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Facilitatin**g** the **A**uthorisation of

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**Technical annex 2 to overall guidance: assessing the quality and safety of donor testing, pathogen reduction and sterilisation steps as part of Preparation Process Authorisation (PPA)**

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# Introduction

Blood and blood components, tissues and cells for clinical use bear the risk of carrying a number of infectious agents, which may result in the unintentional transmission of infectious agents, which could then lead to disease and even death in recipients. There have been several reported cases of infectious disease transmissions through blood, tissues and cells (BTCs). Many took place years ago, when today’s regulations, standards, and improved donor testing were not in place. However, even in the past years there have been cases of viral, bacterial and fungal infections from BTCs. Moreover, new threats have also been identified as for example prions (e.g. in variant Creutzfeldt-Jakob disease; vCJD), emerging viruses (e.g. West Nile virus, dengue virus, Chikungunya virus, Human T-cell leukaemia virus HTLV-I/-II, Zika virus), and parasites (e.g. *Plasmodium falciparum* in malaria, *Trypanosoma cruzi* in Chagas disease, *Babesia* in babesiosis). Emerging of novel pathogens is rather unpredictable, however mathematical models suggest that every 5 years a new transfusion-transmissible infectious agent will emerge (Gallagher LM et al. 2014). The severe acute respiratory syndrome (SARS) virus in 2002/2003, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV, H7N9) in 2012, and the new coronavirus SARS-CoV-2 in 2019/2020 are good examples of how rapidly pathogens can emerge and spread worldwide (Kuiken et al. 2003, Qi et al. 2013, Wang et al. 2020). Therefore the risk of infection transmission through BTC remains a rare but ongoing concern.

Since the 1980s transfusion-transmitted HIV epidemics, the possibility of BTC transmitted HIV, HBV and HCV infections has markedly decreased over the decades due to risk mitigation strategies - for example revised donor selection criteria, testing (including nucleic acid amplification technique (NAT) testing) of donors for HIV, HBV and HCV. Indeed, appropriate and reliable laboratory testing of each donation and/or donor, control of reagents, pathogen reduction, as well as, where appropriate, post-processing microbiological testing of BTCs, can substantially reduce the risk of infection and improve the overall safety of BTCs.

# Aims

This annex provides high level requirements and criteria for verifying that the microbiological quality of blood, tissues and cells is in accordance with current European Blood, Tissues and Cells Directives (EUBTCDs) and other standards and guidelines that ensure the quality and safety of BTCs.

In more detail, this guidance describes aspects which Member States (MS) Competent Authorities (CA) should take into account when assessing:

* competence of laboratories performing donor infectious disease testing and microbiological testing of BTCs
* reliability of the donor infectious disease marker testing kits, assays and other methods
* effectiveness of pathogen reduction during BTC processing
* effectiveness of sterilisation methods during BTC processing
* microbiological status of final products

The safety will be assessed for microbiological pathogens like bacteria, fungi, viruses and other kind of agents (e.g. prions) (as defined by GAPP).

# Scope

The content of this document only applies to BTC and their applications as regulated by the EU Blood, Tissues and Cells Directives (EUBTCD) (ref.2-8), and all novel BTC that are not currently covered by other regulations.

BTCs that are subject to *substantial manipulation* or that are *not intended to be used for the same essential function or functions in the recipient as in the donor* (as defined in Advanced Therapy Medicinal Product Regulation 1394/2007/EC), BTC products classified as Medical Devices and other Medicinal products (such as plasma-derived medicinal products), are not part of the scope of the GAPP Joint Action. Donation, procurement and testing of BTCs intended for ATMP manufacturing fall under the scope of the GAPP Joint Action.

BTC donor and product testing procedures themselves are not in the scope of this guidance.

Furthermore, this guidance does not extend to the assessment of activities such as aseptic working methods, cleanroom maintenance or environmental monitoring, which are assessed by CAs during the Blood Establishment/Tissue Establishment (BE/TE) inspections. Health and safety issues for staff are out of the scope of this guidance, as well.

# General validation requirements

Validation is usually split into two components: qualification and process or method validation. Each part of the process and individual items (including facilities, equipment, computer systems, materials and staff) should be qualified before they are first used in a process and re-qualified at predetermined intervals, or when significant changes are made. Process or method validation should only be performed once all the items used have been qualified. Process or method validation should be performed before a new process or method is used routinely. (Adapted from EDQM T&C Guide, chapter 2.16.1.) Retrospective validation is no longer an acceptable approach (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 4.4.1.2).

The key elements of the site qualification and validation programme should be clearly defined and documented in a validation master plan or equivalent document (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 4.3.2.3-4).

Process or method to be used and acceptance criteria should be documented in a validation plan and approved by the organisation management before qualification or process/method validation commences. The results of the validation compared with the acceptance criteria and any deviation from the validation plan should be recorded and fully investigated during the validation and documented in validation report, including a conclusion. Following validation, the acceptance or rejection of the process by designated organisation management should be documented. (Adapted from EDQM T&C Guide, chapter 2.16.1.) Equipment, facilities, systems and processed should be evaluated at an appropriate frequency to ensure that they are still operating appropriately (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 4.4.1.6).

If processes are contracted to external service providers it is required that responsibilities between BE/TE and service provider are clearly defined, and specifications regarding the whole process are designated. External service providers should meet the requirements of EUBTCD (refs: directives; need to be added). Data supporting qualification and/or validation studies which were obtained from sources outside of the establishment may be used provided that this approach has been justified and there is adequate assurance that controls were in place throughout the acquisition of such data (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 4.3.1.4).

In general, the process or method validation needs to be performed once by each organisation. If the process or method has been successfully validated by any organisation, it may be transferred between organisations. In this case the receiving organisation must repeat the validation to a reduced extent, guided by the sending organisation and in consent with the relevant CA, if applicable in MS in question. This on-site validation should focus on “worst case” conditions. (See WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7.)

Details concerning specificity of validation of donor testing, pathogen reduction and sterilisation will be described in the following chapters.

# Requirements and criteria for laboratories performing donor infectious disease testing and microbiological testing of BTC

Blood Establishments (BE) can perform infectious disease testing of blood donors by themselves or they could contract this work to an appropriately qualified and competent external laboratory selected by the BE (EDQM Blood Guide). Tissue Establishments (TE) mainly contract tissue and cell donor testing to external laboratories, because tissue and cell donor testing must be carried out by a qualified laboratory, authorised as a testing centre by the CA in the MS (Directive 2006/17/EC). These external laboratories may be part of a hospital or private clinic, but may also be an independent enterprises offering the appropriate testing services. In addition to donor testing, the laboratory can examine preparations of BTC to determine, measure or otherwise describe the presence or absence of various micro-organisms (see chapter 6). Whether the laboratory performing these activities is part of the BE/TE or a third party offering its services to BE/TE, it must meet requirements laid down in the EUBTCD (Directive 2002/98, Directive 2004/23).

## Testing and screening of donors

Noun *testing* is used in EUBTCD in the context of tissue and cell donor and donation testing (2006/17/EC), testing of blood and blood components and testing of blood donations (2002/98/EC). Otherwise, instead of *testing*, noun *screening* is widely used in blood sector. In general, there is no difference between screening of blood donors and testing of tissue and cell donors themselves. In contrast, there is difference between donor screening/testing, and diagnostic testing. Diagnostic testing is performed to people, who have a suspected disease or condition to provide a definitive diagnosis, whereas donor screening/testing is performed to presumably asymptomatic people to rule out the presence of infectious diseases. Therefore the reason for the donor screening/testing is the same, to search for the presence of infection markers in donors and assure safety of BTC for recipients. The only difference lie in some infectious markers being tested depending on the type of donor, the interpretation of the results and the subsequent actions (EDQM T&C Guide, WHO 2009, EDCD). Therefore, for the clarity, in the context of this document testing is used to describe a process/ a procedure to determine the presence of infectious disease markers in donor specimen and to assure the quality or safety of BTC (including primary screening of blood donors).

