed to develop a system to review and improve the quality of a product or process.
Quality management system	The organisational structure, with defined responsibilities, procedures, processes and resources, for implementing quality management, including all activities that contribute to quality, directly or indirectly.
Quarantine	The initial status of procured tissues or cells while awaiting a decision on their acceptance or rejection, or tissues or cells isolated physically or by other effective means from other donated material for other reasons until their suitability for use is established.
RABS	Restricted Access Barrier System
Randomised control trial	A study in which samples or subjects are allocated at random into groups, called the 'study' and 'control' groups, to receive or not receive an experimental therapeutic intervention.
Rapid alert	An urgent communication to relevant individuals/organisations to ensure the protection of donors or recipients when an unexpected risk has been identified.
Recall	Removal from use of specific stored or distributed tissues and cells that are suspected or known to be potentially harmful. *See also:* Return; Withdrawal.
Recipient	Person to whom human tissues, cells or reproductive cells and embryos are applied.
Recovery	*See:* Procurement.
Registry	A repository of data collected on tissue, cell and organ donors and/or recipients for the purpose of audit, clinical outcome assessment, quality assurance, validation, healthcare organisation and planning, research and surveillance.
Regulatory authority	*See:* Health Authority.
Release	The act of certifying compliance of a specific tissue or cells of batch of tissues or cells with the requirements and specifications.
Remodelling	Change of the micrometric structure.
Reproductive cells	Oocytes and spermatozoa – in this Guide, oocytes and spermatozoa collected to be used for the purpose of assisted reproduction or fertility preservation.
Resistogram	The result of a test for the sensitivity of an isolated bacterial strain to different antibiotics. Also known as an antibiogram.
Retrieval	*See:* Procurement.
Return	Sending back recalled tissues or cells to the tissue establishment that supplied them for human application.
Risk assessment	Identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm should it occur.
Root cause analysis	A structured approach to investigating and identifying the factors that resulted in the nature, magnitude, location and timing of a harmful or potentially harmful outcome.
Roughness	Quality or state of having an uneven or irregular surface.
Scaffold	A structure made using scaffolding.
Sclera	Fibrous white outer coat of the eye.
Secondary packaging	Any material employed in the packaging of tissues and cells that is not intended to be in direct contact with the graft, and excluding any outer packaging used for transportation or shipment.
Semen analysis	A description of the ejaculate to assess function of the male reproductive tract. Characteristic parameters may include volume and pH, the concentration, motility, vitality and morphology of spermatozoa, and the presence of other cells.
Septicaemia	A systemic disease caused by the spread of pathogenic microorganisms or their toxins via the circulating blood.

Serious adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or to life-threatening, disabling or incapacitating conditions for a patient, or which might result in, or prolong, hospitalisation or morbidity (Directive 2004/23/EC).
Serious adverse reaction	An unintended response, including a communicable disease, in the donor or in the recipient, associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling or incapacitating or which results in, or prolongs, hospitalisation or morbidity (Directive 2004/23/EC).
Shipment	A type of transport where the transfer of tissues or cells from the distributing to the receiving facilities is carried out by means of a contract with a third party, usually a specialised logistics company.
Skin	Thin layer of tissue forming the natural outer covering of the human body. Skin is composed of two primary layers: the epidermis and dermis. These layers are separated by a thin sheet of fibres, the basement membrane. Keratinocytes constitute 95 % of the epidermis. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils and elastic fibres, embedded in proteoglycans.
Somatic cells	Any cell of a living organism other than the reproductive.
Spermatozoon	The mature male reproductive cell.
Split-thickness grafts (STSG)	Grafts composed of epidermis and partial-thickness dermis.
Sporicidal	Refers to a substance, agent or product used for killing bacterial spores.
Standard operating procedure	Written instructions describing the steps in a specific process, including the materials and methods to be used and the expected result. *See also:* procedures.
Sterilisation	Any process that eliminates or inactivates transmissible infectious agents (pathogens) containing nucleic acids, e.g. vegetative and spore forms of bacteria and fungi, parasites or viruses, present on a surface, in a fluid, in medication or in a compound such as biological culture media. Sterilisation can be achieved by applying the proper combinations or conditions of heat, chemicals, irradiation, high pressure and filtration.
Sterility assurance level	Represents the expected probability of a micro-organism surviving on an individual unit of product after exposure to a sterilisation process. SAL 10^{-6} has been established as the standard for allografts and indicates a probability of one chance in a million that one unit of product will be contaminated with a single organism after a sterilisation process. If the product meets or or exceeds this standard, grafts are then considered sterile.
Storage	Maintenance of a product under appropriate controlled conditions until distribution.
Storage temperature	Temperature at which tissues and cells must be stored to maintain their required properties.
Supercritical carbon dioxide	Fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure. It is an alternative for terminal sterilisation of biological materials and medical devices with combination of the paracetic acid.
Supernumerary embryos	Excess embryos after embryo transfer.
Surveillance	Systematic collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health responses, as necessary.
Swim up	A preparation technique based on the ability of spermatozoa to swim in the culture medium.
Tendon	A tough band of fibrous connective tissue that usually connects muscle to bone and which can withstand tension.
Terminal sterilisation	A method for achieving the sterility of a product in its sealed container and with a sterility assurance level of 10^{-6} or better.
Testicular sperm extraction/aspiration	A surgical procedure involving testicular biopsies or needle aspirations to obtain sperm for use in IVF and/or ICSI.
Third countries	Term used within the EU to refer to countries that are not members of the EU.
Third party	Any organisation that provides a service to a procurement organisation or tissue establishment on the basis of a contact or written agreement.
Time-lapse imaging	The photographic recording of microscope image sequences. In this Guide, used for documentation of gametes, zygotes, cleavage-stage embryos or blastocysts at regular intervals.
Tissue	An aggregate of cells joined together by, for example, connective structures and performing a particular function.
Tissue bank	*See:* Tissue establishment.

Tissue establishment	A facility or a unit of a hospital or another organisation where the activities of processing, preservation, storage or distribution of human tissues and cells for human application are undertaken. It may also be responsible for procurement and/or testing of tissues and cells.
Toxicity	Degree to which a substance can damage an organism.
Traceability	Ability to locate and identify a specific tissue/cell during any step from procurement, through processing, testing and storage, to distribution to the recipient or disposal. This implies the ability to identify: the donor; the tissue establishment or processing facility that receives, processes or stores the tissue and cells; and the recipient(s) at the medical facility/facilities applying the tissues and cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues and cells.
Transmissible disease	Comprises all clinically evident illnesses (i.e. characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of micro-organisms in an individual or the transmission of genetic conditions to the offspring. In the context of transplantation, malignancies and autoimmune diseases may also be transmitted from donor to recipient.
Transplantation, implantation or grafting	Transfer (engraftment) of human tissues or cells from a donor to a recipient with the aim of restoring function(s) in the body. *See also:* Implantation (in the context of assisted reproductive technologies).
Transport	The act of transferring a tissue or cellular product between distributing or receiving facilities under the control of trained personnel.
Trophectoderm	Outer layer of cells in a blastocyst (composed of trophectoderm and inner cell mass cells). A group of cells in the blastocyst that do not produce any embryonic structures but give rise to the chorion, the embryonic portion of the placenta.
Unique identification code	A code that unambiguously identifies a particular donor and donation (e.g. a unique donation + tissue product code). *See also:* Coding.
Validation	Documented evidence giving a high degree of assurance that a specific process or system, including pieces of equipment or the environmental conditions, will perform consistently to deliver a product meeting its pre-determined specifications and quality attributes, based on intended use.
Vas deferens, vasa deferentia	Tube(s) that transport(s) sperm from the epididymis to the ejaculatory ducts.
Verification	Preferred term for the validation or qualification of IT systems/software.
Vigilance	Alertness to and/or awareness of serious adverse events, serious adverse reactions or complications related to donation and human application of tissues, cells and organs, involving an established process for reporting at local, regional, national or international level. *See also:* Surveillance.
Viraemia	The presence of viruses in the blood.
Vitrification	Method of ice-free cryopreservation achieved through an extreme elevation in solution viscosity sufficient to suppress the crystallisation of water. Requires rapid cooling and/or high concentrations of solutes, such as the conventional cryoprotectants, to reach the glass transition temperature without ice formation.
Wettability	Tendency of one fluid to spread on, or adhere to, a solid surface in the presence of other immiscible fluids. Wettability refers to the interaction between fluid and solid phases.
Window period	Period of time before infection can be detected by a specific testing method.
Withdrawal	Process instigated by a tissue establishment to recall tissues or cells that have been distributed.
Xenograft	Graft of tissue taken from a donor of one species and grafted into a recipient of another species.
Xenotransplantation	Any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live tissues, cells or organs from a non-human animal source, or (b) human body fluids, tissues, cells or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs.
Zygote	A diploid cell resulting from the fertilisation of an oocyte by a spermatozoon, before completion of the first mitotic division.

Appendix 4. **Example of cleanroom qualification**

Short description of equipment or process being validated.

Qualification of cleanrooms for use in regulated environments.

Details of equipment used in the validation.

An active Environmental Monitoring System (EMS)

Settle plates

Contact/air sampling plates

Particle counter

Active air sampler

Details of testing levels and methods used in validation.

Eudralex Volume 4, "The rules governing medicinal products in the European Union", Annex 1: Manufacture of sterile medicinal products

SOP254 – Environmental monitoring using contact plates

SOP975 – Environmental monitoring using the active air sampling

SOP978 – Environmental monitoring using settle plates

SOP2382 – Environmental monitoring equipment

SOP4007 – SCI cleanroom cleaning

ISO14644 – BSEN14644 and EU GMP cleanroom standards

Installation qualification

No.	Description	Acceptance criteria	Results	Pass/ Fail	Comments	Signature and date
1.	Cleanroom designed in accordance with required operating specifications.	Appropriate specifications available and cleanroom designed to meet specifications. Current drawings for cleanroom layout and air handling unit(s) are available.				
2.	Cleanroom layout, fixtures and finishes are installed according to the current drawings and are of an appropriate standard.	Cleanroom finishes are smooth, impervious, non-shedding and crack and crevice-free. Floor to wall, wall to wall and wall to ceiling junctions are coved and finished in vinyl and are defect-free. All wall and ceiling penetrations are fully sealed with silicone sealant and are defect-free. Light fittings and filter housings are surface-mounted and are fully sealed with silicone sealant and are defect-free. There are no uncleanable recesses and minimal projecting ledges, shelves, cupboards and equipment. Fixtures, fittings and cleanroom furniture are all present, secure and free of rust and defects. Cleanroom entry/exit doors and pass-through hatch doors are interlocked or otherwise controlled to prevent both doors being opened simultaneously.				
3.	Confirm access to the EMS system data is available.	Records must be accessible during the validation process.				
4.	Confirm that particle counters and differential pressure monitoring systems are calibrated and available.	In-date calibration certificates must be available and equipment free for use during the entire validation period.				
5.	Ensure cleanroom and associated air handling unit(s) is registered as an asset in QPulse.	QPulse asset number must be generated.				

Operational qualification

No.	Description	Acceptance criteria	Results	Pass/ Fail	Comments	Signature and date
1.	Particle Challenge Leak Test for testing of each installed HEPA filter using DOP.	External contractor to perform DOP testing of facilities in accordance with ISO 14644-3. Aerosol concentrations must be ≤ 0.01 % of the upstream concentration.				
2.	Air exchange rate testing. Tested in accordance to BSEN 14644-3-2005.	External contractor to measure airflow volume or airflow velocity. Air change rate in compliance with design specification and should achieve > 20 air changes per hour.				
3.	Particle Counting for classification of the cleanroom.	External contractor to perform particle counting in cleanroom to meet EU GMP Annex 1 "at rest" limits for particulates (working to ISO 14644-1).				
4.	Air flow distribution testing using smoke visualisation.	External contractor to perform smoke visualisation test in accordance with ISO 14644-3 demonstrating: • flow distribution is satisfactory within each room; • any dead spots within each room have been identified; • no areas of excessive turbulence below working height exist (that could lead to particulate contamination).				
5.	Perform a weekly clean of the cleanroom as per SOP 4007.	Cleanroom cleaning must be easy to facilitate and unobstructed.				
6.	Perform weekly at rest environmental monitoring as per SOP 254, SOP 975 and SOP 978.	Full set of plates must be exposed and results shown to not exceed EU GMP Annex 1 limits for microbial contamination.				
7.	Perform routine weekly at rest particle counting monitoring as per SOP 2382.	Full set of counts must be obtained in accordance with SOP 2382, and checked for compliance with EU GMP Annex 1 "at rest" limits.				

No.	Description	Acceptance criteria	Results	Pass/ Fail	Comments	Signature and date
8.	Record the differential pressures for cleanroom facilities during "at rest" monitoring.	Daily records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades at rest).				
9.	Perform simulated operation environmental monitoring as per SOP 254, SOP 975 and SOP978.	Full set of plates must be exposed whilst non-clinical cleanroom work is performed, and results shown not to exceed EU GMP Annex 1 limits for microbial contamination.				
10.	Perform simulated operation particle counting monitoring.	Full set of counts must be obtained whilst non-clinical cleanroom work is performed and checked to ensure compliance with EU GMP Annex 1 "in operation" limits.				
11.	Record the differential pressures for cleanroom facilities "simulated operation" monitoring.	Records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades during working).				
12.	Review at rest and simulated operation results and data.	Results should not highlight any problems or trends. All results must not exceed the upper limits for the relevant grades of room, in compliance with EU GMP Annex 1.				
13.	Particulate clean-up rate within stipulated limits.	Particulate air recovery/ clean up rate must be achieved within 20 minutes in the at rest state and after operators/working has left the room (and after simulated operation tests).				

Performance qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Perform a weekly clean of the clean room as per SOP 4007 for a minimum of an 8-week period.	Clean room cleaning must be easy to facilitate and unobstructed.				
2.	Perform environmental monitoring as per SOP 254, SOP 975 and SOP 978 for a minimum of 8 consecutive weeks.	Full set of plates must be exposed at least weekly (in either the at rest or in use state) and results shown not to exceed EU GMP Annex 1 limits.				
3.	Perform particle counting monitoring for a minimum of 8 consecutive weeks.	Full set of counts must be obtained at least once per week and in accordance with SOP 2382, and checked for compliance with EU GMP Annex 1 limits (at rest or in operation, as appropriate for time of monitoring).				
4.	Record the differential pressures for clean room facilities for a minimum of 8 weeks of continued monitoring.	Records must be obtained for differential pressures for each day that environmental monitoring is performed, and checked for compliance with the design specification and EU GMP (10-15 Pa between adjacent rooms of different grades.				
5.	Review results and data. Identify any issues and trends.	Results should not highlight any problems or trends. All results must not exceed the action limits for the relevant grades of room, in compliance with EU GMP Annex 1.				

Note: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live.

Deviations and adverse events

QPulse No.	Details	Date raised	Date closed

Further testing details (if applicable)

Source: National Health Service (NHS), United Kingdom.

Appendix 5. **Example of incubator qualification**

Short description of equipment or process being validated.

Clean room incubators are used within processing to incubate samples at a set temperature for culture. Temperature of each incubator needs to be assessed prior to use in order to evaluate suitability of incubator for use and position of the temperature monitoring system probe.

Details of equipment used in the validation.

Calibrated temperature monitoring devices.

Supplier: _____

Model: _____

Serial No. _____

Details of testing levels and methods used in validation.

See IQ, OQ, PQ description and acceptance criteria.

Temperature mapping carried out as per SOP XXX

Recorded on FRM XXX

Installation qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Site incubator	Undamaged on delivery and fits designated area satisfactorily.				
2.	Ensure that clean-room air flow is not affected	Air flow is satisfactory				
3.	Instruction manual	Manual present				
4.	Certificate of conformance	Certificate of conformance				
5.	Register warranty	Register warranty				
6.	Add to asset register	Add to asset register				
7.	Cleaning instructions provided by supplier	Instructions supplied				

Operational qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Incubator functions	Switches on				
2.	Ensure shelves fitted correctly	Shelves fitted correctly				
3.	Create SOP and FRM for incubator use, cleaning and maintanence.	FRM and SOP created				
4.	Clean incubator as per instructions provided	Batch numbers/expiry of cleaning products recorded				
5.	Swab each shelf onto TSA and SABC agar plates and send for incubation	Swab results clear and appended				
6.	Set temperature to required level	Set temperature to required setting.	Temperature setting: __ °C			
7.	Set CO_2 % level to required level	CO_2 level set to required setting	CO_2 level setting: __ %			

Performance qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Perform initial temperature mapping (EMPTY)	Satisfactory as per SOP XXX				
2.	Site temperature mapping probe	As informed by step 1				
3.	Connected to Environmental monitoring system (EMS) and ensure temperature alarms are set	Connected for both high and low alarms Low alarm limit: High alarm limit: Delay time:	EMS alarm name: Low alarm limit: High alarm limit:		Append EMS record	
4.	Set CO_2 levels on Environmental monitoring system (EMS)	Connected for both high and low alarms. Delay time for alarms calculated by comparing readings on incubator with EMS. Low Alarm Limit: High Alarm limit: Delay Time:	EMS alarm name: Low alarm limit: High alarm limit: Delay time:		Append EMS record	
5.	Perform empty but humidified temperature mapping	Satisfactory as per SOP XXX				
6.	Perform simulated/ full load, humidified temperature mapping	Satisfactory as per SOP XXX			Simulated load details recorded in mapping record	
7.	Enable EMS	EMS alarms enabled				

Note: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live.