## Quality system

In general, based on the EUBTCDs any laboratory undertaking the testing of donors must have a well-managed quality system (Directive 2002/98, Directive 2004/23). As any structure or body, that is responsible for any aspect of testing of human blood or blood components is determined to be a Blood Establishment (except hospital blood banks) (Directive 2002/98), laboratories testing of blood donations and performing microbiological testing of blood components must meet the quality system standards and specifications for BEs as defined in the Directive 2005/62/EC. Similarly, the quality system of tissue and cell donor testing and graft microbiological testing laboratories must meet the requirements laid down in the Directive 2004/23/EC.

## Standard

The EUBTCD are the minimum standards for laboratories performing donor infectious disease testing and microbiological testing of BTC. The EDQM Guide to the preparation, use and quality assurance of blood components is an additional standard for blood donor testing laboratories and EDQM Guide to the Quality and Safety of Tissues and Cells for Human Application could be used for tissues and cells, respectively. In addition to directives, laboratories must meet the relevant national legislation and national standards, which apply to those specific activities.

Many medical laboratories in EU follow EN ISO standards, either voluntarily or under obligation, if required by national legislation. EN ISO 15189, the international standard for all fields in laboratory medicine, is internationally and within EU the most used (Zima 2017, Boursier et al. 2016, Buchta et al. 2018). In some EU MSs other national or international standards which have adopted essential contents of EN ISO 15189 are used (Zima 2017). Whereas in some other EU MSs EN ISO 17025, outlining general requirements for the competence of testing and calibration laboratories, is used as an alternative or additional standard for medical laboratories (Zima 2017). Furthermore, a laboratory using in-house assay for BTC donor infectious disease marker testing must be compliant with standard EN ISO 15189 (Regulation 2017/746). If a laboratory follows ISO standard(s), it must ensure that the applicable requirements as placed in the EUBTCD are met.

## Accreditation, designation, authorisation or licensing of laboratory by competent authorities

Based on the EUBTCDs, any laboratory undertaking donor testing or blood component, tissue and cell testing must be accredited, designated, authorized or licensed by the competent authority or authorities (Directive 2002/98, Directive 2006/17 Annex II 2.1). Furthermore, this should be performed against the relevant standards.

Accreditation according to standards is an effective way to prove competence of the laboratory and it further facilitates accurate and reliable outcomes and reduces errors in the laboratory processes (Allen 2013). CAs should, either themselves, or through a third party, accredit laboratories against standards.

Whatever body is responsible, it must be competent to perform the accreditation/designation/ /authorisation/licensing. Therefore it should itself be accredited, designated or licensed by the national CA or other relevant national/international body, to accredit/license the laboratories in question.

## Information on testing laboratories

For the purposes of evaluating and authorising BTC preparation processes, evidence should be available to show that any laboratory involved in donor infectious disease marker testing and microbiological testing of blood components/tissues/cell grafts has been accredited, designated, licensed and/or authorised by the appropriate authority. Furthermore, to share this knowledge and to increase mutual trust in case of BTC export to other EU MSs, this information should be presented by the applicant as part of Preparation Process Dossier (PPD).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Requirements | Criteria | Additional/Optional information | Specifications | Conform |
| Donor testing laboratory | Laboratory | Quality system(s) of the laboratory | 2005/62/EC / Blood donations  2004/23/EC / T&C donors |  |  |  |
| Name of the laboratory |
| Standard that laboratory follows | Blood Directives  Good Practice Guideline/EDQM  T&C Directives | e.g. ISO15189,  ISO17025,  national standards |  |  |
| Name of the authority that has accredited, designated, authorised or licensed the laboratory | Name of the authority | e.g. Name of the external accreditation body (if applicable) |  |  |
| BTC graft  microbiological testing laboratory | Laboratory | Quality system(s) of the laboratory | 2004/23/EC |  |  |  |
| Name of the laboratory |
| Standard that laboratory follows | T&C Directives | e.g.  Good Practice Guideline/EDQM, ISO15189,  ISO17025 |  |  |
| Name of the authority that has accredited, designated, authorised or licensed the laboratory | Name of the authority | e.g. Name of the external accreditation body (if applicable) |  |  |

# Requirements for selection, validation and performance of donor infectious disease marker testing kits and other methods

Blood transfusion as well as tissues and cells transplantation and reproductive cell transfer may result in transmission of infectious diseases. In order to prevent the transmission of infectious diseases and to ensure an equivalent level of quality and safety, each donor and donation should be tested in conformity with the requirements laid down in European Commission Blood, Tissue and cell Directives (ECBTCDs) (2002/98/EC, 2005/62/EC & 2006/17/EC).

As a minimum requirement, all donors must be tested for HIV, Hepatitis B and Hepatitis C (as summarised in Table I). In addition to the minimum requirements, testing of donors/donations for other agents or markers may be required for specific blood components, tissues or cells, donors or epidemiological situations in any given region or country (2002/98/EC, EDQM T&C 2019 & 2006/17/EC). Furthermore, these additional requirements may vary between the EU Member states based on the national legislation.

**Table I.** Summary of tests required for BTC donors/donations.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Mandatory tests** | **Directive** | **Additional/national tests** |
| Blood donors | HIV 1/2 (Anti-HIV 1/2)  Hepatitis B (HBs-Ag)  Hepatitis C (Anti-HCV) | 2002/98/EC Annex IV | Based on e.g. the donor’s history, the characteristics  of the BTC donated, national epidemiological situation, requirements in national legislation and guidance by European Centre for Disease Prevention and Control (ECDC), additional testing on e.g. HTLV-I, CMV, malaria, toxoplasma, EBV, *Trypanosoma cruzi*, Hepatitis E virus may be required |
| Tissue and cell donors | HIV 1 and 2 (Anti-HIV-1,2)  Hepatitis B (HBsAg, Anti-HBc)  Hepatitis C (Anti-HCV-Ab)  *Treponema pallidum* | 2006/17/EC Annex II |
| Reproductive cell donors | HIV 1 and 2 (Anti-HIV-1,2)  Hepatitis B (HBsAg, Anti-HBc)  Hepatitis C (Anti-HCV-Ab)  *Treponema pallidum* (non partner donors)  Chlamydia (sperm donors; urine sample NAT testing) | 2006/17/EC Annex III |

Effective donor testing for the detection of the high risk transmissible infectious agents can reduce the risk of transmission to a very low level (EDQM 2017 & WHO 2009). In addition to reliable laboratory testing of donors for a range of markers of infectious agents, the choice of assays or platforms together with the quality management systems in place are crucial to ensure the lowest possible infection risk associated with transfusion and transplantation.

Requirements for selection, validation, and performance of donor infectious disease marker testing kits and other methods in the BTC field are summarized below.

## Selection of donor infectious marker testing kits and other methods

The selection of appropriate kit is a critical part of the donor testing. Numerous commercial infectious marker testing kits are available. These are based on various types of assays which detect antibodies, antigens or the nucleic acid of the infectious agent (EDQM T&C 2019, WHO 2009). Different types of assays for testing include:

* Immunoassays (IAs);
* Enzyme immunoassays (EIAs);
* Chemiluminescent immunoassays (CLIAs);
* Haemagglutination (HA)/particle agglutination assays (PAs);
* Rapid/simple single-use assays (rapid tests);
* Nucleic acid amplification technology (NAT) assays (WHO 2009).

However, not all assays are suitable in all situations and each testing system has its advantages and limitations that should be taken into consideration when selecting donor infectious marker testing kits (WHO 2009). As a minimum requirement the following factors should be considered in selecting the most appropriate assays.

As a general requirement infectious disease marker testing of tissue and cell donors must be carried out using CE-marked testing kits where appropriate (2006/17/EC, Annex II, 2.1). Materials, reagents and equipment used in blood sector should also be CE-marked, if relevant (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 4.1.9). Kits used *in vitro* for the examination of pathological state of blood and tissue donors are considered as *in vitro* diagnostic medical devices which must be CE-marked before placing on the market within EU (Regulation (EU) 2017/746). In addition to testing kits, all reagents and reagent products, intended to be used *in vitro* for the examination of blood donors are also considered as *in vitro* diagnostic medical devices and must be CE-marked (Regulation (EU) 2017/746). Therefore infectious disease marker testing of blood donors must also be carried out using CE-marked testing kits where appropriate.

In-house assays may be used for donor infectious disease marker testing only if CE-marked kits are not available, for example when new pathogens emerge (see paragraph 2.3 on Additional technologies and emerging infectious agents).

Test-kit assays must be suitable for the detection of the required markers in the donor types being tested (EDQM T&C Guide, JPAC). Typically infectious-disease marker testing kits are optimised for testing a sample from a living or deceased heart-beating donor (ie. donor after brain death) (EDQM T&C Guide). If there is a need for collection of post-mortem samples (from deceased non-heart beating donor), the testing kit should have been validated for this purpose by the manufacturer. Alternatively, user validation of assays for use with post-mortem samples should be undertaken (EDQM T&C 2019). Additionally, only kits designed and validated for donor testing should be selected. Other systems, such as tests validated for diagnostic purposes only, should not be selected (WHO 2011).

Testing kit should be appropriate in relation to testing volumes/capacity and type of platform available. For example, EIAs, CLIAs and PAs suit to testing from relatively small to large numbers of samples and allow more objective recording and analysis of the results than rapid tests. The use of rapid tests is generally not recommended for donor testing as they are designed for testing of small numbers of samples, mainly for diagnostic purposes. Furthermore, they are performed using manual techniques; the results therefore have to be transcribed by staff and there is a lack of permanent records and traceability. In laboratories where through-put is medium to high fully automated platforms with minimal user involvement (WHO 2009) and accessible donor-testing records should be preferred (WHO 2009, EDQM T&C Guide).

In selecting a specific assay for the testing of BTC donors, both clinical and analytical sensitivity and specificity should be the highest possible (WHO 2009, EDQM T&C Guide). High sensitivity ensures identification of infection and high specificity decreases rates of biological false positives which could result in the wastage of donations and unnecessary deferral of donors (WHO 2009).

## Validation of donor infectious marker testing kits and other methods

Tissue and cell donor infectious marker testing kits and other types of donor testing assays must be validated for the purpose in accordance with current scientific knowledge (2006/17/EC). Furthermore, all testing procedures related to blood donor testing must be validated before use (Directive 2005/62/EC/Annex 6.3.1).

All CE-marked donor infectious disease marker testing kits have undergone assay performance (see section 2.3.) evaluation and validation firstly by manufacturers and secondly by the European Union reference laboratory(ies) designated by the Commission (Regulation (EU) 2017/746). Therefore, only an on-site validation of the CE-marked donor infectious disease marker testing kit is required prior to its routine use in the laboratory (WHO 2011, EDQM GPG Blood 6.3.3.). On-site validation should demonstrate, in addition to qualification, that the performance specifications of the assay established by the kit manufacturer are met in the laboratory (WHO 2011, EDQM GPG Blood 6.3.3.).

Additionally, it is strongly recommended for donor testing laboratories to demonstrate that on routine application of kits/assays the performance specifications are constantly maintained (WHO 2011). The means by which this could be demonstrated are for example:

* Appropriate reactivity with manufacturers’ and any internal and external quality control materials with every series of tests (WHO 2011, JPAC);
* statistically monitoring trends in control measurements on defined control material (WHO 2011, JPAC);
* successful participation in external quality assessment schemes (proficiency testing) by all qualified members of staff (WHO 2011, Blood directive 2005/62, Annex, 6.3.5.).

When the donor testing laboratory intends to use in-house kits/assays instead of CE-marked kits their performance must be validated by the laboratory itself before implementing a test system for routine analysis. This means that the laboratory must demonstrate conformity with the relevant general safety and performance requirements set out in Annex I of Regulation (EU) 2017/746 which apply to it, taking into account its intended purpose. In addition to these, the several conditions as listed in the point 5 of Article 5 of Regulation (EU) 2017/746 must be met (e.g. manufacture and use of the assay under appropriate quality management systems, laboratory´s compliance with standard EN ISO 15189, a justification of their manufacturing, modification and use etc.).

User validation of assays for use with post-mortem samples should be undertaken considering the current EDQM guidance (e.g. Example of validation of screening: infectious disease assays of blood from deceased donors in Appendix 19 of the EDQM T&C guide 2019).

## Performance of donor infectious marker testing kits and other methods

Performance of a donor infectious marker testing kits and assays means the ability of a kit or other methods to achieve its intended purpose as claimed by the manufacturer. It consists of the analytical and the clinical performance (Regulation (EU) 2017/746). The analytical performance means the ability of a donor testing kit/assay to correctly detect a particular analyte (adapted from Regulation (EU) 2017/746). Characteristics describing the analytical performance are such as (Regulation EU/2017/746 Annex I chapter II; WHO 2011):

* analytical sensitivity;
* analytical specificity;
* trueness (bias);
* precision
  + repeatability (replicates of series);
  + reproducibility (replicates of series, variation by operator, by day or by lot of reagents);
* accuracy (degree of closeness of measurements to the true value, resulting from trueness and precision);
* lower and upper limits of detection (serial dilution) and quantitation;
* determination of appropriate criteria for specimen collection and handling;
* control of known relevant endogenous and exogenous interference (e.g. haemolytic sera, lipemic sera).

The clinical performance means the ability of a donor testing kit/assay to yield results that are correlated with a particular pathological state in accordance with the target population (Regulation (EU) 2017/746). Specifically characteristics of the clinical performance are such as (diagnostic) sensitivity, (diagnostic) specificity, positive predictive value, negative predictive value, likelihood ratio, and expected values in normal and affected populations (chapter II of Annex I of Regulation (EU2017/746).

CE-marked donor infectious marker testing kits (which have undergone a performance evaluation by a manufacturer and demonstrated conformity) meet these above mentioned general performance requirements (Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices chapter II Article 5). The above mentioned general performance requirements apply also to in-house donor testing kits/assays and therefore they should also meet these requirements (Regulation (EU) 2017/746).

In addition to general performance requirements, CE-marked donor testing kits are compliant with the common technical specifications for the detection, confirmation and quantification in human specimens of markers of HIV infection (HIV 1 and 2), HTLV I and II, and hepatitis B, C, D (Commission decision 2009/886/EC). Specifically the requirements for sensitivity and specificity of these kits are set out in Table 1 of the Commission decision 2009/886/EC. It is recommended that the minimum evaluated (diagnostic) sensitivity and (diagnostic) specificity levels of all assays used for donor infectious disease marker testing should be as high as possible and preferably not less than 99.5% (Commission decision 2009/886/EC).

## Donor testing of emerging infectious agents

Newly identified infections or emerging known infectious agents in the EU include variant Creutzfeldt Jakob disease, West Nile virus (WNV), dengue, chikungunya virus (CHIKV), Zika virus (ZIKV), Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome-associated viruses (SARS-Cov) (EDQM T&C). Most of these are transmitted through the route of transfusion and transplantation (WHO 2009, Noisakran et al 2010, Schwartz and Albert 2010, Blau et al. 2013). On the other hand, the others pose a theoretical risk of transmission, since no cases of transmission have yet been identified or proven (WHO 2009). Therefore as a precautionary principle a cautious and measured response is needed. In the event of increasing incidence of infection, including to pandemic level, urgent actions are needed to prevent the potential effects of infection on donors and donor sufficiency, potential recipients, and BE/TE staff (WHO 2009). Thus, reliable donor infectious disease marker testing is vitally important during new epidemiological situations.