Deviations and adverse events

QPulse No.	Details	Date raised	Date closed

Further testing details (if applicable)

Source: National Health Service (NHS), United Kingdom.

Appendix 6. Example of process validation – tissue transportation[1]

The example of a process validation outlined below describes a process that will be common to most, if not all, tissue establishments. That is, the need to transport tissues from one place to another (for example, from the site of procurement to the processing facility or from the tissue bank to the end user). Control of the conditions of transportation is critical for ensuring tissue quality. The example provided below refers specifically to the transportation of skin allografts from the procurement site to a tissue establishment at refrigerated temperatures. However, the principles are identical for all types of transportation.

The first stage is to define the process in detail. This was achieved by addressing the following questions:

- Which type of tissue and what maximum volume will be transported?
- How is the tissue contained? What is the nature, volume and temperature of any transport solution to be used? Which type of packaging has been used?
- Which refrigerant has been used and what is its specification and volume?
- What are the specifications of the transport container (i.e. dimensions, insulation, etc.)?
- What are the most extreme transportation conditions allowable in terms of transport time and ambient temperature?

Once the process had been defined, the acceptance criteria needed to be defined. In our example, they were that the:

- Temperature of the skin allograft must remain at 0-10 °C for the duration of the transit.
- Integrity of the tissue packaging must be maintained during transit.
- Integrity of the transport container must be maintained during transit.
- pH of the transportation fluid must be 7.0-7.5 at the end of the transportation.

For some tissues, it may be advisable to go further and validate the quality of the tissue after transit (e.g. assessment of its viability or histological structure).

It was determined that the maximum amount of skin that would be transported would be 6 000 cm^2, immersed in a minimum volume of 300 ml of transport fluid. Specifications of the packaging, transport container, and refrigerant were also documented. The most extreme acceptable transportation conditions were defined as an ambient temperature of 40 °C (e.g. a hot summer day in a vehicle) for a maximum of 12 h, with the minimum volume of refrigerant and transport solution, and the maximum volume of tissue.

A protocol was written and a model prepared using skin obtained from donors unsuitable for clinical donation. This protocol was based on the defined

1 Reproduced with permission from: Winters M, Lomas R. The principles of process validation and equipment qualification. In: Fehily D, Brubaker S, Kearney J, Wolfinbarger L, editors. *Tissue and cells processing: an essential guide*. London, UK: Wiley-Blackwell; 2012.

transport solution, refrigerant, packaging and container specifications. A calibrated data-logging thermometer was used to record the temperature on the external surface of the tissue packaging. The container was placed into a shaking incubator set at an ambient temperature of 40 °C. A shaking incubator was used to model the agitation of the container during vehicular transit (the model should approximate as closely as practically possible real-life conditions).

The study was repeated in triplicate. Acceptable results were obtained on each occasion. All results were well within the pre-defined acceptance criteria, so the process was accepted based on the results of the three replicates.

Note, however, that it may be necessary to find a compromise between an 'ideal' validation and operational practicalities that cannot be avoided. For example, it may not be possible or ethical to obtain and sacrifice large amounts of tissue for validation studies. In these cases, an acceptable compromise should be reached using risk-assessment principles (e.g. use of animal tissue as a substitute).

Application of sufficiently robust process validations (e.g. by challenging a transport process with extremes of time and temperature) obviates the need for routine temperature monitoring of the process. Thus, if the physical conditions identified by the validation study are complied with (e.g. the correct container, containing at least the minimum amount refrigerant, in transit for less than the maximum modelled time), then it can be reliably concluded that the process itself has been carried out correctly. Therefore, to demonstrate compliance with the validated process, all operatives need to do is confirm that they have complied with the relevant standard operating procedures.

Appendix 7. Example of method validation – oocyte vitrification

Preservation of tissues and cells is used where cell viability must be maintained for a long period of time. However, the survival rate after thawing represents a critical step affecting the success of the treatment. The following example refers to the validation of a new method of cryopreservation in human oocytes. For prospective validation, a randomised clinical study with specific endpoints (primary and secondary) and acceptance criteria should be performed. See Figure A7.1.

As an alternative approach, the validation can be performed in parallel by comparing the new method with the established one as follows. See Figure A7.2.

If and when a validation is performed in a randomised clinical study with defined endpoints (see Figure A7.1), usually a multi-centre study is needed and the endpoints (primary and secondary) should be defined and agreed upon between the ART centres.

Figure A7.1. **Validation of a new oocyte vitrification method in a multicentre study**

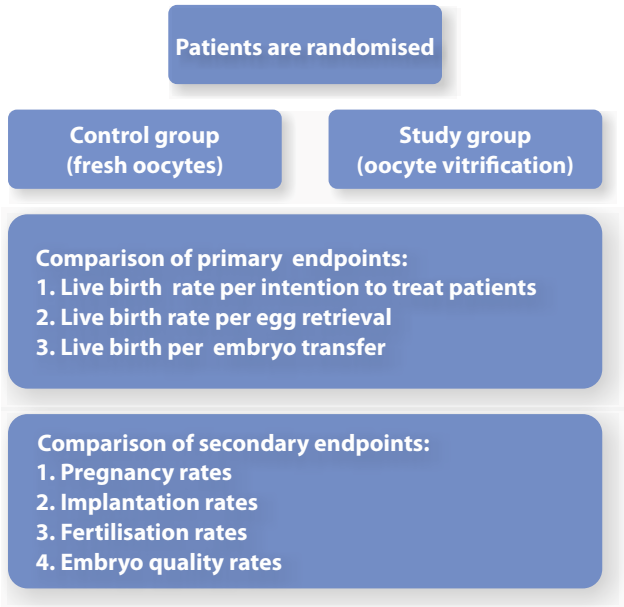
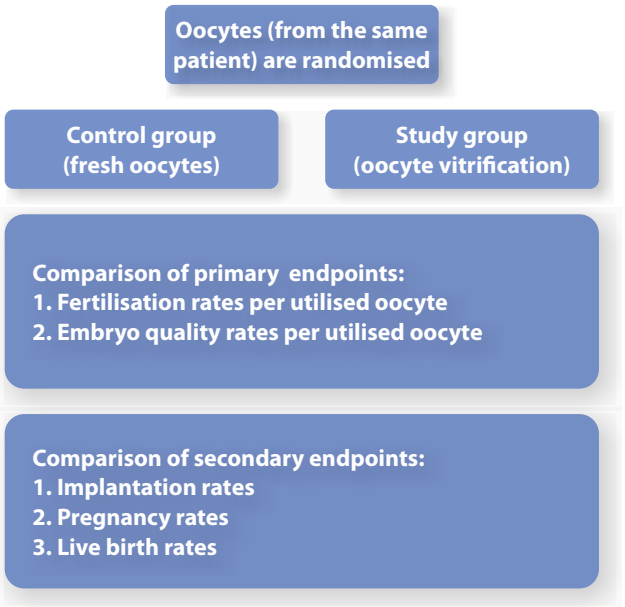
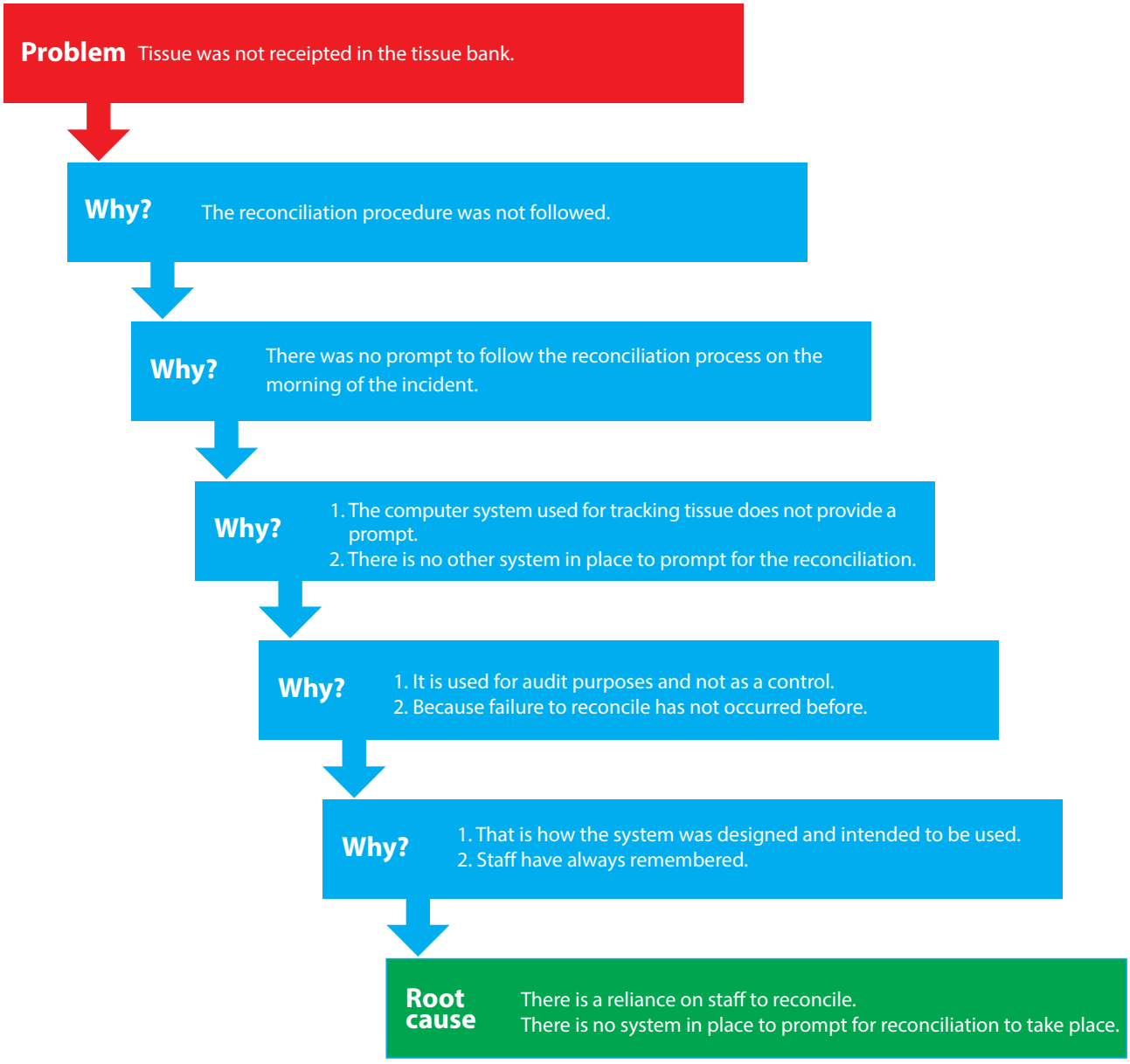


Figure A7.2. **Validation of a new oocyte vitrification method in a single centre**



Appendix 8. **Example of root cause analysis: why, why? – receipt of tissue**

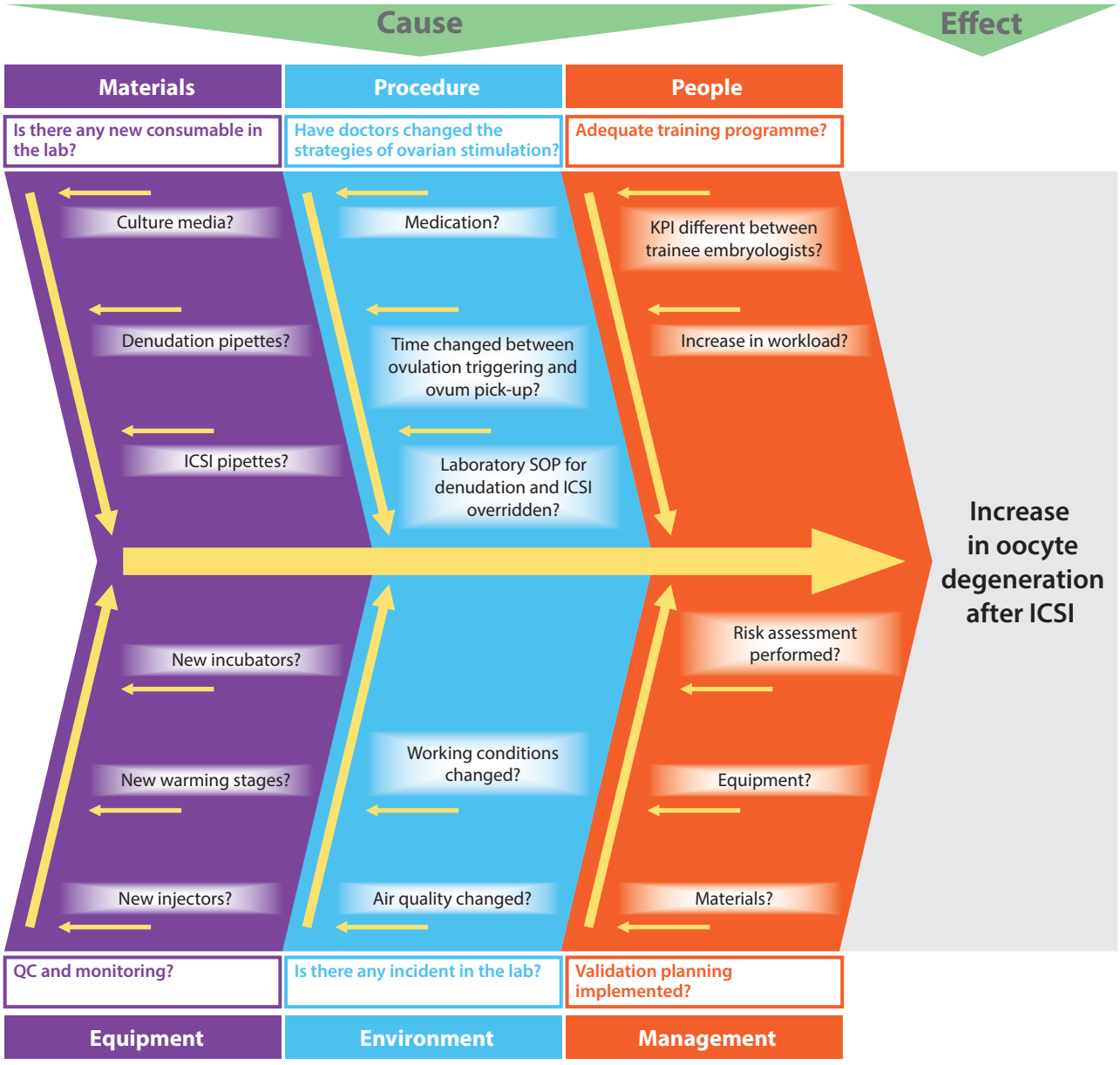


Appendix 9. Example of root cause analysis: fishbone diagram – receipt of tissue

Root causes

1. Reconciliation procedure not followed by tissue bank staff following the receipt and storage of tissue by the porters.
2. Computer system does not alert if reconciliation is not completed.

Appendix 10. **Example of root cause analysis: fishbone diagram – medically assisted reproduction**



Appendix 11. **Example of consent form: female (NHS, UK)**

Women's consent to treatment and storage form (IVF and ICSI)

HFEA
WT form

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about us, visit **www.hfea.gov.uk**.

Who should fill in this form?

Fill in this form if you are a woman and you are having fertility treatment using embryos created outside the body (in vitro) with your eggs. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
  - the different options set out in this form
  - the implications of giving your consent
  - the consequences of withdrawing this consent, and
  - how you can make changes to, or withdraw, your consent.
- an opportunity to have counselling.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. If you do not receive this information before filling in this form, your consent may be invalid.

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your eggs, and embryos created using your eggs, to be used or stored (eg, for IVF or ICSI treatment). If you are storing your eggs or embryos, you must also state in writing how long you consent to them remaining in storage.

You are also legally required to record what you would like to happen to your eggs and embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, your clinic needs to know this so that they only allow your eggs and embryos to be used according to your wishes. If you are unsure of anything in relation to this, please ask your clinic.

Why are there questions about using my eggs and embryos for training purposes?

You may have some eggs and embryos left after treatment which you do not wish to use (eg, because you do not want future treatment or the eggs and embryos are not viable for treatment). On this form, you can consent to donate these for training purposes to allow healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

What if I want to donate my eggs and/or embryos?

Unused eggs and embryos can also be donated for research purposes, helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Your clinic can give you more information about this and provide you with the relevant consent form(s).

You could also think about donating viable unused eggs and embryos to another person for use in their treatment. Before doing this, there are lots of issues to consider. For more information, see **www.hfea.gov.uk/egg-and-sperm-donors.html**. If you decide to donate, you will need to complete a separate form: 'Your consent to donating your eggs' (WD form).

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the consent and information given. When you have completed the form you may request a copy of it from your clinic.

For clinic use only (optional)

HFEA centre reference

☐☐☐☐

Other relevant forms

Date embryos were placed in storage

D D M M Y Y

Date embryos can remain in storage until

D D M M Y Y

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

Version 6, 20 April 2015

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

1 About you

1.1 **Your first name(s)** *Place clinic sticker here*

1.2 **Your surname**

1.3 **Your date of birth** D D M M Y Y

1.4 **Your NHS/CHI/HCN/passport number** (please circle)

2 About your partner

Place clinic sticker here

2.1 **Your partner's first name(s)**

2.2 **Your partner's surname**

2.3 **Your partner's date of birth** D D M M Y Y

2.4 **Your partner's NHS/CHI/HCN/ passport number** (please circle)

3 Your treatment

3.1 **Do you consent to your eggs being used to create embryos outside the body for your treatment (eg, through IVF treatment)?**

In order to create embryos for your treatment you must provide your consent by ticking the yes box below. Please note that the sperm provider also has to give his consent for embryos to be created.