In outbreak situations the actual threat to BTC safety should be properly evaluated. Moreover, the incidence and prevalence of the new infection in the general, BTC donor and patient populations should be determined. In these situations it is recommended to follow the guidance of European Commission and European Centre for Disease Prevention and Control (ECDC) on BTC donor infectious disease marker testing. CE-marked kits should be used, if available.

In-house assays developed for the detection of rare or new emergent diseases can be used when commercial CE-marked assays are not available on the market in the EU. However, they must meet the performance and validation requirements as set in Regulation (EU) 2017/746 and summarized above.

## Information on donor infectious disease marker testing kits

There are MSs in the EU where the donor infectious marker testing kits are not required to be CE-marked and in contrast MSs where in-house assays are not permitted. Furthermore, the requirements for selection, validation and performance of donor infectious marker testing kits and other methods are in some MSs defined within national legislation, national guidance or testing laboratory or BE/TE procedures. For example some MSs require in-house kits/assays to be authorized prior use whereas others do not. Therefore, it would be essential to share this information, especially when BTC are exchanged or BTC recipients are moving between EU MSs. This information should be presented by the applicant as part of Preparation Process Dossier (PPD).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Requirements | Criteria | Additional/Optional information | Specifications | Conform |
| Donor infectious disease marker testing | Infectious disease markers | Infectious disease markers to be tested | 2002/98/EC Annex IV, 2006/17/EC Annex II and III as amended, 2006/17/EC Annex II and III | eg. Additional disease markers to be tested either voluntarily or based on national legislation |  |  |
| Testing kit/assay | CE-marked kit Yes/No | 2006/17/EC  2017/746 |  |  |  |
| In-house assay | 2017/746 |  |  |  |
| Performance |  |  |  |  |  |
| Validation |  |  |  |  |  |

# Criteria for validation of pathogen reduction steps

Even with very sensitive and specific testing, there remains a residual risk of transmission of infection during the window period, undetectable by screening. In addition, mutations occurring in pathogens could result in pathogens remaining undetected by testing. Residual bacterial and fungal contaminations also exist, most likely in BTCs that are stored at or close to room temperature (for example platelets) or due to procurement and processing when open systems are used. New threats have also been identified as for example prions, emerging viruses, and parasites.

With the remaining risks related to BTC recipients, there is pressure to further enhance the quality and safety of BTC. One way to do that is developing pathogen reduction technologies (PRT) that aim to remove or inactivate pathogens in BTC. PRT are also more generalized approach against emerging pathogens. Currently available systems have been demonstrated to inactivate a wide range of viruses, bacteria and parasites but they do not reduce infectivity associated with prion proteins and, hence, for example vCJD risk (ref: EDQM Blood Guide).

This section relates to the MS CA assessment of validation packages that demonstrate the performance of pathogen reduction technologies.

## Validation requirements depend on the type of PRT

According to the Blood Directive 2005/62 (Annex, 6.4), the processing of blood components shall be carried out using appropriate and validated procedures including measures to avoid the risk of contamination and microbial growth in the prepared blood components (Ref. for T&C Directives??). Thus, PRT need to be validated before used at processing of BTCs in order to provide evidence that a chosen PRT process is fit for purpose and can reliably decrease the number of micro-organisms in a given BTC.

Some PRT are already acknowledged and widely used (see Table X), others are just under development or in house PRT systems, meaning that they are developed and used only in the BE/TE/laboratory in question. For blood products, acknowledged PRT are in many cases authorized by CA and commercial products. At the time of writing this guidance, it has not been possible to treat whole blood donations, so blood must be separated into its component parts before use of PRT (EDQM Blood Guide). For tissues and cells, acknowledged PRT are not necessarily commercial products but acknowledged protocols. Validation requirements in these two cases are different and described below:

* **On-site validation of acknowledged PRT systems.** A reduced validation strategy is usually sufficient when using a PRT system/device that has already been authorized by a relevant CA and recognised in EU (CE marked devices for e.g. platelets or routinely used systems for e.g. plasma components). Validation of PRT systems 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 (EDQM T&C Guide, chapter 2.16.1.). For example spiking studies (see later) do not need to be performed. Both data from the PRT supplier as well as relevant literature can be referred to. A comprehensive assessment of the relevance of these data by the BE/TE is required to ensure it is directly applicable to the treatment process to be used and the operational conditions at the site. However, changes in sample processing procedures, instruments and equipment or the BTC itself should be partially validated.
* **Validation of novel or in-house PRT systems.** The use of an in-house PRT requires an extensive validation, covering parameters of a primary validation study (Ph. Eur. 5.1.1., EMA/CHMP/CVMP/QWP/850374/2015). Aspects like pathogen reduction limit/capacity, specificity and robustness should be addressed. The PRT systems should be validated using “worst case” scenario. This will usually involve spiking the material with a larger-than normal level of the undesirable micro-organisms and demonstrating its effective removal, or reduction to safe levels, by the process. Strains of micro-organism that are known to be resistant to antimicrobial treatment, e.g. spore-forming, heat-resistant bacteria, may be used for spiking (EDQM T&C Guide, chapter 2.16.9.).

**Table X.** Examples of existing Pathogen Reduction Technologies.

|  |  |  |
| --- | --- | --- |
| **PRT mechanism** | **BTC for which is used** | **Specific considerations when assessing a validation study** |
| Blood products | | |
| Amotosalen + UVA light (320-400 nm) (*Intercept by Cerus*) | Platelets (whole blood or apheresis derived)  Plasma (whole blood or apheresis derived)  Red cells (in trial) |  |
| Riboflavin + UVB light (280-360 nm) (*Mirasol by Terumo BCT*) | Platelets (whole blood or apheresis derived)  Plasma (whole blood or apheresis derived)  Whole blood |  |
| Solvent/Detergent (*Octaplas S/D by Octapharma*) | Large-pool of plasma (whole blood or apheresis derived) |  |
| UVC light  Filtration + Methylene Blue + visible light (400-700 nm)  (*Theraflex by Macopharma*) | Platelets  Fresh frozen plasma |  |
| Solvent/Detergent (*by VIPS*) | Single donation or mini-pool of plasma (whole blood or apheresis derived) |  |
| Tissue and cell grafts | | |
| Antibiotic/anti-mycotic treatments | Amniotic membrane, musculoskeletal tissue, adipose tissue, cardiovascular tissues, ocular tissues |  |
| High concentrate glycerol | Skin, amniotic membrane | glycerol solutions used should be sterile and of high  quality (e.g. see European Pharmacopoeia monograph 0497 – Glycerol 85 %) |
| Decellularisation | Skin, cardiovascular tissues |  |
| Chemical decontamination (e.g. peracetic acid, iodophors, ethanol) | Musculoskeletal tissue |  |

(Ref. EDQM T&C Guide 2019)

## Aspects of PRT validation

Application should describe all relevant information which CA needs to review:

* **Starting material**. The effectiveness of a PRT should be shown in the BTC preparation itself and not only in an aqueous solution (EDQM T&C Guide, ch. 7.6.2).
* **Specification of reduction capacity.** Prior to the PRT validation it is necessary to assess the bioburden usually present in the BTC material as well as defining worst case scenarios. Latter can be critical for a successful pathogen reduction and should be also addressed in the study.
* **Target organisms**. Appropriate model organisms for the spiking studies include typical contaminants likely to be found in the BTC material as well as micro-organisms that might represent a challenge due to an increased resistance towards the PRT. In addition, model organisms should be stable in the presence of the matrix. Ideally, known and well characterized reference organisms should be used (ref: WHO, EDQM). The applicant should justify the choice of micro-organism in accordance with the aims of the validation study.
  + Suitable spike stocks.To demonstrate high magnitude reduction ability, BE/TE or contracted testing laboratory must source representative high titre stocks of pathogens (JPAC Guidance, WHO Bacterial reference strains, EMA CPMP/BWP/2016). A panel with relevant characteristics should be included. Where WHO bacteria reference strains are available they should be used. The quantitation range of the assay should cover the bioburden concentration range expected in the BTC product.
  + Key bacteria against which PRT must demonstrate effective reduction: see Table 1 in the JPAC guidance “Validation of Plasma and Platelet Pathogen Inactivation”.
  + Viruses that may contaminate BTCs include viruses with a DNA or RNA genome, with and without a lipid membrane, and range in size from small to larger ones. In addition, the pathogenicity of a virus may depend on the patient group and on the BTC being administered. PRT should therefore be shown to be able to remove or inactivate a wide range of viruses if they are to be considered satisfactory. Typically, validation studies have involved at least three virus types, chosen to represent different kinds of agent. A robust, effective and reliable process will be able to remove or inactivate substantial amounts of virus, typically 104 or more. Guidance is available for the selection and assay of model viruses (CPMP/BWP/268/95, Table I).
* **Interfering factors.** Factors which might have an effect on the reduction capacity have to be considered in the validation study (e.g. hemoglobin concentration in UV inactivated platelet concentrates). A monitoring system of these factors should be in place to ensure a level within an acceptable and valid range.
* **Critical process parameters (CPP).** CPPs are used to measure the performance of the PRT treatment unit, and relate to the reduction performance of the target pathogen (PRT treatment efficacy). Continuous monitoring of CPPs provides assurance that the system is under control and alerts operators and control systems if PRT treatment efficacy is reduced to an unacceptable level.
* **Quantitative assays for each model pathogen.** To determine the reduction capacity accurately, validated quantitative assays for each model pathogen have to be in place at the BE/TE or contracted testing laboratory. These must detect live pathogen. NAT testing will not differentiate between live and inactivated pathogens but can be used in the validation of removal processes.
* **Model process.** Suitable model process verifies the PRT model and its comparability with the proposed/current preparation process.
* **Controls.** Suitable controls demonstrate the model of pathogen reduction.

Additional aspects for the CA to consider:

* All validation experiments should be performed in the original matrix.
* Critical reagents and materials must be CE-marked, when applicable (Directive 2006/86/EC, Annex I, C.6; and Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 4.1.9). Materials that are rare or expensive can be replaced by an appropriate surrogate.
* PRT should be carried out at as short interval after BTC donation as possible (usually within 24 hours). If commercial PRT kit is used, manufacturer gives instructions on the maximum interval and these instructions should be followed. If PRT is carried out later during which time bacteria have multiplied, the level of bacteria may be significantly higher. Additionally, growth of bacteria may lead to the formation of pyrogenic agents and endotoxin. (JPAC Transfusion guidelines, Validation of plasma and platelet pathogen inactivation, 2019).
* The product matrix and its components might have a significant effect on the model organisms and its behavior (e.g. complement killing of bacteria). Bacteria can start to grow in the product after spiking thereby altering the initial spiking concentration. A non-inactivated control should be performed in parallel.
* In order to be able to provide evidence that a chosen PRT process can reliably decrease the number of contaminants in a given BTC, deliberately contaminated BTC need to be prepared by adding known and defined micro-organisms. Certain requirements (e.g. EU GMP Guidelines chapter 5.18) might restrict the deliberate use of potential contaminants in the production facilities. For validation purposes, it should be avoided to share equipment and facilities due to the risk of cross-contaminations. Exemptions can be made in cases in which validation procedures are performed in closed systems.
* Re-validation should be performed in case of change of facility, change of process or any relevant new knowledge.

## Validation criteria

The methodology for the statistical assessment of pathogen reduction assays and limitations of such studies have been described previously (CPMP/BWP/268/95; CPMP/ICH/295/95).

In summary, the validation will result in a set of data for each pathogen or representative model used in the spiking study.

The titre of the spiked test material before undertaking the scale model PRT can be compared to the titre of the sample(s) collected from the test material when the scale model process has been completed, in order to determine the overall pathogen reduction achieved. Sample titres and reduction figures achieved are normally reported on a logarithmic scale. The reduction achieved for each pathogen or appropriate model should be reported and include the 95% confidence intervals wherever possible.

Although it is considered that the level of bacterial contamination in blood donation which may result in clinically significant levels of bacteria in stored platelet components is no more than 20 cfu/ml, a higher minimum proven level of pathogen reduction must be demonstrated: PRT must reduce any bacterial contamination by the amount specified i.e. 104 to ensure maximum effectiveness. (JPAC guidance “Validation of Plasma and Platelet Pathogen Inactivation”)

Validation reports should include a discussion of the suitability of the scale model system, taking into consideration the results from appropriate assay control samples, and the degree to which these support the proposed mechanism of pathogen reduction.

## Methods for control of microbiological quality

The method for control of microbiological quality of BTC needs to be chosen depending on whether the BTC is defined sterile or non-sterile. In case of sterile BTC, the conventional method of sterility assay is described in *Ph. Eur.* 2.6.1. If the final BTC product is not required to be sterile or cannot be made sterile, the method is described in *Ph. Eur.* 2.6.12 (bioburden). The method 2.6.12 should be used together with 2.6.13 if BTC risk assessment defines the absence of predetermined specified micro-organisms. The test samples for sterility testing must be representative of all of the components, but if this is not possible surrogate testing may be performed (EDQM T&C Guide 10.4). This testing may require use of validated methods employing special media and/or conditions to enable growth of such micro-organisms and their detection.

Several BTC derived preparations are short lived and of small quantity. Conventional compendial methods, e.g. growth based microbiological methods (*Ph. Eur.* 2.6.1), are increasingly outperformed by so called alternative rapid microbiological methods (RMM) in terms of sensitivity, speed and width of information. By the use of RMM, final test results are available much faster allowing a timely and often more substantiated final BTC product release. (EDQM T&C Guide 10.4)

The use of RMM for testing of BTC preparations is still limited. One reason is the considerable effort for the control laboratory to validate new methods with respect to method performance in comparison to the compendial reference method. *Ph. Eur*. chapters 5.1.6 “Alternative Methods for Control of Microbiological Quality” and 2.6.27 “Microbiological examination of cell-based preparation” provide the current EU framework for RMM validation. EDQM provides an online resource in which information on exemplary RMM validation procedures for a particular application are made available to control laboratories. This resource is currently regarded as a starting point for users without being peer-reviewed and exhaustive.

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, based on risk assessment.

Deviations from the *Ph. Eur*. standards should be justified, and alternative test methods must be validated in accordance with *Ph. Eur.* 5.1.6.

## Effect of PRT on BTC quality

PRT must not change the properties of the BTC as to making it unacceptable for the clinical use. The benefits of PRT in reducing microbiological risk must be balanced against any potential loss of potency or efficacy of the BTC and this should be assessed as part of the validation (JPAC guidance “Validation of Plasma and Platelet Pathogen Inactivation”). A framework to assess this is provided in the WP6 section/Annex I (*include more detailed reference here*). A framework to assess this after prion removal procedures is provided in the JPAC guidance “Validation of Blood Component Quality Following Prion Removal Procedures for Red Cell Components”.

# Criteria for validation of sterilisation methods

Key for microbiological and viral safety of BTC is the confirmation of validated processes for pathogen reduction (see section 3 of this guidance) or sterilisation, where applicable. The sterilisation methods and criteria described here apply mainly for bacteria and fungi. If a risk assessment points out a viral contamination possibility, it is necessary to demonstrate the process capability of removing/inactivating relevant viruses during the process.