☐ Yes

4 Storing embryos

4.1 **Do you consent to the embryos (created outside the body with your eggs) being stored?**

Please note that embryos can only be stored if the sperm provider has also given his consent.

☐ Yes ▸ after signing the page declaration below, continue on the next page.

☐ No ▸▸ now sign the page declarations on this page and the next page then go straight to section five.

▸▸▸▸ Continues on the next page

Page declaration

Your signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

4 Storing embryos *continued*

Embryo storage periods

You may wish to store any embryos left after treatment so they can be used in future treatment. To be stored, embryos are frozen or 'vitrified'. When considering how long to store for, you may want to think about how far in the future you might want/be able to use your stored embryos and the costs of storing – ask your clinic if you are unsure. The law permits you to store for any period up to 10 years but in cases where you or your partner are prematurely infertile, or likely to become prematurely infertile, you may store for longer, up to 55 years.

Please note that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store your embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

4.2 Have you, or your partner, been diagnosed as prematurely infertile or likely to become prematurely infertile?

Causes of premature infertility can include chemotherapy treatment and early menopause. Please speak to your clinic if you are unsure. If your circumstances change and either you or your partner become prematurely infertile, or are likely to become prematurely infertile, you and your partner can change your consent to store your embryos for up to 55 years.

☐ No ▸ go to 4.3.

☐ Yes ▸▸ go straight to 4.4.

4.3 For how long do you consent to store your embryos?

You can consent to store your embryos for up to 10 years. Please note that the sperm provider also has to give his consent to storage.

☐ For 10 years

☐ For a specific period (up to a maximum of 10 years) ▸ specify the number of years:

☐ years

The consent period will start from the date of storage. Remember you can always change the time period you consent to by completing this form again and specifying the new total time period you would like your embryos to be stored for. For example, if you consented to five years' storage on the original form and wish to consent for a further five years (10 years in total), you should complete another copy of this form but tick the box for 10 years. This second form would supersede the first form you completed. ▸▸ **Now sign the page declaration below and go straight to section five.**

4.4 Premature infertility

If you or your partner are prematurely infertile, or likely to become prematurely infertile, you can consent to store your embryos for up to 55 years. Although you can consent up to a maximum of 55 years on this form, after the first 10 years your medical practitioner will need to certify in writing that the medical criteria for premature infertility have been met for storage to continue for more than 10 years.

▸▸▸▸ Continues on the next page

Page declaration

Your signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

4 Storing embryos *continued*

When the criteria have been met, the storage period will be extended by 10 years from the date the criteria are met. The storage period can then be extended by further 10 year periods (up to a maximum of 55 years) at any time within each extended storage period if it is shown that the criteria continue to be met. For more information about this, please ask your clinic.

For how long do you consent to store your embryos?

Please specify the number of years you consent to store your embryos for (up to a maximum of 55): ☐ years.

Clinic staff: please attach all relevant medical practitioners' statements to this form.

5 Using eggs and embryos for training

5.1 **Do you consent to your eggs being used for training purposes?**

☐ Yes ☐ No

5.2 **Do you consent to embryos (already created outside the body with your eggs) being used for training purposes?**

Please note that embryos can only be used if the sperm provider has also given his consent.

☐ Yes ☐ No

6 In the event of your death or mental incapacity

As part of your consent, you also need to decide what you would like to happen to your eggs, or embryos created outside the body with your eggs, if you die or lose the ability to decide for yourself (become mentally incapacitated). Please note your embryos may only be used within the storage period you consented to above. **If you do not give your consent in the below section, your eggs or embryos must be allowed to perish in the event of your death or mental incapacity and cannot be used for treatment.**

6.1 **Do you consent to your eggs being used for training purposes?**

If you die	If you become mentally incapacitated
☐ Yes ☐ No	☐ Yes ☐ No

6.2 **Do you consent to embryos (already created outside the body with your eggs) being used for training purposes?**

Please note that embryos can only be used if the sperm provider has also given his consent.

If you die	If you become mentally incapacitated
☐ Yes ☐ No	☐ Yes ☐ No

Other uses for your eggs or embryos

If you wish your eggs or embryos to be used in someone else's treatment if you die or become mentally incapacitated, please speak to your clinic for more information. Depending on your circumstances, you will need to complete one of the following: • 'Your consent to donating your eggs' (WD form), • 'Your consent to donating embryos' (ED form), or • 'Women's consent to the use and storage of eggs or embryos for surrogacy' (WSG form).

Page declaration

Your signature ✗ _____ **Date** D D M M Y Y

For clinic use only (optional) Patient number _____

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

7 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
  - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling
  - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me, and
  - I understand that I can make changes to, or withdraw, my consent at any point until the time of embryo transfer, use of eggs or embryos in training, or the eggs or embryos have been allowed to perish.
- I declare that the information I have given on this form is correct and complete.
- I understand that information on this form may be processed and shared for the purposes of, and in connection with, the conduct of licensable activities under the Human Fertilisation and Embryology Act 1990 (as amended) in accordance with the provisions of that act.

Your signature ✗

Date D D M M Y Y

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because she is unable to sign for herself due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with her direction.

Representative's name

Representative's signature ✗

Relationship to the person consenting

Date D D M M Y Y

Witness's name

Witness's signature ✗

Date D D M M Y Y

D

For clinic use only (optional) Patient number

Source: Human Fertilisation and Embryology Authority (HFEA), United Kingdom.

Appendix 12. **Example of consent form: female (CNPMA, Portugal)**

cnpma conselho nacional de procriação medicamente assistida

CRYOPRESERVATION OF OOCYTES AND/OR OVARIAN TISSUE

Informed Consent

Oocytes are female reproductive cells that, in their immature form, are already present in the ovaries at the moment of birth. From puberty onwards, during each normal menstrual cycle, groups of oocytes undergo maturation phenomena that results in the release by the ovary of an oocyte that is mature and can therefore be fertilised.

In certain clinical situations - when essential treatments threaten the survival of reproductive cells, for example cryopreservation of oocytes has been proposed in an attempt to protect future fertility.

The cryopreservation of oocytes may also be justified in other clinical situations.

A number of key points should be highlighted:

- Cryopreservation of oocytes is a technique the global implications of which are not yet a matter of consensus in the scientific world.
- Cryopreservation of oocytes does not guarantee that a pregnancy will be obtained; it only guarantees a reserve of female reproductive cells that can be used in future. Currently, the rate of pregnancy achieved with *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) of cryopreserved oocytes is lower.
- Given the small number of children born as a result of the use of cryopreserved oocytes there is no reliable information as regards a possible increased risk of foetal abnormalities.
- Cryopreservation of oocytes does not establish any obligation on this centre in relation to the future use of those cells, nor does it grant the person from whom they originated any type of preferential treatment; at all times, the clinical criteria of good clinical practice appropriate to each situation will be applied.
- You alone, and no one else, have the right to use the cryopreserved oocytes.
- Unforeseen accidents, such as fires or calamities of other types, may, despite the safety precautions taken, lead to loss or destruction of the cryopreserved oocytes.

CONSENT

I, the undersigned, declare that:

- I have read and understood this document and the additional information provided.
- The queries and questions I have raised have been answered.
- I recognise that this text cannot describe exhaustively all the situations that could arise in the future.
- I have understood and I accept that the oocytes and/or ovarian tissue will be cryopreserved for a maximum period of five years and that I may revoke this authorisation at any time during this period.
- I am aware that I alone have the right to use these frozen oocytes and/or ovarian tissue.
- I have understood that, under the prevailing legislation, at the end of this five-year period, I will have to travel to the centre to sign a consent form for this freezing to continue. In the absence of a signed declaration requesting a further period of cryopreservation, I declare that I have been clearly informed that the oocytes and/or ovarian tissue will be thawed and destroyed, unless I hereby express authorisation for their use for scientific purposes. In those circumstances (write **Yes** or **No**):

  - I consent to use of my oocytes in scientific research projects

- I fully understand and accept the conditions, risks and limitations set out above.

cnpma conselho nacional de procriação medicamente assistida

Therefore, having been fully informed, I freely assume the obligations arising from conclusion of this agreement and give my consent for the use of this technique in preserving my oocytes.

NAME _____

SIGNATURE _____

CIVIL ID/PASSPORT NO. _____

Clinician: _____ ___/___/_____

Source: Cryopreservation of oocytes and/or ovarian tissue, Portugal (CNPMA).

Appendix 13. **Example of consent form: male (NHS, UK)**

Men's consent to treatment and storage form (IVF and ICSI)

HFEA
MT form

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about us, visit **www.hfea.gov.uk**.

Who should fill in this form?

Fill in this form if you are a man and your partner is having fertility treatment using embryos created outside the body (in vitro) with your sperm. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
  - the different options set out in this form
  - the implications of giving your consent
  - the consequences of withdrawing this consent, and
  - how you can make changes to, or withdraw, your consent.
- an opportunity to have counselling.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. If you do not receive this information before filling in this form, your consent may be invalid.

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you. However, if you are consenting to being registered as the legal father of any child born as a result of treatment after your death (see section 6.5), you **must** sign the form yourself.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your sperm, and embryos created using your sperm, to be used or stored (eg, for IVF or ICSI treatment). If you are storing your sperm or embryos, you must also state in writing how long you consent to them remaining in storage.

You are also legally required to record what you would like to happen to your sperm and embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, your clinic needs to know this so that they only allow your sperm and embryos to be used according to your wishes. If you are unsure of anything in relation to this, please ask your clinic.

Why are there questions about using my sperm and embryos for training purposes?

You may have some sperm and embryos left after treatment which you do not wish to use (eg, because you do not want future treatment or the sperm and embryos are not viable for treatment). On this form, you can consent to donate these for training purposes to allow healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

What if I want to donate my sperm and/or embryos?

Unused sperm and embryos can also be donated for research purposes, helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Your clinic can give you more information about this and provide you with the relevant consent form(s).

You could also think about donating viable unused sperm and embryos to another person for use in their treatment. Before doing this, there are lots of issues to consider. For more information, see **www.hfea.gov.uk/egg-and-sperm-donors.html**. If you decide to donate, you will need to complete a separate form: 'Your consent to donating your sperm' (MD form).

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the consent and information given. When you have completed the form you may request a copy of it from your clinic.

For clinic use only (optional)

HFEA centre reference

☐☐☐☐

Other relevant forms

Date embryos were placed in storage

D D M M Y Y

Date embryos can remain in storage until

D D M M Y Y

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

Version 4, 1 April 2015

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

1 About you

1.1 **Your first name(s)** *Place clinic sticker here*

1.2 **Your surname**

1.3 **Your date of birth** D D M M Y Y

1.4 **Your NHS/CHI/HCN/passport number** (please circle)

2 About your partner

2.1 **Your partner's first name(s)** *Place clinic sticker here*

2.2 **Your partner's surname**

2.3 **Your partner's date of birth** D D M M Y Y

2.4 **Your partner's NHS/CHI/HCN/passport number** (please circle)

3 Your treatment

3.1 **Do you consent to your sperm being used to create embryos outside the body for your partner's treatment (eg, through IVF treatment)?**

In order to create embryos for your partner's treatment you must provide your consent by ticking the yes box below. Please note that the egg provider also has to give her consent for embryos to be created.

☐ Yes

4 Storing embryos

4.1 **Do you consent to the embryos (created outside the body with your sperm) being stored?**

Please note that embryos can only be stored if the egg provider has also given her consent.

☐ Yes ▸ after signing the page declaration below, continue on the next page.

☐ No ▸▸ now sign the page declarations on this page and the next page then go straight to section five.

▸▸▸▸ Continues on the next page

Page declaration

Your signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

4 Storing embryos *continued*

Embryo storage periods

You may wish to store any embryos left after treatment so they can be used in future treatment. To be stored, embryos are frozen or 'vitrified'. When considering how long to store for, you may want to think about how far in the future you might want/be able to use your stored embryos and the costs of storing – ask your clinic if you are unsure. The law permits you to store for any period up to 10 years but in cases where you or your partner are prematurely infertile, or likely to become prematurely infertile, you may store for longer, up to 55 years.

Please note that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store your embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

4.2 **Have you, or your partner, been diagnosed as prematurely infertile or likely to become prematurely infertile?**

Causes of premature infertility can include chemotherapy treatment and early menopause. Please speak to your clinic if you are unsure. If your circumstances change and either you or your partner become prematurely infertile, or are likely to become prematurely infertile, you and your partner can change your consent to store your embryos for up to 55 years.

☐ No ▸ go to 4.3.

☐ Yes ▸▸ go straight to 4.4.

4.3 **For how long do you consent to store your embryos?**

You can consent to store your embryos for up to 10 years. Please note that the egg provider also has to give her consent to storage.

☐ For 10 years

☐ For a specific period (up to a maximum of 10 years) ▸ specify the number of years:

☐ years

The consent period will start from the date of storage. Remember you can always change the time period you consent to by completing this form again and specifying the new total time period you would like your embryos to be stored for. For example, if you consented to five years' storage on the original form and wish to consent for a further five years (10 years in total), you should complete another copy of this form but tick the box for 10 years. This second form would supersede the first form you completed. ▸▸ **Now sign the page declaration below and go straight to section five.**

4.4 **Premature infertility**

If you or your partner are prematurely infertile, or likely to become prematurely infertile, you can consent to store your embryos for up to 55 years. Although you can consent up to a maximum of 55 years on this form, after the first 10 years your medical practitioner will need to certify in writing that the medical criteria for premature infertility have been met for storage to continue for more than 10 years. When the criteria have been met, the storage period will be extended by 10 years from the date the criteria are met.

▸▸▸▸ Continues on the next page

Page declaration

Your signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

4 Storing embryos *continued*

The storage period can then be extended by further 10 year periods (up to a maximum of 55 years) at any time within each extended storage period if it is shown that the criteria continue to be met. For more information about this, please ask your clinic.

For how long do you consent to store your embryos?

Please specify the number of years you consent to store your embryos for (up to a maximum of 55): ☐ years.

Clinic staff: please attach all relevant medical practitioners' statements to this form.

5 Using sperm and embryos for training

5.1 **Do you consent to your sperm being used for training purposes?**

☐ Yes ☐ No

5.2 **Do you consent to embryos (already created outside the body with your sperm) being used for training purposes?**

Please note that embryos can only be used if the egg provider has also given her consent.

☐ Yes ☐ No

6 In the event of your death or mental incapacity

As part of your consent, you also need to decide what you would like to happen to your sperm, or embryos created outside the body with your sperm, if you die or lose the ability to decide for yourself (become mentally incapacitated). Please note that if you would like your partner to use your sperm or embryos in the event of your death or mental incapacity, your partner should be named on this form. Your embryos may only be used within the storage period you consented to above.

If you do not give your consent in the below section, your sperm or embryos must be allowed to perish in the event of your death or mental incapacity and cannot be used for treatment.

6.1 **Do you consent to your sperm being used to create embryos outside the body for your partner's treatment?**

Please note that the egg provider also has to give her consent for embryos to be created.

If you die ☐ Yes ☐ No

If you become mentally incapacitated ☐ Yes ☐ No

6.2 **Do you consent to embryos (already created outside the body with your sperm) being used for your partner's treatment?**

Please note that embryos can only be used if the egg provider has also given her consent.

If you die ☐ Yes ☐ No

If you become mentally incapacitated ☐ Yes ☐ No

▸▸▸▸ Continues on the next page

Page declaration

Your signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number ☐

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

6 In the event of your death or mental incapacity *continued*

6.3 **Do you consent to your sperm being used for training purposes?**

If you die ☐ Yes ☐ No

If you become mentally incapacitated ☐ Yes ☐ No

6.4 **Do you consent to embryos (already created outside the body with your sperm) being used for training purposes?**

Please note that embryos can only be used if the egg provider has also given her consent.

If you die ☐ Yes ☐ No

If you become mentally incapacitated ☐ Yes ☐ No

Other uses for your sperm or embryos

If you wish your sperm or embryos to be used in someone else's treatment if you die or become mentally incapacitated, please speak to your clinic for more information. Depending on your circumstances, you will need to complete one of the following:

- 'Your consent to donating your sperm' (MD form)
- 'Your consent to donating embryos' (ED form), or
- 'Men's consent to the use and storage of sperm or embryos for surrogacy' (MSG form).

Consent to birth registration

Complete this part of section six if you consented to your sperm, or embryos created outside the body with your sperm, being used in your partner's treatment after your death.

If you have given your consent to your sperm or embryos (to be created outside the body with your sperm) being used after your death, you may also wish to consent to being registered as the legal father of any child that is born as a result of your partner's treatment.

6.5 **Do you consent to being registered as the legal father of any child born as a result of your partner's treatment after your death?**

By ticking yes, you consent to the following:

- I consent to my name, place of birth and occupation being entered on the register of births as the legal father of any child born from my partner's treatment.

  This register is kept under the Births and Deaths Registration Act 1953, or the Births and Deaths Registration (Northern Ireland) Order 1976, or the Registration of Births, Deaths and Marriages (Scotland) Act 1965.
- I also consent to information about my or my partner's treatment being disclosed to my partner and one of the following registrars:
  - the Registrar General for England and Wales
  - the Registrar General for Scotland
  - the Registrar for Northern Ireland.

Please note that being recorded in the register of births as the legal father of a child born from your partner's treatment does not transfer any inheritance or other legal rights to the child.

☐ Yes ☐ No

Page declaration

Your signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

7 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
  - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling
  - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me, and
  - I understand that I can make changes to, or withdraw, my consent at any point until the time of embryo transfer, use of sperm or embryos in training, or the sperm or embryos have been allowed to perish.
- I declare that the information I have given on this form is correct and complete.
- I understand that information on this form may be processed and shared for the purposes of, and in connection with, the conduct of licensable activities under the Human Fertilisation and Embryology Act 1990 (as amended) in accordance with the provisions of that act.

Your signature ✗ **Date** D D M M Y Y

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because he is unable to sign for himself due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form. However, if the person consenting consented to being registered as the legal father after his death (that is if he ticked yes to question 6.5), he **must** sign the form himself.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with his direction.

Representative's name

Representative's signature ✗

Relationship to the person consenting

Date D D M M Y Y

Witness's name

Witness's signature ✗

Date D D M M Y Y

For clinic use only (optional) Patient number

Source: Human Fertilisation and Embryology Authority (HFEA), United Kingdom.

Appendix 14. **Medical and social history questionnaire (NHS, UK)**

NHS
Blood and Transplant

Tissue Donor Number ☐☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

Medical and Social History Questionnaire

Directions for completion

1 This form must be completed in **black or dark blue ink** by the Specialist Nurse – Organ Donation (SNOD)/Specialist Nurse – Tissue Donation (SNTD)/Tissue Donor Co-ordinator (TDC) and signed where required.

2 The original copy should be retained by the **SNOD/SNTD/TDC** for the donor file.

3 In the event of organ and tissue donation, a legible copy should be sent to the relevant **Tissue Establishment**, where required.

NOTE: The term patient is used throughout the form to refer to the potential donor.

The term relative is used throughout the form to refer to the relationship between the patient and the interviewee.

NHS
Blood and Transplant

Tissue Donor Number ☐☐☐☐☐☐☐☐☐ ODT Donor Number ☐☐☐☐☐☐

In order to ensure the safety of organs and tissue for transplant I will need to ask you some questions about *(name of patient)* medical and lifestyle history. Some of the questions are of a sensitive and personal nature. They are similar questions to those asked when someone donates blood. I will read and discuss each question with you and ask that you answer to the best of your knowledge with either a "Yes" or "No."

PATIENT INFORMATION

Patient's Forename(s) Please print ____ Patient's Surname Please print ____

Donating Hospital ____

NHS/CHI Number ☐☐☐☐☐☐☐☐☐☐ Cause of Death ____

Hospital Number ☐☐☐☐☐☐☐☐☐☐

Date of Birth (dd/mm/yyyy) ☐☐ ☐☐ ☐☐ Occupation ____

Country of Birth ____ Country of Residency ____

INTERVIEWEE INFORMATION

Information discussed with

Name Please print ____ Relationship Please print ____

For patients under the age of 18 months, or those who have been breast-fed or fed breast milk by a donor in the last 12 months, the mother is required to answer these questions with regard to her own and her child's health.

For children: has your child been breast-fed in the past 12 months? Yes ☐ No ☐ Unknown ☐

NOTE: for all patients under the age of 18 months and any child who has been breast-fed in the last 12 months, a blood sample for microbiological testing is required from the mother, as well as from the patient.

For ALL female patients between 13 and 53 years of age: Is there a possibility that your relative could be pregnant? Yes ☐ No ☐ Unknown ☐

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GENERAL HEALTH INFORMATION

1. Did your relative visit a general practitioner in the last two years? Yes ☐ No ☐ Unknown ☐

If YES, give details

2. Was your relative currently seeing or waiting to see a general practitioner or any other healthcare professional? Yes ☐ No ☐ Unknown ☐

If YES, give details

3. Did your relative ever take regular medication? Yes ☐ No ☐ Unknown ☐

If YES, give details of any current or previous medication including any medication for acne, prostate or psoriasis

4a. Did your relative have a history of allergies to medication, food or other substances? Yes ☐ No ☐ Unknown ☐

If YES, please provide details of the substance they were allergic to and describe the reaction

4b. Did your relative have any health problems due to exposure to toxic substances such as pesticides, lead, mercury, gold, asbestos, cyanide, agent orange etc? Yes ☐ No ☐ Unknown ☐

If YES, please provide details of the toxic substance and treatment

5a) Was your relative a diabetic? Yes ☐ No ☐ Unknown ☐

If YES, were they on insulin? Yes ☐ No ☐ Unknown ☐ N/A ☐

5b) Is there a family history of diabetes? Yes ☐ No ☐ Unknown ☐

If YES, is it insulin-dependent diabetes? Yes ☐ No ☐ Unknown ☐ N/A ☐

6. Did your relative suffer from any chronic or autoimmune illness or disease of unknown cause? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

7. Did your relative ever suffer from any bone, joint, skin or heart disease? Yes ☐ No ☐ Unknown ☐

If YES, specify which and give details

8. Did your relative ever have hepatitis, jaundice or liver disease? Yes ☐ No ☐ Unknown ☐

If YES, give dates, diagnosis, treatment and hospital /clinic name if known

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GENERAL HEALTH INFORMATION

9. Did your relative recently suffer from significant unplanned weight loss? Yes ☐ No ☐ Unknown ☐

If YES, give details

10. Did your relative ever undergo any investigations for cancer or were they ever diagnosed with cancer? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

11. Did your relative have a history of eye disease, receive any medications for eye problems (e.g. eye drops), or undergo eye surgery or laser treatment? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

12. Did your relative ever have any operations? ***If NO go to question 15*** Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

13. Did your relative ever have any surgery on the brain or spine? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details including hospital name and dates of treatment if possible. **Surgery before 1993** is particularly significant

14. Did your relative ever have an organ or tissue transplant? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details including hospital name and dates of treatment if known

15. Was your relative ever told not to donate blood? Yes ☐ No ☐ Unknown ☐

If YES, give details of where, when and the reason

16. Did your relative receive a transfusion of blood or blood product(s) at any time? Yes ☐ No ☐ Unknown ☐

If YES, give details including country, hospital name, dates and reason for transfusion

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GENERAL HEALTH INFORMATION

17. Did your relative suffer from any type of brain disease such as Parkinson or Alzheimer disease or dementia? Yes ☐ No ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

18. Did your relative suffer from any one or more of the following problems: memory problems or confusion, change in personality or behaviour, or were they unsteady on their feet? If **NO** go to Question 19, if **YES** Yes ☐ No ☐ Unknown ☐

18a. Were you aware of a condition causing these symptoms? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, please specify condition

18b. When did these symptoms start?

Please give details

18c. Did they worsen noticeably over time?

Please give details

18d. Was your relative able to live independently?

Please give details

19. Did your relative have a family history of prion disease, such as CJD, or were they ever told that they were at risk of prion disease? Yes ☐ No ☐ Unknown ☐

If YES, please give details

20. Did your relative ever receive human pituitary extracts, e.g. growth hormones or fertility treatment or test injections for hormone imbalance? Yes ☐ No ☐ Unknown ☐

If YES, give details including dates and hospital/clinic name if known

21. Did your relative ever have any significant infection? Yes ☐ No ☐ Unknown ☐

If YES, give details, and any treatment received and hospital/clinic name if known

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RECENT HISTORY

22. Did your relative come into contact with an individual with an infectious disease within the last month? Yes ☐ No ☐ Unknown ☐

If YES, please specify details, dates, symptoms, diagnosis, and treatment

23. Did your relative have any signs of infection, e.g. colds, flu, fever, night sweats, swollen glands, diarrhoea, vomiting or skin rash within the last month? Yes ☐ No ☐ Unknown ☐

If YES, please specify dates, symptoms, diagnosis, and treatment

24. Did your relative have any immunisations within the last 2 months? Yes ☐ No ☐ Unknown ☐

If YES, give details including travel vaccinations and flu vaccination or flu nasal spray

25. Did your relative have tattooing, body piercing, botox injections, acupuncture, colonic irrigation, faecal transplantation, or any other cosmetic treatments or injuries that involve piercing the skin in the last 3 months? Yes ☐ No ☐ Unknown ☐

If YES, give details including where and when including unlicensed clinics in UK or abroad

26. In the last 12 months has your relative been bitten or scratched by any animal (strays, pets, wild, farm or ticks) or been bitten by a human. Or, has your relative ever been bitten or in close contact with bats anywhere in the world or been bitten by a mammal outside the UK? Yes ☐ No ☐ Unknown ☐

If YES, give details of incident, circumstances, animal, place, dates and treatment

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TRAVEL HISTORY

27. Did your relative ever travel or live outside the UK (including business trips)? Yes ☐ No ☐ Unknown ☐
If NO go to question 33

28. In the last 12 months did your relative go outside the UK (including business trips)? Yes ☐ No ☐ Unknown ☐ N/A ☐

Give details of dates and destinations visited

29. Did your relative ever have malaria or an unexplained fever which they could have picked up whilst abroad? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give date of fever/illness, places visited, duration and dates

30. Was your relative ever unwell whilst abroad or in the first month of their return to the UK? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details

31. Did your relative ever live or travel outside the UK for a continuous period of 6 months or more? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details of dates and destinations

32. Did your relative ever go to Central America, Mexico or South America for a continuous period of 1 month or more? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details of dates, places (remote/rural/urban areas), nature of visit

33. Was your relative's mother born in Central America, Mexico or South America? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details

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BEHAVIOURAL RISK ASSESSMENT

34. Did your relative
(a) Consume alcohol? Yes ☐ No ☐ Unknown ☐

If YES, give details

(b) Smoke tobacco or any other substance? Yes ☐ No ☐ Unknown ☐

If YES, give details of substance, frequency, history of smoking time and time elapsed since giving up

(c) Take any recreational drugs? Yes ☐ No ☐ Unknown ☐

If YES, give details of route of administration and dates

35. Is it possible that any of the following apply to your relative?

(a) Was, or may have been infected with HIV, hepatitis or HTLV? Yes ☐ No ☐ Unknown ☐

(b) Within the last 12 months have they injected, or been injected, with non-prescription drugs, including performance enhancing drugs or injectable tanning agents? Yes ☐ No ☐ Unknown ☐

(c) Been in prison or a juvenile detention centre for more than 3 consecutive days in the last 12 months? Yes ☐ No ☐ Unknown ☐

(d) Taken medication to prevent HIV infection e.g. (PrEP Pre/Post exposure prophylaxis)? Yes ☐ No ☐ Unknown ☐

If YES to any of the above questions a-d, give details, including dates for question c

36. Has your relative **ever** had sex – consensual or otherwise?
If no, go to question 38. Yes ☐ No ☐ Unknown ☐

If **YES**, is it possible that your relative:

(a) Was given payment for sex with money or drugs in the last 3 months? Yes ☐ No ☐ Unknown ☐ N/A ☐

(b) Ever had a sexually transmitted disease? Yes ☐ No ☐ Unknown ☐ N/A ☐

If YES, give details, including hospital/clinics, dates, treatments.

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37. Did your relative have sex, consensual or otherwise in the last 3 months?	Yes		No		Unknown		N/A	
If no, go to question 38. If yes, is it possible that in the last 3 months your relative had sex with:								
(a) *(for male patients only)* another man?	Yes		No		Unknown		N/A	
(b) *(for female patients only)* a man who has ever had sex with another man?	Yes		No		Unknown		N/A	
(c) Anyone who is HIV or HTLV positive?	Yes		No		Unknown		N/A	
(d) Anyone who has hepatitis?	Yes		No		Unknown		N/A	
(e) Anyone who had a sexually transmitted disease?	Yes		No		Unknown		N/A	
(f) Anyone who has ever been given payment for sex with money or drugs?	Yes		No		Unknown		N/A	
(g) Anyone who in the last 12 months has injected or been injected with non-prescription drugs including performance enhancing drugs or injectable tanning agents?	Yes		No		Unknown		N/A	
If YES, give details								
(h) Anyone who could have had sex, in any part of the world, where AIDS/HIV is very common (this includes most countries in Africa)?	Yes		No		Unknown		N/A	
(i) Anyone who has developed an illness related to travel such as Zika?	Yes		No		Unknown		N/A	
38. Having answered all the previous questions, is there anyone else who you think may provide more information?	Yes		No					
If YES, please specify								

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Question number	Relevant additional information. If any questions have been answered as unknown, give an explanation

Signature of healthcare professional obtaining information

Please print name

Designation of healthcare professional obtaining information

Date of interview | | | | | 2 | 0 | | | Time of interview | | | | |

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Source: National Health Service, United Kingdom.

Appendix 15. **Physical assessment form (Dutch Transplant Foundation)**

Donor identification:

Donor number:

Date of birth:

Gender: M ☐ F ☐

Date recovery:

Identification verification: No ☐ Yes ☐

Consent: No ☐ Yes ☐

Recovery team members:

Start time recovery:

Eye tissue | Skin

Heart valves | MS tissue

Thoracic aorta | Femoral arteries

Complications during procedure: No ☐ Yes ☐

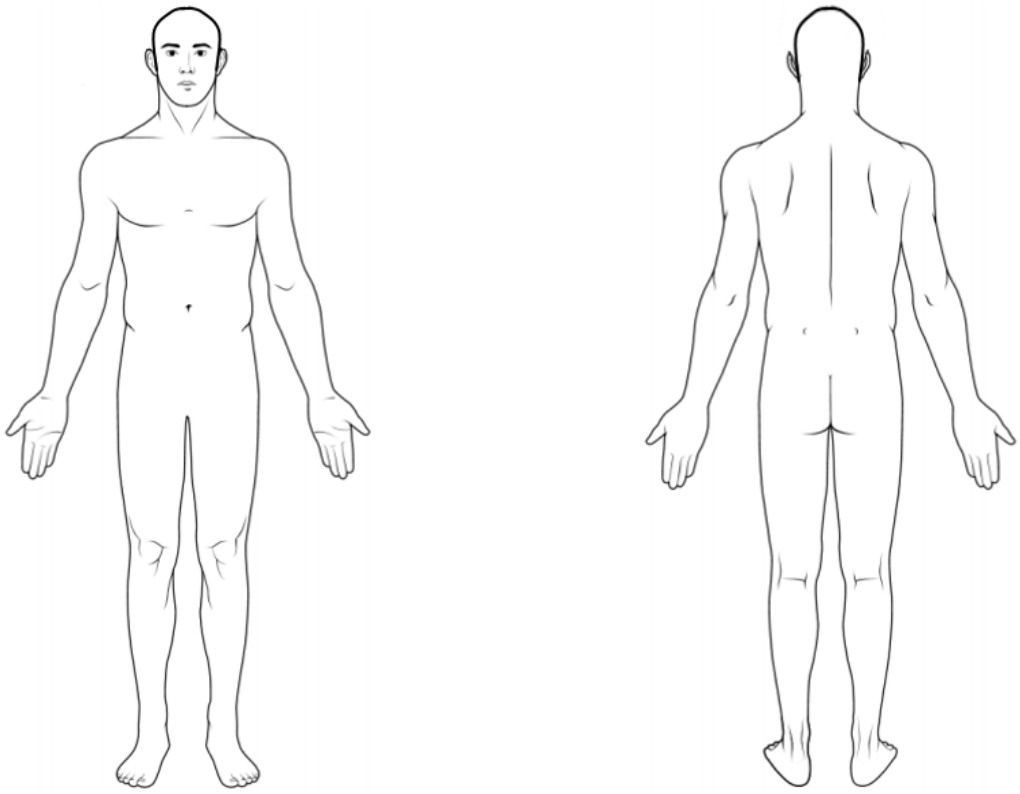
General appearance: Good ☐ Moderate ☐ Poor ☐

Height: cm Weight: kg

(O)	Ocular abnormalities	No ☐	Yes ☐	Unable to visualise ☐	
(WS)	White spots in the mouth	No ☐	Yes ☐	Unable to visualise ☐	
(J)	Jaundice	No ☐	Yes ☐		
(LN)	Abnormal lymph node(s)	No ☐	Yes ☐	Location? Size? Consistency?	
(L)	Enlarged liver	No ☐	Yes ☐		

(H)	Haematoma/bruises	No ☐	Yes ☐				
(GL/PL)	Genital and/or perianal lesions	No ☐	Yes ☐				
(NMI)	Non-medical injection sites	No ☐	Yes ☐				
(SL)	Skin lesions	No ☐	Yes ☐	Requires description			
(S)	Scars	No ☐	Yes ☐	Old ☐	Recent ☐		
(Ta/Pi)	Tattoos/piercings	No ☐	Yes ☐	Old ☐	Recent ☐		

(IV)	IV/Arterial line	(P)	Pacemaker/ICD	(BN)	Bone needle
(MP)	Needle entry site (medical procedures)	(D)	Drainage	(St)	Stoma
(BC)	Needle site blood collection	(C)	Cast	(Ca)	Catheter
(B)	Bandage	(I)	Autopsy/organ recovery incision	(De)	Decubitus



Describe findings/tattoos	
Consultation	No ☐ Yes ☐
Photos taken?	No ☐ Yes ☐
Notes	

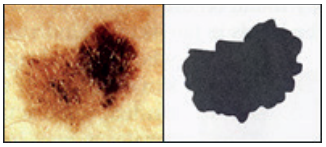
Appendix 16. **Evaluation of pigmented skin lesions**

A careful physical examination of the donor should be conducted, paying particular attention to the skin, looking for potential neoplasms or scars of previous surgical procedures (see Chapter 4).