Sterilisation is defined as a process that results in the state of complete absence of all cell-based micro-organisms capable of replication (sterility) (Ph Eur 5.1.1.; EDQM T&C Guide 8.6.1.). According to directive 2006/17 (Annex IV, 1.3) sterile, wherever possible CE marked, instruments and devices must be used for tissue and cell procurement. Where possible, single-use instruments for procurement are recommended. When re-usable instruments are used, a validated cleaning, disinfection, packaging and sterilisation process for removal of infectious agents has to be in place (Directive 2006/17/EC, Annex IV, 1.3.9). 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 (EDQM T&C guide 8.6).

Consistently, Directive 2005/62 sets the standards for using sterile CE-marked blood bag systems for the collection and processing of blood and blood components (Directive 2005/62/EC/Annex 6.2.2). In addition, BE shall use sterile and pyrogen-free connective devices, anticoagulants and containers (EDQM Blood guide, Good practices guidelines, 4, 6).

The conventional test for sterility is described in Ph. Eur 2.6.1. In addition, use of a validated automated culture system may be advantageous if available (Ph. Eur 2.6.27). If sterility test is not feasible, sterility needs to be assured by the use of suitably designed, validated and controlled processes. Previous chapters 4.1 General validation requirements and 4.4 Methods for control of microbiological quality apply also to this chapter 5.

## Objects of sterilisation

Tissues (e.g. bone and amniotic membrane) can in some cases be subject to sterilisation methodologies (EDQM T&C guide 1.3). Sterilisation to tissue grafts should be applied in their final packaging without subsequent exposure (JPAC General guidelines for tissue processing, 21.5.3.2). For cells and blood components, sterilisation is not applicable. However, pathogen reduction methods can be applied to blood components (see chapter 4). Wherever possible, sterilisation methods can be applied to instruments, procurement devices and materials (e.g. raw materials, reagents, excipients, single-use components, containers, gowning and cloth) which are in contact with starting materials, process intermediates or final products (Directive 2006/17/EC, article 2, section 7 and Annex IV 1.3.8).

## Sterilisation methods

Whenever possible, sterilisation should be done using methods described in the European Pharmacopoeia (5.1.1). Main points are also presented in European Medicines Agency guideline (EMA/CHMP/CVMP/QWP/850374/2015). These methods are based on moist heat (steam), dry heat, gas, irradiation or membrane filtration. Selection of the sterilisation method must be based on the characteristics of the object of the sterilisation and its associated bioburden (Table X). Modifications or combinations of the described methods may be used, provided that the procedure(s) is validated.

**Table X.** Sterilisation methods according to Ph. Eur. 5.1.1 “Methods of preparation of sterile products”

|  |  |  |
| --- | --- | --- |
| **Sterilisation Method** | **Application examples** | **Reference conditions** |
| Steam | Instruments, materials, cloth and media | terminal steam sterilisation at ≥121 °C for 15 min |
| Dry Heat | Glass and metal instruments/tools | terminal dry heat sterilisation at ≥160 °C for ≥2 h |
| Ionisation radiation (irradiation) | Bone, skin, amniotic membrane (1), (EDQM T&C Guide)  Containers, equipment and gowns | terminal ionising radiation of absorbed dose ≥ 25 kGy |
| Gas (chemical agent) (2) | Containers and equipment | depends on chemical, no general conditions predefined |
| Membrane filtration | Fluid or gas products that are not amenable to other sterilisation methods | nominal pore size ≤ 0.22 µm |

1. Radiation sterilization of tissue allografts: A review; Rita Singh, Durgeshwer Singh, and Antaryami Singh; World J Radiol. 2016 Apr 28; 8(4): 355–369.
2. Acceptable only if no other sterilisation methods are feasible (EMA/CHMP/CVMP/QWP/850374/2015).

## General validation requirements for sterilisation

Validation must be performed in order to demonstrate consistent efficacy of method chosen and assurance of sterility. Whenever a sterilisation step is introduced, the following general validation requirements need to be addressed (Ph. Eur. 5.1.1; JPAC 21.5.3).

### 5.3.1. Sterility Assurance Level (SAL)

For sterilisation processes with a well-defined dose/kill relationship, a very high level of sterility assurance can be achieved (EDQM T&C Guide 8.6.1). This is quantified by the SAL value which is an experimentally-derived number expressing the likelihood of a contaminant to survive the process. In order to determine the SAL, the bioburden of the respective matrix must be known. Frequently, sterilisation processes are validated to assure the SAL ≤ 10-6 for sterile products or equipment. For validation of sterilisation technique, SAL of 10-6 should be achieved for the most resistant micro-organism (often bacterial spores). This is a “worst-case” validation and will guarantee a significant overkill for more sensitive microbes. SAL means that the likelihood of non-sterile item is 1 in 1 million. The SAL 10 -6 cannot be applied to membrane filtration method or to quantify the efficacy of virus inactivation/removal. (EDQM T&C Guide 2.16.9, Process validation; Ph. Eur 5.1.1; JPAC 21.5.3; EDQM T&C Guide 8.6.1.2; 10.3.6).

### 5.3.2. Biological indicators

Biological indicators are test systems (e.g. inoculated carriers) containing viable micro-organisms (usually spores of bacteria, e.g. *Bacillus* or *Clostridium* sp.) that provide a defined worst case challenge to verify the required effectiveness of a specified sterilisation process. Commercially available biological indicators are recommended, but if suitable are not available, custom-made may be used. (EDQM T&C Guide 2.16.9.; Ph.Eur. 5.1.2.)

Bioburden (and where relevant, bacterial endotoxins) should be specified prior to sterilisation. Bioburden is usually expressed as a measure of the numbers and identification of the species of micro-organisms in the material to be sterilised (EDQM T&C Guide 10.3.6; 10.4.3; Ph. Eur. 2.6.12 and 2.6.13). Validation of sterilisation potency requires that the maximum predicted level of microbiological contamination can be eliminated by determining the elimination capacity as the number of log scale reductions of the spiked micro-organism. The micro-organisms should verify the required effectiveness of selected sterilisation method by covering all relevant micro-organisms commonly found on the object including for example vegetative Gram positive and negative bacteria, vegetative fungi, fungal and bacterial spores and viruses, if applicable (T&C guide 10.3.6; Ph Eur 5.1.2; EMA/CHMP/CVMP/QWP/850374/2015; CPMP/BWP/268/95.).

Viral indicators should be chosen to resemble viruses which may contaminate the BTC product. Further detailed recommendations of viral safety (Ph. Eur. 5.1.7) as well as examples of the used virus indicators are listed in CPMP/BWP/268/95 *Virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses.*

## Specific validation criteria for sterilisation methods

Depending on the sterilisation method in question, more specific validation data needs to be evaluated for removal of micro-organisms. As a main principle, validation of the method effectiveness should be done using a combination of physical (e.g. thermo couples in moist heat sterilisation) and biological indicators, which shall be placed at the locations where sterilising conditions are most difficult to achieve (e.g. cold spots when using heat, difficult to penetrate areas when using gas, minimum/maximum load). (This principle is not applicable to membrane filtration). Parameters to achieve the required SAL and an example of the most widely accepted biological indicators are described under the relevant sterilisation method below.

Conditions of the sterilisation methods should be developed and validated in compliance with Ph. Eur. 5.1.1 and 5.1.2. In addition, guidelines for validation of sterilisation methods are explained e.g. in the publication by European Medicines Agency (EMA/CHMP/CVMP/QWP/850374/2015).

### 5.4.1. Steam sterilisation (Autoclaving)

Steam (moist heat) sterilisation is performed in saturated steam under pressure in autoclaves (Directive 2014/68/EU for pressure equipment) and the critical parameters are pressure, time and temperature. When using the method, equal distribution and adequate penetration of steam should be verified. The reference cycle for steam sterilisation is 15 min at 121 °C. Depending on the product and load, another combination of time and temperature may be adopted based on cycle validation, with a minimum acceptable temperature 110 °C. The sterilisation effectiveness may be calculated by F0 concept. F0 is the time in minute for the specified temperature that causes the same lethality as one minute at 121 °C, with minimum F0 not less than 8 min. (Ph.Eur. 5.1.5).