The 'ABCDE rule' is an easy guide to detecting usual signs that may be indicative of melanoma [1-2].

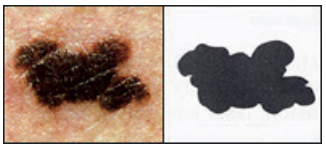
A. Asymmetry

If one half is not identical to the other half, suspect melanoma.



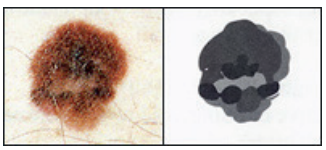
B. Border irregularity

Notched, scalloped, ragged or poorly defined borders should lead us to suspect melanoma.



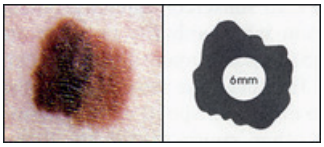
C. Colour

Naevi usually have a uniform colour; if there is colour variability from black-brown to red-blue-grey or white suspect melanoma.



D. Diameter/Difference

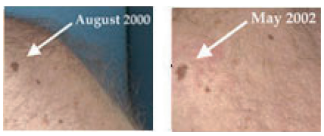
If the diameter is > 6 mm, suspect melanoma. Small lesions with some of the previous characteristics should also lead us to suspect melanoma.



If there are multiple lesions with a more or less regular aspect but among them there is one that has a very "ugly" aspect compared to the rest (ugly duckling sign), suspect melanoma.

E. Evolution

If there has been an evolution or change in appearance of a lesion, suspect melanoma. Any change – in size, shape, colour, elevation or another trait, or any new symptom such as bleeding, itching or crusting – points to danger.



Source of the images: www.dermatology.ucsf.edu/skincancer/general/types/melanoma.aspx and www.skincancer.org/skin-cancer-information/melanoma.

Other warning signs are:

- Naevi are the most powerful predictor of risk of melanoma. An individual with more than 100 common naevi or more than two atypical naevi has a 5- to 20-fold increased risk of melanoma;
- People with a first-degree relative with melanoma are at increased risk of developing melanoma; 5-10 % of individuals with melanoma

have a family history of melanoma. If there is a suspicious lesion and there is family history of melanoma, suspect melanoma.

References

1. Friedman RJ, Rigel DS, Kopf AW. Early detection of malignant melanoma: The role of physician examination and self-examination of the skin. *CA Cancer J Clin* 1985; 35(3):130-51.
2. Whited JD, Grichnik JM. The rational clinical examination. Does this patient have a mole or a melanoma? *JAMA* 1998; 279(9):696-701.
3. Gachon J *et al.*: First prospective study of the recognition process of melanoma in dermatological practice. *Arch Dermatol* 2005; 141(4):434-8.

Appendix 17. Evaluation of malignancies for risk assessment in tissue and cell donors

Table A17.1. **Grading of selected central nervous system tumours (WHO 2016 classification)**

Diffuse astrocytic and oligodendroglial tumours	I	II	III	IV
Diffuse astrocytoma, IDH-mutant		•		
Anaplastic astrocytoma, IDH-mutant			•	
Glioblastoma, IDH-wildtype				•
Glioblastoma, IDH-mutant				•
Diffuse midline glioma, H3K27M-mutant				•
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted		•		
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted			•	

Other astrocytic tumours	I	II	III	IV
Pilocytic astrocytoma	•			
Subependymal giant cell astrocytoma	•			
Pleomorphic xanthoastrocytoma		•		
Anaplastic pleomorphic xanthoastrocytoma			•	

Ependymal tumours	I	II	III	IV
Subependymoma	•			
Myxopapillary ependymoma	•			
Ependymoma		•		
Ependymoma, RELA fusion-positive		•	•	
Anaplastic ependymoma			•	

Other gliomas	I	II	III	IV
Angiocentric glioma	•			
Chordoid glioma of third ventricle		•		

Choroid plexus tumours	I	II	III	IV
Choroid plexus papilloma	•			
Atypical choroid plexus papilloma		•		
Choroid plexus carcinoma			•	

Tumours of the pineal region	I	II	III	IV
Pineocytoma	•			
Pineal parenchymal tumour of intermediate differentiation		•	•	
Pineoblastoma				•
Papillary tumour of the pineal region		•	•	

Meningiomas	I	II	III	IV
Meningioma	•			
Atypical meningioma		•		
Anaplastic (malignant) meningioma			•	

Embryonal tumours	I	II	III	IV
Medulloblastoma (all subtypes)				•
Embryonal tumour with multi-layered rosettes, C19MC-altered				•
Medulloepithelioma				•
CNS embryonal tumour, NOS				•
Atypical teratoid/rhabdoid tumour				•
CNS embryonal tumour with rhabdoid features				•

Neuronal and mixed neuronal-glial tumours	I	II	III	IV
Dysembryoplastic neuroepithelial tumour	•			
Gangliocytoma	•			
Ganglioglioma	•			
Anaplastic ganglioglioma			•	
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	•			
Desmoplastic infantile astrocytoma and ganglioglioma	•			
Papillary glioneuronal tumour	•			
Rosette-forming glioneuronal tumour	•			
Central neurocytoma		•		

Neuronal and mixed neuronal-glial tumours	I	II	III	IV
Extraventricular neurocytoma		•		
Cerebellar liponeurocytoma		•		

Tumours of the cranial and paraspinal nerves	I	II	III	IV
Schwannoma	•			
Neurofibroma	•			
Perineurioma	•			
Malignant peripheral nerve sheath tumour (MPNST)		•	•	•

Mesenchymal, non-meningothelial tumours	I	II	III	IV
Solitary fibrous tumour/haemangiopericytoma	•	•	•	
Haemangioblastoma	•			

Tumours of the sellar region	I	II	III	IV
Craniopharyngioma	•			
Granular cell tumour	•			
Pituicytoma	•			
Spindle cell oncocytoma	•			

Source: adapted from: Louis DN, Ohgaki H, Wiestler OD *et al.* (2016) *World Health Organization histological classification of tumours of the central nervous system*. Geneva: WHO Press/Lyon: International Agency for Research on Cancer.

Table A17.2. **Recommendations on the use of organs from donors with CNS tumours**

Absolute contraindications
• Primary cerebral lymphoma
• All secondary intracranial tumours

Intracranial tumours with an intermediate risk of cancer transmission
(2.2 % with an upper 95 % CI of 6.4 %) include WHO grade 4 tumours and equivalents:
• Glioblastoma
• Giant cell glioblastoma
• Gliosarcoma
• Pineoblastoma
• Medulloblastoma
• CNS primitive neuroectodermal tumour
• Medulloepithelioma
• Ependymoblastoma
• Atypical teratoid/rhabdoid tumour
• Malignant peripheral nerve sheath tumour (MPNST)
• Germinoma
• Immature teratoma
• Teratoma with malignant transformation
• Yolk sac tumour
• Embryonal carcinoma
• Choriocarcinoma

Intracranial tumours with a low risk of transmission
(< 2 %) include WHO Grade 3 and equivalents:
• Anaplastic astrocytoma
• Anaplastic oligodendroglioma
• Anaplastic oligoastrocytoma
• Ependymoma
• Choroid plexus carcinoma
• Anaplastic gangliomyoma
• Pineal parenchymal tumour of intermediate differentiation
• Papillary tumour of the pineal region
• Malignant peripheral sheath tumour
• Anaplastic/malignant meningioma
• Papillary meningioma
• Rhabdoid meningioma
• Haemangiopericytoma

Source: Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO). *Transplantation of organs from deceased donors with cancer or a history of cancer*. London: Department of Health and Social Care, April 2014.

Table A17.3. **Recommendations on the use of organs from donors with non-CNS cancers**

Absolute contraindications
• Active cancer with spread outside the organ
• Active haematological malignancy

High risk (>10% risk of transmission)
• Melanoma: without spread (except as below)
• Breast: cancer other than those identified below
• Colon: cancer other than those identified below
• Kidney: renal cell cancer >7 cm or stages 2-6
• Sarcoma: >5 years previously and resected
• Small cell cancer: lung/neuroendocrine
• Lung cancer: stage I to IV

Low risk (0.1-2% risk of transmission)
• Melanoma: superficial spreading type with tumour thickness <1.5 mm with curative surgery and cancer-free period of >5 years
• Breast: stage 1, hormone receptor negative with curative surgery and cancer-free period of >5 years
• Ovary: curative surgery and cancer-free >10 years
• Colon: adenocarcinoma with curative surgery and cancer-free period of >5 years
• Thyroid: solitary papillary carcinoma 0.5-2.0 cm
• Thyroid: minimally invasive follicular carcinoma 1.0-2.0 cm
• Kidney: resected solitary renal cell carcinoma >1.0 cm and <2.5 cm and Fuhrman grade 1/2
• Prostate: Gleason >6
• Treated gastrointestinal stromal cancers

Minimal risk (<0.1% risk of transmission)
• Skin: basal cell carcinoma
• Skin: squamous cell carcinoma with no metastases
• Skin: non-melanoma skin cancer *in situ*
• Uterine cervix: *in situ* cancer
• Thyroid: solitary papillary carcinoma (<0.5 cm)
• Thyroid: minimally invasive follicular carcinoma (<1.0 cm)
• Bladder: superficial non-invasive papillary carcinoma
• Kidney: Resected solitary renal cell carcinoma <1.0 cm and Fuhrman grade 1/2
• Prostate: Gleason <6, or >6 with curative treatment and cancer free >3 years

Note. Only those cancers where evidence is available for analysis have been classified.

Source: Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO). *Transplantation of organs from deceased donors with cancer or a history of cancer*. London: Department of Health and Social Care, April 2014.

Appendix 18. **Sample haemodilution algorithm**

This sample haemodilution algorithm is based on the US Food and Drug Administration Guidance document cited below. N.B: This guidance gives 'colloid' a different definition from that used in Europe; note particularly point 2 (2) below.

The following definitions, as used in this sample haemodilution algorithm, are adapted from the US FDA Guidance, Chapter V, p. 31.

1. Blood component means a product containing a part of human blood separated by physical or mechanical means (§1271.3i).
2. Colloid means: (1) a protein or polysaccharide solution, such as albumin, dextran or hetastarch, that can be used to increase or maintain osmotic (oncotic) pressure in the intravascular compartment; *or* (2) blood components such as plasma and platelets (§1271.3j).
3. Crystalloid means an isotonic salt and/or glucose solution used for electrolyte replacement or to increase intravascular volume, such as saline solution, Ringer's lactate solution, 5 % dextrose in water (§1271.3k), or total parenteral nutrition.
4. Plasma dilution means a decrease in the concentration of the donor's plasma proteins and circulating antigens or antibodies resulting from the transfusion of blood or blood components and/or infusion of fluids (§1271.3p).

DONOR ID # _

Date and Time of Specimen Collection _ _ _ _ _ _ _

Donor's weight in kg _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

A = Total volume of blood transfused in the 48 hours before death or sample collection, whichever comes first

B = Total volume of colloid infused in the 48 hours before death or sample collection, whichever comes first

C = Total volume of crystalloid infused in the 1 hour before death or sample collection, whichever comes first

BV = donor's blood volume

Calculated blood volume = donor's weight (kg)/0.015 *or* donor's weight (kg) × 70 mL/kg

PV = donor's plasma volume

Calculated plasma volume = donor's weight (kg)/0.025 *or* donor's weight (kg) × 40 mL/kg

Calculate both:

1. Is B + C > PV?

2. Is A + B + C > BV?

[Enter a zero if a category (A, B, or C) was not transfused/infused.]

Determination of sample acceptability for infectious disease tests

- If the answers to both 1 and 2 are NO, the post-transfusion/infusion sample is acceptable.
- If the answer to either 1 or 2 is YES, the post-transfusion/infusion sample is not acceptable; use a pre-transfusion/infusion sample or reject the donor.

When calculating haemodilution of donors that have received plasma, the end result might differ according to the definition used:
For example: a donor 70 kg:
Donor plasma volume (PV): 2 800 mL
Donor blood volume (BV): 4 667 mL
Total volume of blood transfused (48 h): 600 mL = A
Total volume of plasma transfused (48h): 600 mL
Total volume of colloid transfused (48h): 1500 mL
Total volume of crystalloid infused (1h): 1000 mL =C

FDA guidance

1. (600 + 1 500) + 1 000 mL = 3 100 mL > PV
2. 600 + (600 + 1 500) + 1 000 mL = 3 700 mL < BV

European interpretation

1. 1 500 + 1 000 mL = 2 500 mL < PV
2. (600 + 600) + 1 500 + 1 000 mL = 3 700 mL < BV

Source: US Food and Drug Administration. Guidance for industry: eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps), August 2007, Appendix 2 at www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm091345.pdf, accessed 21 March 2019.

Appendix 19. Example of validation of screening: infectious disease assays of blood from deceased donors

The reliability of the results of screening for infectious diseases in blood samples from deceased individuals is critical and can be enhanced substantially if appropriately validated assays are used. Assays for infectious diseases specifically labelled for use in screening blood donors are considered suitable for use in screening living donors of tissues and/or cells. However, few of these assays have been validated specifically for use with blood collected after the donor's heart has stopped beating. If an assay's manufacturer has not validated the assay for use with blood samples from deceased donors, no claims are made in regard to the performance or reliability of the test results generated with such samples. Consequently, screening laboratories are expected to specifically validate these assays with such blood samples to support performance of deceased donor screening in their laboratory.

Before any validation work, potential issues associated with screening blood from deceased individuals must be understood. There are three key issues:

a. occurrence of *post mortem* degradation, or fall in detectable level, of a screening target (a marker of infectious disease such as an antigen or antibody related to the infectious agent);

b. inhibition of the assay by substances accumulating from *post mortem* changes in blood;

c. the potential for a blood sample from a deceased donor to be haemodiluted.

In all three cases there is a possibility for a false-negative test result. Although sensitivity and specificity are important for any screening assay, whether samples from living or deceased individuals are tested, sensitivity is the more important of the two because a false-negative result is the major threat to the safety of the tissue or cell transplant. In general, specificity is not as important because algorithms can be employed to effectively discriminate non-specific and specific reactivities in screening assays.

To validate assay performance when using blood samples from deceased donors, the following recommendations apply:

a. the collection times for blood samples from deceased donors used for assay validation must be representative of the full range of time points typically encountered during tissue procurement, specifically from immediately after death up to 24 h after death (see 5.3.1.1);

b. all information about storage and handling conditions for blood tubes from time of blood collection to time of testing must be documented and meet any assay sample-handling requirements stated;

c. each blood sample from a deceased donor used for validation must be evaluated for haemodilution using an approved algorithm;

d. use a dilution series prepared in deceased donor material; or use spiked specimens inoculated with the relevant infectious-disease marker at a potency near the assay's cutoff and vary sources used for spiking. In both cases, test in parallel with the same material diluted in serum or plasma from a living individual;

e. test a sufficient number of samples from different deceased donors (≥ 20);

f. include haemolysed samples;
g. sample storage methods (i.e. refrigerated, frozen) used for validation should mimic the method of storage that is routine for that laboratory.

Assay evaluation is undertaken to determine the overall performance of an assay, specifically including its core sensitivity and specificity.

Validation is done to determine the suitability of an already evaluated assay for use for a specific purpose in a laboratory; for example, for use with a blood specimen type (i.e. from a deceased donor) not specifically stated as known to be acceptable by the manufacturer. Evaluations are undertaken using panels of provenanced samples of known status, and importantly include seroconversion panels (i.e. sets of sequential samples from infected individuals following the course of infection from pre-exposure to late infection). However, such samples are almost all obtained from living patients. Suitable comparable 'positive' specimens in a series from deceased individuals are not available, making this specific type of evaluation not possible. An alternative, less realistic approach but one that has been accepted by licensing authorities is to spike non-reactive blood specimens from deceased donors with known and varying levels of virus to attempt to mimic a 'true' positive specimen [1, 2, 3].

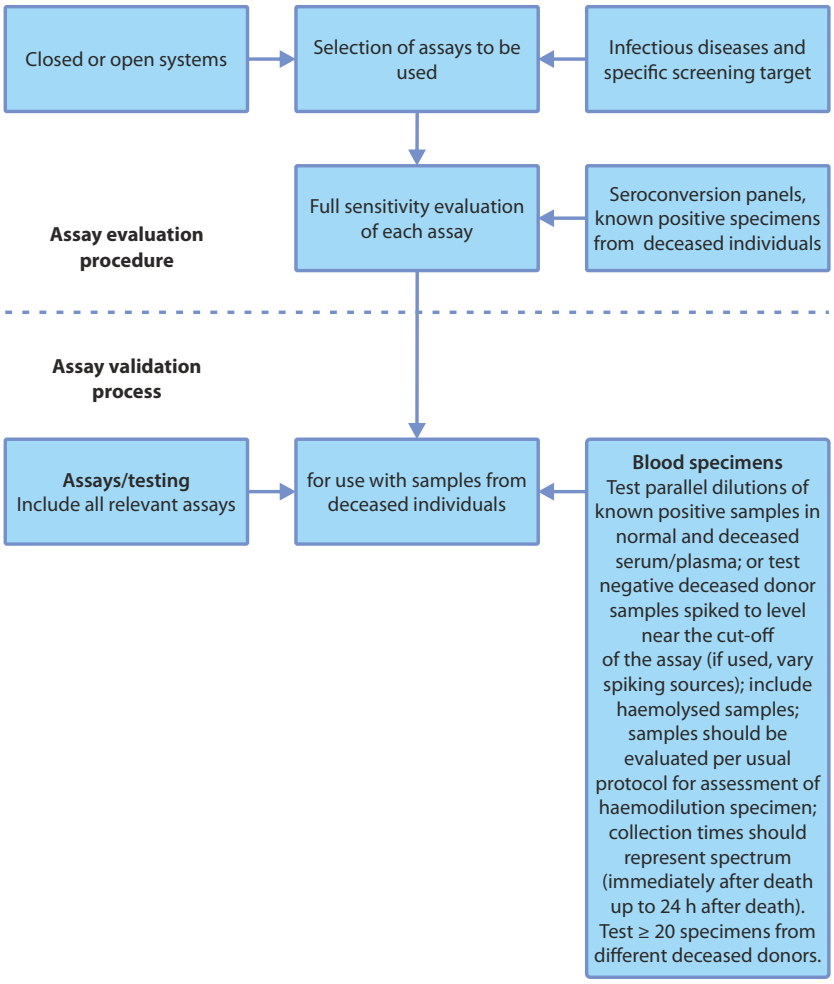
Nonetheless, before using assays with blood specimens from deceased individuals, the performance of the assays must be assessed in some way to make attempts to demonstrate there is no loss of, or other change in, the expected performance of the assay.

Validation of assays for use with blood from deceased individuals is critical, both for serological assays that detect antigens or antibodies and for molecular assays that detect viral nucleic acid. However, simultaneously, this is problematic due to key issues stated above, and a lack of suitable blood samples from deceased individuals to support such work. The issue of the degradation of any screening target that may be present is the hardest to deal with due to lack of suitable published studies. However, if the timeframe for sample collection *post mortem* is limited by those carrying out tissue procurement, it can be theorised that it would be unlikely for any markers of a previously unidentified and relevant infection to have been degraded to a level that is undetectable using the high-quality assays available from major international diagnostics manufacturers. If this aspect is controlled, the next issue to be considered is the potential 'inhibitory effect' of the sample as a result of any *post mortem* changes. To some degree the same argument applies in regard to degradation of the screening target *post mortem* because specimen collection within a suitable timeframe minimises the extent of any *post mortem* changes, whatever they are (e.g. red cell haemolysis, precipitants/byproducts of cell death, etc.). There are ways in which an inhibitory effect can be examined in serological and molecular assays. Known positive serum/plasma samples can be diluted in serum plasma from living individuals in parallel with serum/plasma from deceased individuals and from living individuals, either to a fixed point or to extinction, and the outcomes compared. In this aspect, validation of molecular assays is slightly easier than that of serological assays because the inclusion of an internal control in molecular assays validates each test result with respect to the presence of any 'inhibitory substance' that may be present in a sample. If the internal control is not amplified, the test result for the sample is deemed to be invalid. Therefore, although not definitive, such approaches would identify any gross effects, which would most likely result in false-negative results.

To meet donor/donation screening expectations, validation of infectious-disease assays for use with blood from deceased individuals must be undertaken. If the assays have been evaluated appropriately by the testing laboratory, then there is clear understanding of the baseline performance of each assay, and this can be used as the basis of additional assay validation work for use with blood samples from deceased donors. Lack of suitable samples to mirror evaluation panels is a particular problem, leaving comparative dilutional studies and/or spiked sample studies as feasible approaches to determine any problems associated with testing blood from deceased individuals.

Validation scheme

Flowchart courtesy of Dr Alan Kitchen with input from Susan Best.



References

1. Edler C, Wulff B, Schroeder AS *et al.* A prospective time course study on serological testing for human immunodeficiency virus, hepatitis B virus and hepatitis C virus with blood samples taken up to 48 hours after death. *J Med Microbiol* 2011;60:920-6.
2. Meyer T, Polywka S, Wulff B *et al.* Virus NAT for HIV, HBV, and HCV in post-mortal blood specimens over 48 h after death of infected patients – first results. *Transfus Med Hemother* 2012 Dec;39(6):376-80.
3. Commission Directive 2012/39/EU of 26 November 2012 amending Directive 2006/17/EC with regard to certain technical requirements for the testing of human tissues and cells, available at https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:327:0024:0025:en:PDF, accessed 7 August 2019.

Appendix 20. *Treponema pallidum* testing

The algorithms that are recommended for the serological diagnosis of syphilis are challenging due to the inherent complexity of these methods. Thus, these tests are subdivided into treponemal and non-treponemal tests and the interpretation of the results obtained is often particularly difficult, hence the need for further confirmatory testing (see Table A20.1). See also the website of the European Centre for Disease Prevention and Control at www.ecdc.europa.eu.

Non-treponemal tests

Non-treponemal tests are tests that search for IgG and IgM directed against the lipids that are released from the damaged human cells during an early stage of the disease. The goal of these tests is therefore to search for antibodies to antigens that are not specific to an infection with species of the genus *Treponema*, as reflected in the term reaginic antibodies. The non-specific nature of this category of serological tests is also reflected in the fact that many other causes, such as advanced age, pregnancy, various types of malignant tumours, autoimmune diseases and other unrelated infections may result in the formation of anti-lipoid antibodies, thus generating false-positive results.

Consequently, a positive result obtained with a non-treponemal test should always be confirmed by means of a treponemal test. Moreover, non-treponemal tests usually display a low sensitivity in the detection of early syphilis, and the first positive results are not obtained until some 4-8 weeks after infection. The tests belonging to this category have mainly a diagnostic purpose as part of the therapeutic follow-up of patients with syphilis. Thus, a declining titre over a certain period of time is indicative of a favourable response to treatment. As a rule, successful treatment leads to negative results for these tests. The Venereal Diseases Research Laboratory (VDRL) test and Rapid Plasma Reagin (RPR) test belong to this group of non-treponemal tests used for serological syphilis screening.

Treponemal tests

Treponemal tests are serological screening tests that search for specific antibodies directed against species of the genus *Treponema*. No distinction can be made between the different treponematoses due to immunological cross-reactions. These tests usually remain positive after the initial infection, which means that they cannot be used to monitor the response to treatment or diagnose reinfections. Treponemal serological tests include the *T. pallidum* haemagglutination (TPHA) test, the *T. pallidum* particle agglutination (TPPA) test, treponemal enzyme immunoassays (EIA), chemiluminescence immunoassays (CLIA) and immunoblotting.

Algorithms in use

New developments, especially in the optimisation of treponemal immunoassays, offer new possibilities due to the earlier detection of syphilis and the shorter diagnostic window, but do not necessarily simplify the assessment of the overall serological picture. According to recent international recommendations the following screening algorithms can be used for serological syphilis screening.

Only the treponemal screening test

This screening strategy is commonly used in European blood banks and laboratories due to its potential for large-scale automation. This algorithm identifies both individuals in whom syphilis has been treated successfully as well as those who have not received any treatment. It is better suited to detect the early stages of infection than the sole use of a non-treponemal test. Given the fact that this strategy is mainly used for populations with a low prevalence of syphilis, it suffers from a considerable number of false-positive results.

Only the non-treponemal screening test

Ideally, a non-treponemal test carried out for screening purposes should be quantitative in nature in order to rule out the prozone effect when using undiluted blood samples. (This concerns < 2% of samples, usually during the secondary phase of syphilis. These patients display extremely high titres of antibodies that interfere with the formation of antigen-antibody complexes, which are necessary to visualise flocculation when interpreting the non-treponemal test.) This algorithm can only detect active (infectious) syphilis, which means that it can miss the early stage of syphilis.

Treponemal and non-treponemal tests

This algorithm is especially useful to screen high-risk populations as well as to screen for the early stages of syphilis.

In the serological diagnosis of syphilis and independently of the screening algorithm used, a confirmatory test will always need to be carried out, regardless of which of the screening tests turned out positive.

- If the initial screening test only included a treponemal test, the results should be confirmed by means of a second treponemal test based on a different analytical method as well as a quantitative non-treponemal test if this second treponemal test also turns out positive.
- If the initial screening test only included a non-treponemal test, the positive result needs to be confirmed by means of a treponemal test, whereas the non-treponemal test should be performed in a quantitative manner if this was not initially the case.
- If the initial screening was performed using a treponemal test as well as a non-treponemal test, the non-treponemal test should be performed in a quantitative manner. A second treponemal test based on a different analytical method may be used to rule out a false-positive result for the initial treponemal test only if the non-treponemal test is negative.

Table A20.1. **Interpretation of the results of biological screening tests for syphilis**

Treponemal test	Non-treponemal test	Interpretation	Consequence for the donation	Further management
positive	positive[1]	active infection	reject	contact the attending physician
positive	negative	treated (past) infection *or* early stage of infection *or* false-positive[2]	reject	contact the attending physician[2]
negative	not carried out or negative	no infection	release possible	no further action
negative	positive	false-positive result for the non-treponemal test *or* false-negative result for the treponemal test	release potentially possible[3]	no further action

1 Given the fact that in the vast majority of cases in which the non-treponemal test yielded false-positive results the titres were ≤ ¼, a 'positive treponemal test' is considered to be with a titre ≥ ⅛.

2 In such a case a confirmatory treponemal test needs to be carried out. If this confirmatory treponemal test yields a negative result, the initially positive result of the treponemal test is not confirmed and therefore looked upon as false-positive, which justifies releasing of tissues and cells intended for donation and requires no contact with the attending physician concerned.

3 The TE administrator can still accept the tissues and cells after having consulted the clinical biologist, possibly after carrying out additional tests, and having received the informed consent of the recipient and the medical transplant team.

Appendix 21. **Sample form to assess working environment (NHS, UK)**

(extracted from NHS FRM3831/5.1) Effective: 16/12/13

Tissue Services Tissue Donation From Deceased Donors

Venue:	Donation Number and Donor Number:

The intent of the below is to assess the suitability of the working environment for safety to both staff and tissue.

Minimum Requirements:

	Yes	No	Comments
Are there a minimum 2 authorised people present?			
Is there good lighting and a sink with running water available?			
Do you know the evacuation procedure?			
Is there suitable access to minimise carrying and handling e.g. parking.			
Is the support equipment working and used where applicable? e.g. trolleys, control panels, 'in use' signs etc.			
Can unauthorised people view the donation? e.g. doors open, blinds open, clear glassed windows			
Is there unauthorised filming / photography equipment in use?			

Site Assessment:

	Yes	No	Comments / Action
Are the floors wet?			
Do any surfaces appear dirty / contaminated?			
Are there any sharp objects/dangerous equipment/clutter around i.e. hazards to avoid/move?			
Is the donation area a clean environment (if necessary clean with detergent prior to use)?			
Is a post mortem being carried out at the same time as the donation?			
Do you believe that tissue can be retrieved with minimal or no environmental contamination?			
Are there specific donor related risks and actions taken to mitigate these risks e.g. large donor?			

	Yes	No	Comments / Action
Was the Donors face protected prior to moving to donation area?			If No, explain.
Person Responsible for moving donor to / from donation area:	Delete as applicable: **Porters / APTs / TS Staff / Others** Others please specify:		

Authorisation to proceed:

Are you satisfied that this is a safe / clean working environment? Yes / No
If No, contact the Duty Manager ASAP
Name of Manager contacted:

(Template Version 07/10/08)

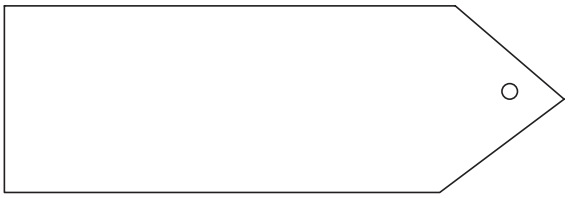
Cross-Referenced in Primary Document: MPD557

Source: National Health Service (NHS), United Kingdom.

Appendix 22. **Sample donor-identification form (NHS, UK)**

(extracted from NHS FORM FRM3831/5.1) Effective: 16/12/13

Tissue Services Tissue Donation From Deceased Donors

Donation Number:		
Confirm correct donor by transcribing identification details directly from identity band/label. You **must** have 3 points of matched ID (e.g. name, dob, hospital number, address, or (exceptionally) circumstances of death). Method of ID: Toe tag/Identity band/Mortuary label/Other…….  **COPY TAG**		
Identification and Examination performed by (PRINT NAME)	Sign:	Date:
Identification Double checked by (PRINT NAME)	Sign:	Date:

Check Authorisation. Do you have 3 points of matched ID that correspond with the referral information and donor identification?			
Yes / No	Print name:	Sign:	Date:
If No, can you justify your rationale for proceeding with the donation below? **Yes / No / NA** **If No, contact Duty Manager ASAP** Name of Manager contacted:			

ADDITIONAL INFORMATION	SIGNATURE AND DATE

(Template Version 07/10/08)

Cross-Referenced in Primary Document: MPD557

Source: National Health Service, United Kingdom.

Appendix 23. **Checklist for revision of computerised systems**

Establishment . . . . . .

Date . . . . . .

Signature of person responsible for the revision . . . . . .

1. Identification of the system and function		Comments
Name of the system		
Version		
Supplier		
Platform		
Function		
Connected with other computerised systems		

2. Organisation	Comments
☐ Is the organisation for function and maintenance described in the QM system (system owner, system manager, person responsible)? ☐ Are these functions placed in an organisation scheme?	
☐ Is the responsibility of the supplier described in QM system and in written agreements? ☐ Is it clear that the user is responsible for validation when data are transferred between different systems?	
☐ Have the responsible persons (functions) received enough and documented training in case of malfunction of the system?	
☐ Is the computerised system included in the scheme for internal revision?	

3. Written agreements	Comments
☐ Are responsibilities for support (software and hardware) clearly defined? ☐ Are functions/responsibilities of subcontractors included?	
☐ Are instructions for documentation of unexpected events included? ☐ Is time-limit for corrective actions by responsible support defined?	
If data are transferred between different computerised systems: ☐ Are platforms and protocols described? ☐ Are obligations to inform each other about changes and events that may influence information transfer included? ☐ Are responsibilities for the different parts in the chain between the systems clearly defined?	

4. Documentation of the system	Comments
☐ Is complete and updated documentation of the system accessible? ☐ Does the documentation contain measures for managing malfunctions and fallbacks? Is a user guide with version number accessible: ☐ in paper copy? ☐ as electronic 'help-function'?	

5. Maintenance	Comments
☐ Are standard operating procedures available for measures in case of malfunction/total downtime? ☐ Back-up system? ☐ Reset of data? ☐ Are back-up system and read-back functions tested?	

6. Changes	Comments
☐ Is a test environment available? ☐ Are validation procedures defined and performed before updates, changes, new versions in the system?	

7. Information security	Comments
☐ Is access to the computers protected by locked doors (physical data protection)?	
☐ Is a virus protection system active (if applicable)?	
Is access to the computerised system protected by personal login? ☐ Single-level login ☐ Double-level login	
☐ Is access to the system (and login) associated with a certificate of authorisation? ☐ Who decides on, and keep records of, access to the system? ☐ Are records for access to the system updated (i.e. access removed when no longer needed)?	
☐ Does the system provide traceability of the user? ☐ Does the system provide traceability of changes in manually added data/text (with the original text still readable)?	
If data are manually inserted/transferred from another system: How is correctness of the data verified: ☐ by data insert of two individuals independently? ☐ by saving the original (paper) result? ☐ by signature(s) of the individual(s) inserting the data?	
If data are automatically transferred from another computerised system: ☐ Are 'check points' to verify the correctness of data transfer available in the system or as standard operating procedures?	

Adapted from SWEDAC DOC 10:5, 2010 Guidance for information security managers (available from www.isaca.org/Knowledge-Center/Research/ResearchDeliverables/Pages/Information-Security-Governance-Guidance-for-Information-Security-Managers.aspx) with interpretation of ISO/IEC 17025, 'General requirements for the competence of testing and calibration laboratories' (available from www.iso.org/iso/catalogue_detail?csnumber=39883) and ISO/IEC 27007:2011 (available from www.iso.org/obp/ui/#iso:std:iso-iec:27007:ed-1:v1:en).

Appendix 24. Serious adverse reaction or event: impact assessment form

This impact assessment tool assists practitioners and regulators in planning their response to a given serious adverse reaction or event (SARE), taking into account the broader consequences beyond the individual patient affected or potentially affected. The assessment should be based on available data, past experience and scientific expertise.

Step 1: Assessment of the likelihood of occurrence/ recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		**On individual(s)**		**On the system**		**On tissue/cell supply**
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

Step 3: Application of the impact matrix

Likelihood of recurrence → / Impact of recurrence ↓	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/ almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Step 4

The response of a tissue establishment or health authority to a specific SARE should be proportionate to the potential impact, as assessed by the matrix shown in Step 3 above and described below.

White The tissue establishment is to manage the corrective and preventive actions; the Health Authority is to file the report and keep a 'watching brief'.

Pale shading Requires interaction between the tissue establishment and the Health Authority, which may request an inspection that focuses on the SARE and the corrective and preventive actions to be followed up, including evidence of effective recall, where necessary. Written communication to professionals working in the field might be appropriate.

Dark shading The Health Authority will, in general, designate representatives to participate in developing or approving the corrective and preventive action plan (possibly a task force to address broader implications). Inspection, follow-up and written communication should be done as at the previous level; and possibly notification of health authorities in other countries where relevant.

Effectiveness of the response can be assessed by re-applying the impact matrix following implementation of corrective and preventive actions. The impact can be reduced by:

- reducing the probability of recurrence through preventive measures;
- increasing the detectability of the risk; or
- reducing the severity of the consequences, if it should recur.