* + Suitable test micro-organism: *Geobacillus stearothermophilus* (e.g. strains ATCC 7953, NCTC 10007, CIP 52.81, NCIMB 8157, ATCC 12980)
  + Specific standard to be considered: ISO 17665-1:2006*: Sterilization of health care products* - Moist heat - Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices.

### 5.4.2. Dry heat

For dry heat the critical parameters are time and temperature. Reference conditions are minimum of 160 °C for at least 2 h. Other combinations may be used if validated and SAL ≤ 10-6 is demonstrated. Validation should be done using a combination of temperature mapping and biological indicator.

* + Suitable test micro-organism: *Bacillus atrophaeus* (e.g. strains ATCC 9372, NCIMB 8058, NRRL B-4418, CIP 77.18), at temperatures between 160 °C and 180 °C.
  + Specific standard to be considered: ISO 20857:2010 *Sterilization of health care products* — Dry heat — Requirements for the development, validation and routine control of a sterilization process for medical devices

### 5.4.3. Irradiation

Sterilisation by irradiation is achieved by gamma rays, accelerated electron beams or x-rays. The reference absorbed dose is 25 kGy and dose should never be less than 15 kGy. Depending on bioburden, ≥ 25 kGy irradiation dose may be required for sterilisation of bacteria and fungi. Quite often ≥ 34 kGy may be required for virus inactivation, since many viruses are resistant to irradiation and therefore viral inactivation data must be supported by appropriate marker viruses (EDQM T&C guide 8.6.2.1). Validation is usually performed by using dosimeters placed throughout the load.

* Suitable test micro-organism: *Bacillus pumilus* (e.g. strains ATCC 27142, NCTC 10327, NCIMB 10692, CIP 77.25) For this method, biological indicators are not always necessary, but may be required for the validation of BTC products (Ph. Eur. 5.1.2).
* Specific standards to be considered: ISO 11137-2: *Sterilization of health care products* -- Radiation -- Part 2: Establishing the sterilization dose

ISO 11737-2: *Sterilization of medical devices* -- Microbiological methods -- Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

### 5.4.4. Gas sterilisation

Multiple gas sterilisation processes are currently used and they are divided in two categories: alkylating agents (e.g. ethylene oxide) and oxidizing agents (e.g. hydrogen peroxide and peracedic acid). With all options, sufficient gas and moisture penetration is essential and thus gas concentration, exposure time, temperature and humidity are the parameters to follow. It is the responsibility of the user to define the suitability of the biological indicator for reactive chemical in question. It should be noted that the levels of residual toxic substances after sterilisation must be minimised (e.g. residual ethylene oxide in the product should not exceed a limit of 1 ppm, CPMP/QWP/159/01)

* Suitable test micro-organisms:

Ethyleneoxide: Bacillus atrophaeus (e.g. strains ATCC 9372, NCIMB 8058, NRRL B-4418, CIP 77.18); Hydrogen peroxide: *Geobacillus stearothermophilus*

* Specific standard to be considered: ISO 11135:2014: *Sterilization of health care products* - Ethylene oxide - Requirements for the development, validation and routine control of a sterilization process for medical devices

### 5.4.5. Filtration

In contrast to other methods, the principle of membrane filtration is not inactivation but removal/reduction of micro-organisms. As a sterilisation method, the nominal pore size of the microporous membrane is not greater than 0.22 µm. Before filtration, filter capacity should be validated and the method should retain microbial challenge of at least 107 cfu/cm2 on filter surface using suitable micro-organism (Ph. Eur 5.1.2). It should be noted that filtration is not suitable sterilisation method for viruses and mycoplasma.

* + Suitable test micro-organism: *Brevundinomas diminuta* (with nominal pore size ≤0.22 µm) (ATCC 19146, NCIMB 11091, CIP 103020) as single cells suspension. In addition, if possible, a suspension of vegetative bacterial cells representing the natural flora in question.
  + Specific guidance to be considered: Filter integrity testing (bubble point test), GMP Guide, Annex 1

## Information on sterilisation validation

Validation should be planned and reported. Plan/report should include the following relevant information:

* + The object(s) of sterilisation.
  + The used sterilisation method and justification for selection why that particular method is chosen. Method selection should be based on the properties of the sterilisation object(s).
  + Selected biological indicator by which SAL is determined.
  + Validation procedure considering the above described critical parameters. Specific for the used sterilisation method.

## Effect of sterilisation on tissue quality

Sterilisation must not render tissues clinically ineffective or harmful to the recipient neither adversely affect the essential properties. (ref. WP6/Annex 1 and JPAC General guidelines for tissue processing 21.5.3.2)

# Requirements and criteria for microbiological quality of the final BTC product

BTC collection and processing procedures are intended to produce non-infectious final BTC products for the recipients but in some cases microbiological contamination still occurs. Microbiological contaminations of BTC depend on for example the origin, procurement method and processing steps of the BTC (ref.: EDQM Guides?). As an example, causes of bacterial contamination in blood products include occult bacteraemia in the donor, inadequate or contaminated skin preparation at the phlebotomy site, coring of a skin plug by the phlebotomy needle and breaches of the closed system from equipment defects or mishandling (EDQM Blood Guide).

Microbiological quality criteria form part of final release criteria for the BTC. Release is the act of certifying compliance of specific BTC or batch of BTC with the requirements and specifications. Before any BTC are released for clinical use, 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 standard operating procedure (SOP) and national regulations.

Requirements and criteria for microbiological quality of the final BTC product differ from each other based on the level of microbiological purity needed:

* For some tissues (e.g. bone and amniotic membrane), terminal sterilisation can be applied and the aim is to reach sterile tissue products. Parametric release with acceptance criteria for the control of identified process parameters can replace microbiological testing of the tissue grafts. Validated procedures for all critical production steps (procurement of tissues, transportation, all processing steps, packaging and storage) and a fully validated sterilisation method must be applied (see chapter 5) (EDQM T&C Guide, 10.3.5.2).
* Some BTC are procured and/or processed aseptically but will not require further disinfection or sterilisation (e.g. sperm, oocytes, embryos).
* Many BTC cannot be sterilised and in those cases, aspects of microbiological testing need to be considered in order to ensure the microbiological quality of the final BTC product. Those aspects are described in chapters below.

BTC procurement and processing should be performed using aseptic techniques and/or in aseptic environment. Requirements for these and of environmental microbiological monitoring are not in scope of this guidance (see EudraLex Vol 4 Annex I for air quality requirements and for example JPAC General guidelines for tissue processing, section 21.5.1).

Previous chapter 4.4 apply also to this chapter 6.

## Common requirements applicable to all BTC

The aim in BTC processing is to produce non-infectious final BTC products which contain as few as possible micro-organisms, but a very high degree of “purity” can be difficult and expensive to obtain and might not even be necessary in all instances.

Differences in BTC make it difficult to establish a general rule for microbiological testing requirements (see Table X). Therefore, for each procedure, risk assessment 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.

Microbiological safety of BTC is guaranteed by microbiological testing using validated methods of known sensitivity and specificity. Currently such methods are primarily compendial microbiological methods of the European Pharmacopoeia (*Ph. Eur.*). Microbiological testing of final BTC products should be performed by an authorised testing laboratory (see chapter 2) and in compliance with Ph. Eur. requirements (see chapter 4.4).