Source: SOHO V&S Guidance for Competent Authorities.

Appendix 25. **Serious adverse reaction: notification form – for ocular tissues (Agence de la Biomédecine, France)**

Notification form for SAR on ocular tissues

SEC | Recipient code | Date of birth *(dd/mm/yyyy)*

Date of transplant *(dd/mm/yyyy)* | Sex ☐ M ☐ F | Eye involved ☐ Right ☐ Left

Type of graft ☐ DALK ☐ DSAEK ☐ DMEK ☐ PK

SAR

1. Short term (pre-graft until 1 month post-graft)

- ☐ Primary graft failure (endothelial decompensation)
- ☐ Ocular infection (from bacterial, fungal, parasitic or viral origin including endophthalmitis)
- ☐ Irreversible rejection (specific immunologic response)
- ☐ Systemic infection (compatible with a donor-recipient transmission)
- ☐ Persistent ulceration or corneal perforation

2. Mid- to long term	1 month to 1 year	> 1 year
☐ Any ocular pathology that could suggest transmission from the donor (for example: tumoral pathologies of the anterior segment)	☐	☐
☐ Any systemic pathology that could suggest transmission from the donor (transmissible viral diseases (rabies, hepatitis, etc.), malignant diseases, prion disease …)	☐	☐
☐ Unrecognised donor corneal diseases (history of refractive surgery, corneal dystrophy including FUCHS, keratoconus, scars …)	☐	☐
☐ Endothelial cell decompensation including cornea guttata	☐	☐
☐ Chronic endophthalmitis	☐	☐
☐ Late-onset local infection including bacterial, fungal, viral, or parasitic keratitis or kerato-endotheliitis	☐	☐

☐ Failure (leading to re-graft). Specify		☐
☐ Persistence of complicated epithelial defects (epithelial ulcerations) (visual decline, infection, rejection, stromal ulceration, perforation …) or development of epithelial tumour, dysplasia or epithelial hyperplasia	☐	☐
☐ Defect of corneal transparency (corneal opacification, calcifications, corneal transparency delay, inflammatory infiltrate …)	☐	☐
☐ Astigmatism induced by the graft or giant astigmatism (> 8 D) linked or likely to be linked to recurrence or appearance of keratoconus on the graft	☐	☐
☐ Loss of eyeball (anatomical or functional)	☐	☐
☐ Death of patient linked to the ocular graft	☐	☐

Risk factors

☐ No risk factor identified

Risk factor identified (tick the one concerned)

☐ Neovascularisation – History of autoimmune disease (scarring pemphigoid)	☐ Neurotrophic history	☐ Atopic ground
☐ Graft size outside the range of 8.5 diameter	☐ Palpebral alterations (statics/kinetics)	☐ Paediatric patient or less than 16 years old
☐ Hypertonia/glaucoma	☐ Emergency cornea grafting or therapeutic keratoplasty	☐ Re-graft – how many? ☐
☐ Ocular inflammation/uveitis	☐ History of rejection on either ipsi or contralateral eye	☐ Herpes virus infection (HSV, VZV)
		☐ Dry eye syndrome
		☐ Chemical burn

☐ Other – give details

Appendix 26. **Serious adverse reaction or event: notification form – for ocular tissues (NHS, UK)**

FORM FRM4159/2 Effective: 17/07/17

Ocular Tissue Transplantation - Serious Adverse Reactions/Events

Part A – NOTIFICATION OF ADVERSE REACTIONS AND EVENTS

(See reverse side of this page for important additional information)

Please complete this Part A form to notify NHSBT of an Adverse Reaction/Event:

- Contact Tissue and Eye Services Customer Care on Tel: 08456076819
- Send one copy of this form to Tissue and Eye Services Customer Care either by e-mail to tscustserv@nhsbt.nhs.uk, or by fax to 08456076820, or by post to Tissue and Eye Services Customer Care Dept., NHSBT, 14 Estuary Banks, Speke, Liverpool L248RB; you can also report online at https://www.organdonation.nhs.uk/IncidentSubmission
- Retain original in the patient's notes

DONOR NUMBER/ EYE BANK REFERENCE .....

REPORTING DATE ..... **RECIPIENT INITIALS & DATE of BIRTH** .....

DATE OF TRANSPLANT ..... **RECIPIENT NHS/CHI NUMBER** .....

CONSULTANT (please print) ..... **RECIPIENT HOSPITAL** .....

CONTACT DETAILS Tel ..... **Email** .....

INDICATION / URGENCY of TRANSPLANT.....

EYE BANK SUPPLYING THE TISSUE please tick)
□ Filton/Bristol □ Manchester □ Moorfields □ East Grinstead □ other

TYPE OF TISSUE OR CELLS (please tick)
□ Cornea □ Sclera □ Limbal
□ Other - please specify.....

ADVERSE REACTION

DATE OF SUSPECTED ADVERSE REACTION .....

TYPE OF SERIOUS ADVERSE REACTION (please tick)

1. Primary graft failure (corneal transplant never cleared)
2. Endophthalmitis or other serious ophthalmic infection
3. Graft failure due to donor tissue which was out of date, scarred or had evidence of previous surgery.
4. Malignancy likely to be attributable to the transplanted tissue
5. Systemic infection possibly attributable to the transplanted tissue
6. Other, please specify .....

ADVERSE EVENT

TYPE OF SERIOUS ADVERSE EVENT (please tick)

1. Wrong material supplied.
2. Tissue supplied is out of date.
3. Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery.
4. Other, please specify .....

Was there any adverse impact on the patient, e.g. patient already anaesthetized?.....

WAS THIS TISSUE USED? YES / NO
PLEASE RETURN ALL UNUSED TISSUE IN THE ORIGINAL CONTAINER TO THE ISSUING EYE BANK FOR INVESTIGATION; PLEASE DO NOT USE FORMALIN

(Template Version 01/11/13)

Cross-Referenced in Primary Document: Standalone Page 1 of 2

FORM FRM4159/2 Effective: 17/07/17

Ocular Tissue Transplantation - Serious Adverse Reactions/Events

Ocular Tissue Transplantation - Adverse Reactions/Events

NHSBT SERIOUS ADVERSE REACTIONS/EVENTS REPORTING MECHANISM
Part A (i.e., this form) – To be completed by the person notifying NHSBT of an Adverse Reaction/Event
Serious Adverse Reactions/Events will be reviewed by NHSBT and OTAG[1]

DEFINITIONS[2]
'Serious adverse reaction'– means an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity

'Serious adverse event'– means any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalisation or morbidity.

Serious Adverse Reactions
Corneal Transplantation
1. Primary graft failure (graft never cleared)
2. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue[3]
3. Graft failure due to donor tissue which was out of date, damaged, scarred or had evidence of previous surgery
4. Malignancy likely to be attributable to the transplanted tissue
5. Systemic infection likely to be attributable to the transplant tissue
6. Other[4]

Ocular Tissue Stem Cell Graft
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue/cells[3]
2. Graft failure due to donor tissue/cells that were out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue/cells
4. Systemic infection likely to be attributable to the transplanted tissue/cells
5. Other[4]

Scleral Grafts
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue
2. Graft failure due to donor tissue which was out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue
4. Systemic infection likely to be attributable to the transplanted tissue
5. Other

Serious Adverse Events
There are potentially numerous serious adverse events. Specific serious adverse events that are peculiar to ophthalmology include
1. Wrong material supplied
2. Tissue supplied is out of date
3. Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery
4. Other[5]

Notes:
1. OTAG Adverse Reactions and Events Reporting subgroup
2. EU Tissues and Cells Directive 2004/23/EC
3. This relates to the development of a severe infection likely to be attributable to the transplanted tissue. It does not include for example the occurrence of a microbial keratitis related to a suture abscess.
4. Other refers to any unexpected adverse reaction which is considered by the surgeon to be serious and possibly attributable to the transplanted tissue
5. Other refers to any adverse event which is considered to be serious and could potentially lead to a serious adverse reaction if the tissue is used in a patient.

(Template Version 01/11/13)

Cross-Referenced in Primary Document: Standalone Page 2 of 2

Source: National Health Service, United Kingdom.

Appendix 27. **Sample forms for the evaluation of heart valves**

Pulmonary Heart Valve Information

GRAFT INFORMATION	
Heart Valve Bank:	Donor Number:
ODT Number(if applicable):	Valve Number:
Date Dissected:	Date Cryopreserved:
Expiry Date of Valve:	Photograph Available on request: Y / N

L R R L

Pathology noted on cusps:

Key (please annotate the diagram above)
Atheroma = ……
Fenestration = 000
Fibrosis = XXXX
Other:

Condition: Excellent / Good / Fair

Comments:

Pulmonary Inner Annular Diameter	mm	Left artery inner annular diameter	mm
Length of Vessel	mm	Left artery length	mm
Length of muscle skirt (Min / max)	mm	Right artery inner annular diameter	mm
Total Length	mm	Right artery length	mm

STERILITY REPORT	
Hepatitis B:	HCV PCR:
Hepatitis B Core Antibody:	HIV PCR:
Hepatitis C:	HBC PCR :
HIV I and II:	Microbiology Culture:
Syphillis:	Mycobacteria:
HTLV :	Other :

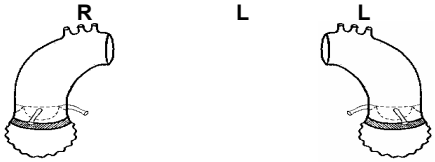
DONOR INFORMATION	
Age:	Sex:
Date of Death:	Cause of Death:

Information Entered By:	Date:	Signature:

Aortic Heart Valve Information

GRAFT INFORMATION	
Heart Valve Bank:	Donor Number:
ODT Number (if applicable):	Valve Number:
Date Dissected:	Date Cryopreserved:
Expiry Date of Valve:	Photograph available on request: Y / N

R L L R



Pathology noted on cusps:

NCC
LCC RCC

Key (annotate diagram above)	
Atheroma = ……	LCC = Left Coronary Cusp
Fenestration = 000	RCC = Right Coronary Cusp
Fibrosis = XXXX	NCC = Non Coronary Cusp
Other:	

Aorta Inner Annular Diameter	mm
Length of Aorta	mm
Length of muscle skirt (Min / max)	mm
Total Length	mm

Condition: Excellent / Good / Fair

Comments:

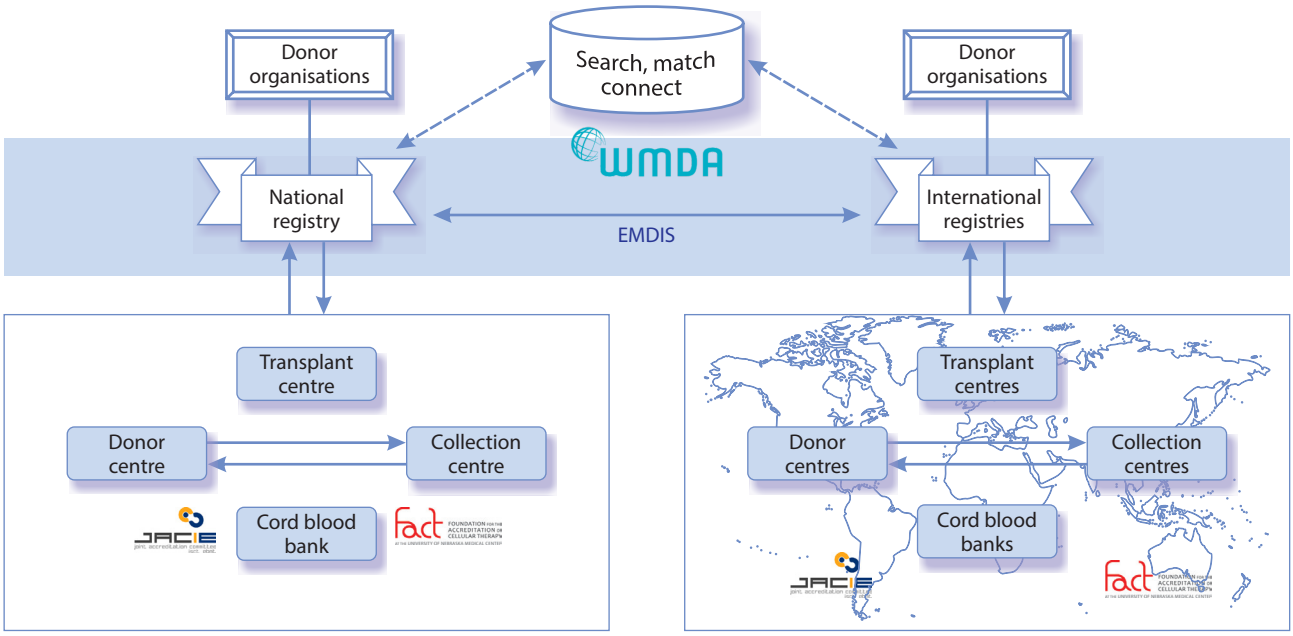
SEROLOGY / BACTERIOLOGY STATUS		
Hepatitis B:	Hepatitis B Core Antibody:	Hepatitis C:
HIV I and II:	HIV PCR:	HCV PCR:
HBC PCR:	Syphilis:	HTLV:
Mycobacteria:	Microbiology Culture:	
ABO Blood group (if known):	Other:	

DONOR INFORMATION	
Age	Sex:
Date of Death:	Cause of Death

Information Entered By:	Date	Signature

Source: National Health Service, United Kingdom.

Appendix 28. **Donor search through registries for haematopoietic progenitor cell transplantation**



WMDA: World Marrow Donation Association.
EMDIS: European Marrow Donor Information System.
JACIE: Joint Accreditation Committee ISCT-Europe & European Society for Blood and Marrow Transplantation.
FACT: Foundation for the Accreditation of Cellular Therapy.

Appendix 29. **Health assessment questionnaire for cord blood donor**

Mother's name. . . . . .

Collection facility . . . . . *ISBT code* . . . . . .

Date of birth . . . . . *Questionnaire date*. . . . . .

Post-birth questionnaire

Pharmacological treatment history	Yes	No	Observations
Did you take any medication in the last seven days before delivery?			
Did you take Finasteride or similar medication for hair loss during pregnancy?			
Did you take Etretinate or similar medication for skin problems during pregnancy?			

In the last two weeks before birth	Yes	No	Observations
Did you have fever with headache and nausea?			
Did you visit the dentist?			

In the last month before birth	Yes	No	Observations
Did you get vaccinated?			
Were you in contact with someone who had a contagious/infectious disease?			

In the last four months before birth	Yes	No	Observations
Did you go to a doctor or were you in hospital?			
Did you have any endoscopy: colonoscopy, gastroscopy, cystoscopy or other such treatment?			
Did you have acupuncture with non-disposable equipment or materials?			
Did you get a tattoo and/or skin piercing?			
Did you have contact with the blood of another person by an accidental needle-stick puncture or splash?			
Have you lived with or maintained close contact with a person with hepatitis or jaundice, or a hepatitis virus carrier?			
Did you have surgery?			

In the last six months before birth	Yes	No	Observations
Did you travel outside Spain?			
Where?			
Have you ever live abroad (outside Spain)?			
Where?			
Have you ever (at any time) travelled abroad?			
Where?			
Have you lived for more than one year – in total, adding all periods of stay – in the United Kingdom (England, Wales, Scotland, Northern Ireland, Channel Islands, Isle of Man) during the 1980-1996 period?[1]			
During pregnancy, did you reside in or travel to a risk area for the Zika virus?			
During pregnancy, did you have a medical diagnosis of Zika virus infection?			
During pregnancy, did you have sexual contact with a male who: • was diagnosed with a Zika virus infection in the 6 months prior to the sexual contact? • travelled to or resided in a risk area for the Zika virus in the 6 months prior to the sexual contact?			
Have you ever been excluded from being a blood donor?			
Have you ever had any serious disease requiring regular medical checks?			
Have you ever had any hepatitis, jaundice or liver problems?			
Have you ever been affected by any infectious disease such as malaria, Chagas disease, leishmaniasis, infectious tuberculosis, mononucleosis, syphilis or gonorrhea, among others?			
Have you ever had any serious illness of lungs, brain, kidneys, thyroid, digestive system or in any other part of the body?			
Have you ever had heart or blood-pressure problems?			
Are you diabetic and being treated with insulin, excluding gestational diabetes?			
Have you had any type of cancer?			
Have you ever had any severe illness or severe allergic reaction?			
Have you had any bleeding problem or blood disease, such as anaemia or excessive red blood cells?			
Have you ever received a blood transfusion or clotting factor treatment?			
Did you before 1987 receive a growth hormone of human origin?			
Have you ever received an organ or tissue (*dura mater*, cornea or other)?			
Have you or anyone related to you suffered from spongiform encephalopathy (Creutzfeldt–Jakob disease)?			
Have you ever had or do you now have infection with human T-lymphotropic virus (HTLV-I/II)?			
Have you ever been diagnosed with autoimmune disease?			

1 Transmissible spongiform encephalopathies transmission risk should be considered in persons who lived in the United Kingdom between January 1980 and December 1996 for longer than 6 months (for residents of countries other than the UK).

Baby history	Yes	No	Observations
Is your child in good health?			
Has he/she had any problem or disease?			
How well did the paediatrician find him/her during the last visit?			
Has there been any analysis or investigation of the child?			
Birthplace of the child's maternal grandmother			
Birthplace of the child's maternal grandfather			
Birthplace of the child's paternal grandmother			
Birthplace of the child's paternal grandfather			

Obstetrical and consanguinity history	Yes	No	Observations
Was this your first son/daughter?			
If not, how many children do you have?			
Are you related to the child's father (cousin or other relation of consanguinity)?			
Do you have any questions or doubts?			

Conclusions

Reviewed by (name and signature) . . . . . .

Source: adapted from the Concordia Programme (Barcelona Cord Blood Bank).

Appendix 30. **Data collection for cord blood donor**

Summary of diseases that contraindicate a donation

Addiction to non-legal drugs

Parenterally excludes the donor even if it was only once. Addiction to other drugs, including alcohol, excludes because the donor's faculties may have changed so as to impair the ability to give full consent.

Anaemia

Homozygous or heterozygous father or mother for haemoglobinopathies or hereditary enzymopathies.

Severe eclampsia

Coagulopathies

Father to mother with hereditary coagulation problems that require treatment and that can be transmitted by the progenitor cells (example: platelet disorders).

Depression, manic-depressive psychosis not treated with regularity, dementia

Not in a position to give consent with full autonomy.

Haemochromatosis, cirrhosis, Wilson disease

Hepatitis, except Hepatitis A, CMV and EBV after 6 months of treatment

The sexual partners of people with hepatitis B or C will not be able to donate up to 4 months after the last sexual contact. People who live together (direct domestic contact), including the vaccinated, with carriers of the Hepatitis B virus cannot donate up to 4 months after the end of the cohabitation. People who have received transfusions cannot donate until 4 months afterwards.

Infertility (in treatment)

If subject has been treated with gonadotropins of pituitary origin before 1987.

Autoimmune disease

Rheumatoid arthritis, Behçet's disease, esclerodermia, amyotrophic lateral sclerosis, multiple sclerosis and its variety Devic disease, spondyloarthropathy, fibromyalgia, rheumatic fever, Wegener granulomatosis, Systemic lupus erythematosus polymyositis, dermatomyositis, chronic inflammatory demyelinating polyradiculoneuropathy, extensive psoriasis in treatment with Tigasón or with arthritis, immune thrombocytopenic purpura, sarcoidosis, Chronic Fatigue Syndrome, Guillain–Barré syndrome, Sjögren syndrome, systemic vasculitis, vitiligo, Type I diabetes, Reiter syndrome, vasculopathies.

CNS diseases

Neurodegenerative diseases, neurofibromatosis, Parkinson's.

Dermatological diseases

Dermatitis herpetiformis, bullous dermatitis, polymorphic drug erythema, Kaposi syndrome, urticaria pigmentosa, mycosis fungoides, cutaneous leukosis, Sézary's disease.

Creutzfeldt–Jakob disease

Diseases suspected of being caused by viruses or prions must be excluded. Definitive exclusion of pituitary hormone receptors of human origin (those administered before 1987). Stay in the United Kingdom: this excludes people who have lived in the United Kingdom for more than 12 months during the period 1980-1996 on a continuous or intermittent basis. The United Kingdom consists of: England, Wales, Scotland, Northern Ireland, Isle of Man and Channel Islands. The Republic of Ireland (Eire) is not part of the United Kingdom.

Neoplastic haematological diseases, deposit and genetic diseases

Kidney diseases

Chronic renal failure.

Oncological diseases

Neoplasms of any type except carcinoma *in situ*, provided that it is not receiving treatment or has unhealed wounds.

Lung diseases

Sarcoidosis.

Thyroid diseases

Treatment with radioactive iodine excludes up to 6 months after completion. Exclusion if it is a neoplasm or is part of a multisystem disease. Apart from the above situations, a person can be accepted as a donor even if being treated with thyroxine.

Tropical diseases

Donors returning from a trip through tropical countries at risk should not be accepted as donors until after 6 months, and then only if they have been free of disease during this period.

Myasthenia/myopathy

Infectious diseases

Donors with a history of tuberculosis or malaria will be excluded for 5 years after the cure. They will be excluded for 2 years after the cure of brucellosis, Q fever (negative serology), rheumatic fever and osteomyelitis. After an episode of fever > 38 °C, potential donors will be excluded for at least 2 weeks from the date of clinical recovery. A history of hepatitis C, hepatitis B, AIDS, visceral Leishmaniosis (Kala-azar), babesiosis, Chagas disease or infection per HTLV-I/II is cause for exclusion. A history of measles, mumps, rubella or varicella excludes up to 4 weeks after cure. In cases of infectious mononucleosis, the exclusion will be 6 months.

Data collection form

Preprinted label	CB label	Mother donor label

Collection centre

Donor name ID

Geographical origin

Mother ☐ Spain ☐ EU ☐ Europe (other) ☐ North Africa ☐ Sub-Saharan Africa ☐ Asia ☐ Latin America ☐ Other

Father ☐ Spain ☐ EU ☐ Europe (other) ☐ North Africa ☐ Sub-Saharan Africa ☐ Asia ☐ Latin America ☐ Other

Travel to endemic areas _____

Inclusion questionnaire	**Yes**	**No**
Infectious transmissible diseases	☐	☐
Transmissible genetic disorders found in newborn's father, mother and/or siblings	☐	☐
Severe maternal anaemia (Hgb < 7 g/dL)	☐	☐
Fever (temperature > 38 °C) (with suspected infection)	☐	☐
Haemolytic disease of the newborn	☐	☐
High-risk delivery	☐	☐

Obstetrical history ☐ ☐ ☐ ☐ T = term births; P = preterm births < 37 weeks;
T P A L A = abortions < 20 weeks; L = living children

Medical background of the mother _____

Medical background of the father _____

Check if pregnancy from ☐ egg donation ☐ and/or sperm donation

Negative viral serology during pregnancy for ☐ HBV ☐ HCV ☐ HIV ☐ Chagas

Donor's date of birth d d m m y y y y Age ☐☐ Weeks of gestation ☐☐

Delivery type ☐ eutocic ☐ instrumental ☐ programmed Caesarean ☐ urgent Caesarean

Time for cord clamping ☐☐☐ seconds APGAR: minute 1 _____ minute 5 _____

Drugs administered during childbirth _____

Incidents, adverse effects detected in the donor _____

Date and time of cord blood collection d d m m y y y y h h : m m Sex (M/F) ____ Weight _____ g

Problems detected in the newborn _____

Trained collector

ID

Surname

First name

E-mail

Signature

Source: adapted from the Concordia Programme (Barcelona Cord Blood Bank).

Appendix 31. Informed legal consent for cryopreserving and storing semen from a minor

as a precautionary measure under medical circumstances with a risk of reduction of fertility

Informed consent declaration (Spanish Fertility Society – SEF) model

	Date dd mm yyyy		History no.
Mr	Name and surname of father		
ID/Passport no.			Age
Resident at			

	Date dd mm yyyy		History no.
Mrs	Name and surname of mother		
ID/Passport no.			Age
Resident at			

	Date dd mm yyyy		History no.
Mr	Name and surname of child		
ID/Passport no.			Age
Resident at			

Due to the present circumstances of the under-age patient (illness, intervention or medical treatment that may cause sterility, and/or the advisability of preserving a sample of his semen for use in an assisted reproduction treatment), we wish to cryopreserve (freeze) a sample of his semen, which, through this act, we put at the disposal of the clinic so that it may be used at a later date.

For these purposes, we, and in particular the minor, who is intellectually and emotionally capable of understanding the scope of the circumstances, have been informed of the following dispositions: Law 14/2006 of 26 May on Assisted Reproduction Techniques and Law 41/2002 of 14 November, governing the independence of the patient and the rights and obligations to clinical information and documentation.

The cryopreserved semen may be kept in authorised gamete banks during the lifetime of the male to whom it belongs.

The present commitment to preserve the cryopreserved semen is agreed upon for a period of ☐ years, during which Instituto Valenciano de Infertilidad (IVI) is committed to adequately maintaining the samples. On expiry of the period agreed upon, we agree to pay the annual amount that we have been informed of through the corresponding financial information, in order to cover maintenance and preservation costs.

Should we be interested in possessing the cryopreserved samples, we are committed to contacting IVI to inform them of our intentions. If we do not inform them of our desire to maintain the samples longer, IVI will understand that we are no longer interested in maintaining them, the cryopreservation and storage commitment, as well as all the other obligations that IVI has assumed, then being automatically dissolved, so resulting in the destruction of the sample.

Furthermore, we are committed to informing IVI of any change of address that we, or the minor, may make in the future for the appropriate purposes.

Finally, we have been informed of the absence of risk to the gametes, resulting from their cryopreservation (with the exception of catastrophe or other justified causes outside the control of this centre) and of the economic cost of their cryopreservation and storage.

We (and in particular the minor) have understood the explanations that have been provided to us in clear and simple language and the physician who has attended to us has allowed us to ask as many questions as we like and has clarified all the doubts that we have expressed.

The information has been provided to us in sufficient time for us to think it over calmly and to reach a decision freely and responsibly, and it has been communicated to us 24 hours before the standard procedure.

We have been informed that pursuant to Royal Decree 9/2014, following blood collection, serum shall be stored for a specific period of time as provided in the applicable regulations.

We also understand that at any moment and without the need for any explanation, we may revoke the consent which we are now presenting.

We, therefore, declare ourselves to be satisfied with the information received and that we understand the scope and risks of the treatment.

Authorisation

We authorise the Reproduction Unit staff to cryopreserve the semen sample that, through this act, we put at the disposal of the clinic, from our son Mr ☐, ☐ years of age, ID no. ☐ for the purposes referred to and during the maximum time stated.

Signed at . . . . . .

Date . . . . . .

Parents' signatures . . . . . .

. . . . . .

Patient's signature . . . . . .

Laboratory director's signature . . . . . .

This consent form must be signed by the patients on **all** pages and on both sides.

Consent revocation

For the exercise of patients' rights and for the revocation of this consent, patients may fill in a form requiring the enforcement of such rights at any time during the procedure. This fact will be reflected in the medical history.

Source: *Reproducción humana asistida, protocolos de consentimiento informado de la Sociedad española de fertilidad*, Editorial Comares año 2002. (Human assisted reproduction – informed consent models, Spanish Fertility Society Model, 2002.)

Appendix 32. **Decellularisation**

Several procedures can be applied to decellularise human material for clinical application, but the chosen method should take into account the final specific clinical use of the scaffold and the specific properties needed for this application, since the preserved properties may differ, depending on the method used [1, 2]. Clinical-grade extra-cellular matrix (ECM) may be used to preserve the intrinsic structural and biological properties of the substances of human origin (SoHO), while removing cells, cellular debris and alloantigen (to minimise immunogenicity). It is also important to achieve the elimination of toxins. However, it is clear that any decellularisation process brings some ECM denaturation.

Decellularisation agents

As ECM properties vary between tissues and organs, the main properties to safeguard must be clearly identified and determined in order to choose the correct decellularisation protocol. There are three general methods used to decellularise tissues or organs: physical, biological (enzymatic) and chemical [3, 4]. Each of these methods has a different mode of action and effects on the ECM [1, 5] (see Table A32.1), which can be critical for the functionality of the final decellularised ECM. These methods are usually combined to ensure complete decellularisation and can be also combined with different techniques to improve their effectiveness (see Table A32.2). Aside from the selected decellularisation agents, the efficiency of tissues or organs decellularisation will depend on the intrinsic tissue properties, such as the specific cell density, thickness, compaction and lipid content. The maintenance of each scaffold's mechanical properties is directly related to the maintenance of one or more ECM components. For example, structural 3D maintenance is related to structural molecules such as collagens, elasticity depends on elastic fibres [6-9] and tensile strength is associated with structural proteins and chondroitin sulfate, while the ability to resist compressions is also associated with structural proteins conformation and hyaluronic acid [10].

Table A32.1. **Techniques used to apply decellularisation agents**

Technique	Advantages	Disadvantages
Perfusion	Facilitates chemical exposure and removal of cellular material	Pressure associated with perfusion can disrupt ECM
Pressure gradient across tissue	Facilitates chemical exposure and removal of cellular material	Pressure gradient can disrupt ECM
Supercritical fluid	Pressure can burst cells – facilitates chemical exposure and removal of cellular material	Pressure necessary for supercritical phase can disrupt ECM
Agitation	Can lyse cells – facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM

Table A32.2. **Modes of action and the effect of different decellularisation agents**

Physical			
Method		***Mode of action***	***Effects on ECM***
Snap freezing		Intracellular ice crystals disrupt cell membranes	Rapid freezing can disrupt or fracture ECM
Mechanical force		Pressure can burst cells, and tissue removal eliminates cells	Mechanical force can damage the ECM
Mechanical agitation		Cell membrane lysis; facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Non-thermal irreversible electroporation		Selective damage on cell membrane while sparing the other tissue components.	ECM preservation and produces a functional recellularisable scaffold.
Biological (enzymatic)			
Method		***Mode of action***	***Effects on ECM***
Trypsin		Cleaves peptide bonds on the C-side of Arg and Lys	Prolonged exposure can disrupt ECM structure; removes laminin, fibronectin, elastin and glycosaminoglycans (GAG)
Endonucleases		Catalyse the hydrolysis of the interior bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Exonucleases		Catalyse the hydrolysis of the terminal bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Chemical			
Method		***Mode of action***	***Effects on ECM***
Alkaline/acid		Solubilises cytoplasmic components of cells; disrupts nucleic acids	Removes GAG
Hypo/hyperosmotic solutions		Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively remove cellular remnants
Alcohols		Dehydrate and lyse cells. Lipid removal	Fixative properties, protein precipitation and ECM ultrastructure damage.
Other solvents (acetone)		Lipid removal	Fixative properties and ECM ultrastructure damage
EDTA/EGTA		Chelating agents that bind divalent metallic ions, thereby disrupting cell adhesion to ECM	Typically used with enzymatic methods
Non-ionic detergents	Triton X-100	Disrupt lipid–lipid and lipid–protein interactions, while leaving protein–protein interactions intact	Mixed results; efficiency dependent on tissues; removes GAG
Ionic detergents	Sodium dodecyl sulfate (SDS)	Solubilise cytoplasmic and nuclear cellular membranes; tend to denature proteins	Removes nuclear remnants and cytoplasmic proteins; tends to disrupt native tissue structure, remove GAG and damage collagen
	Sodium deoxycolate		More disruptive to tissue structure than SDS and GAG removal
	Triton X-200		Achieves efficient cell removal when used with zwitterionic detergents
Zwitterionic detergents	3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS)	Exhibit properties of non-ionic and ionic detergents	Efficient cell removal with ECM disruption similar to that of Triton X-100
	Sulfobetaine-10 and -16		Achieves cell removal and mild ECM disruption with Triton X-200
	Tri(*n*-butyl)phosphate	Organic solvent that disrupts protein–protein interactions	Variable cell removal; loss of collagen content, although effect on mechanical properties is minimal

Physical methods (such as agitation, pressure, freeze/thaw steps, sonication, etc.) can be applied but have limited efficacy and should be carefully evaluated to assess any possible damage to the ECM.

Usually, they are combined with chemical and enzymatic methods. For the maintenance of ECM structure, ionic detergents could be the optimal choice, and enzymatic or alkaline-acid methods should be avoided, because the damage of the collagen can be limited with time and temperature using an ionic detergent; but the disruption provoked by enzymatic or alkaline-acid methods is highly aggressive. This consideration is valid for the preservation of any protein. On the other hand, osmotic buffers are a milder method to obtain decellularised ECM but they are slower and cannot penetrate into thicker, compact organs. For dense tissues or intact organs, detergents can help buffers to penetrate, but they will affect the protein ultra-structure due to disruption of protein–protein interactions. Furthermore, any residual detergents can have cytotoxic effects and it is imperative to assure their elimination. With the aim of removing specific proteins, enzymatic treatment should be used. However, it may cause the unspecific digestion of desired constituents and may not be sufficient for complete decellularisation of the whole tissue. Alcohols and other solvents can remove lipids very efficiently, but in the process they also crosslink proteins and modify the ECM ultrastructure. A compromise between lipid removal and crosslinking should be achieved to decide time and temperature of the treatment with these solvents.

In conclusion, specific combinations of mild physical, biological and chemical methods, along with the type of administration, should be tested, validated and controlled to obtain the best results for each tissue or organ with the aim to obtain a scaffold which presents the desired characteristics needed for a specific clinical application. Moreover, if the scaffold is a future starting material for a medicinal product, the manufacturer should refer to pharmaceutical guidelines for the development, validation and controls of its products.

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Appendix 33. **Active members of the working group for the elaboration of the *Guide to the quality and safety of tissues and cells for human application* (4th Edition) and other members and contributors**

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Human tissues and cells can save lives or restore essential functions, but their use also raises questions of safety and quality since only tissues and cells recovered, processed and stored following strict quality and safety standards are likely to function satisfactorily. Careful evaluation of donors is essential to minimise the risk of transmission of diseases. In addition, since human tissues and cells can currently only be derived from the body of a person, strong ethical principles need to be associated with their donation and use. The Council of Europe approaches the donation and human application of human tissues and cells in compliance with the principles of non-commercialisation and voluntary donation of materials of human origin.

This 4th Edition of the *Guide to the quality and safety of tissues and cells for human application* contains information and guidance for all professionals involved in identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood collection centres, fertility clinics, tissue establishments processing and storing tissues and cells, testing laboratories, organisations responsible for human application, inspectors auditing any of these establishments and Health Authorities responsible for tissues and cells for human application.

For matters dealing with the use of organs and blood components, see the Council of Europe *Guide to the quality and safety of organs for transplantation* and *Guide to the preparation, use and quality assurance of blood components*, respectively.

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