Wherever possible, representative samples of BTC should be removed and tested for bacterial and fungal contamination using protocols authorised by designated personnel. Swabs, contact solutions or other validated non-destructive sampling methods should be used where it is impossible to remove sample without damaging the BTC graft. (EDQM T&C Guide 10.3.1, JPAC General guidelines for tissue processing 21.5.2). Aseptic techniques to obtain sample are required in order to minimise the risk of false positive cultures due to contamination at the time of sampling or upon inoculation in culture. Additionally, a sample is recommended to be retained that can be used for repeat culture to validate a positive result. (EDQM Blood Guide, ch. 12)

In general, testing is recommended to be performed for both pre-processing samples of the procured BTC and on post-processing samples of every final BTC product. Sampling should be conducted immediately before packaging or as late as possible during the procurement or processing. (EDQM T&C Guide, 10.3.1).

For some BTC, pathogen reduction can be applied (see chapter 4) prior of having the final BTC product. Further processing after the pathogen reduction step should be conducted without antimicrobial agents. Methods for testing of final BTC products must be evaluated carefully with respect to possible inhibition of microbiological growth due to decontaminating agents or their residues (EDQM T&C Guide, 10.3.5.3).

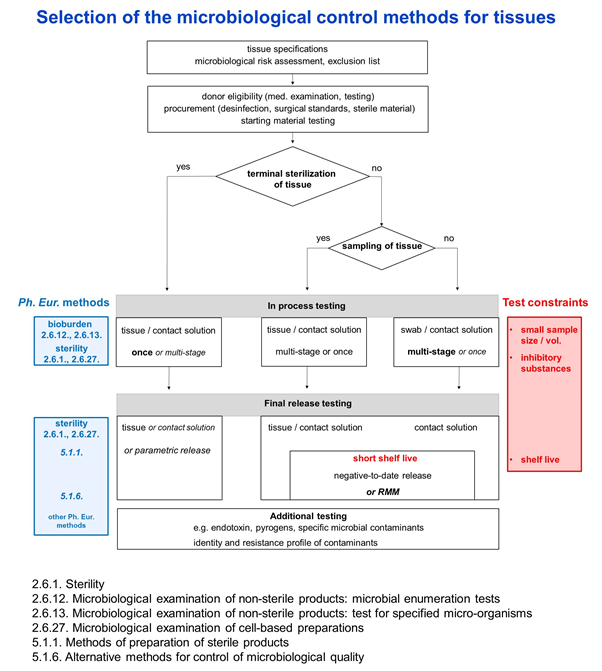
BTC with a short shelf-life may be released based on negative-to-date results. In this context, implementation and documentation of sufficient assurance of the microbiological quality of the final BTC product when released is essential. This will include in-process microbiological tests that have been established on the basis of risk analysis, usually including sterility testing of the starting material and/or of samples from the intermediate product at critical steps, if applicable. Final testing is still ongoing after the BTC is released and will be completed. Procedures for handling positive results after release must be in place, including potential recall, notifying the clinician caring for the recipients and identification of the microbial species and resistogram (EDQM T&C Guide 10.4.2.1).

In general, if any microbiological contamination is detected, it is necessary to identify the strain and to investigate its source. In this case the final BTC product should not be used for clinical application unless a risk–benefit analysis indicates that it is the best option for the patient.

**Table x**. Factors affecting the microbiological quality and thus should be considered when determining the microbiological testing protocol (sample types, sampling times, analyses) (modified from table 7.2. Risks of contamination that should be considered when determining air-quality specifications of processing facilities, EDQM T&C guide)

|  |  |  |
| --- | --- | --- |
| **Phase** | **Risk factors** | **Examples/information** |
| Procurement | Tissue type | Skin vs femoral head  Tissue type specific micro-organisms must be taken account when validating the microbiological analyses |
| Procurement environment | Funeral home, operating theatre etc. |
| Processing | Tissue or cell contamination during open *versus* closed processing | Closed processes are less prone to contamination during processing than processes where BTC are exposed to the environment |
| Effectiveness of the pathogen reduction method to remove contaminants | Some BTC, even though not terminally sterilised, can be treated with pathogen reduction which reduces the risks of transferring any microbiological contamination |
| Sampling | 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 |
| Batch size | text needs to be added |
| Storage | Storage method of the final product | text needs to be added |
| Shelf-life of the final product | Limited time for testing, 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). |
| Transplantation | 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. |

**Figure X**. (below)



## Specific requirements and criteria depending on the type of BTC processing

Different requirements for testing of microbiological quality of BTC apply for cases where BTC has been procured and processed in closed or open systems. BTC procurement systems can be closed with equipment designed and operated in such way that the BTC are not exposed to the environment, or open that expose the BTC to the environment.

Chapter 4.4 Methods for control of microbiological quality apply to both cases.

### 6.2.1. BTC with processing in closed systems

Use of closed systems is strongly recommended for all steps in blood component processing. Open systems may exceptionally be necessary due to local constraints and should be undertaken in an environment specifically designed to minimise the risk of contamination (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines 6.6.3). Processing in closed systems are generally used also for haematopoietic progenitor cells procured by apheresis and mononuclear cells (EDQM T&C Guide, 22.3).

For BTC with processing in closed systems, repeated testing steps do not yield additional information on the microbiological status of the BTC and are thus not required. In such cases, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point may be applicable. (EDQM T&C Guide, 10.3.5.1)

According to the Directive 2004/33/EC (Annex V, 2.2), appropriate bacteriological control of the collection and processing of blood products must be performed. Bacterial cultures of platelet components provide the best indication of the overall rate of contamination of whole blood donation provided that the sample for culture is obtained in a suitable volume and at a suitable time after collection. Platelet components are more likely than other blood components to be associated with sepsis due to their storage at room temperature, which facilitates bacterial growth. Data on routine bacterial monitoring should be analysed using statistical process control techniques to ensure that the process remains in control. (EDQM Blood Guide, ch. 12)

For blood products, it is recommended to perform bacterial screening:

* on 100% of platelet components;
* using an increased sample volume aiming at increasing test sensitivity;
* delaying sampling time at least ≥24h after blood donation giving time for low level contamination to grow
* using aerobic and anaerobic media
* performing routine QC test to check performances of the method in use (WHO / PEI platelet contamination reference strains)

### 6.2.2. BTC with processing in open systems

Most tissues and cells, including those for which pathogen reduction has been applied to, are exposed to the environment at certain processing stages between procurement and packaging. If terminal sterilisation cannot be used, the 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 (EDQM T&C Guide 10.3.5.4).

Sampling and microbiological assessment should include the starting material, the transport solution and any solutions used to wash BTC (EDQM T&C Guide, 10.3.5.4).

Microbiological testing of tissues and cells should be performed according to the tissue-specific requirements in Part B of EDQM T&C Guide and general criteria described in chapter 10.3. Tissue-specific requirements describe the minimum standards to control microbiological safety of each BTC type and microbial contaminants that should result in BTC discard, if applicable.

# Final considerations

(To be drafted later, if needed)

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# Acronyms

**Ab** Antibody

**Ag** Antigen

**BE** Blood Establishment

**BTC** Blood, Tissues and Cells

**CA** Competent Authority

**CPP** Critical Process Parameter

**ECDC** European Centre for Disease Prevention and Control

**EUBD**  European Blood Directives

**EUBTCD** European Blood, Tissues and Cells Directives

**EUTCD**  European Tissues and Cells Directives

**GAPP** Facilitating the Authorisation of Preparation Process for blood, tissues and cells

**HIV** Human Immunodeficiency Virus

**HTLV** Human T-cell leukaemia virus

**JA** Joint Action

**MAR** Medically Assisted Reproduction

**MS** Member State

**NAT** nucleic acid amplification technique

**PPA** Preparation Process Authorisation

**PPD** Preparation Process Dossier

**PRT** Pathogen Reduction Technology

**RMM** rapid microbiological methods

**SAL** Sterility Assurance Level

**TE** Tissue Establishment

**vCJD** variant Creutzfeldt-Jakob disease

**VISTART** Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation

**WHO** World Health Organization

**WP** Work Package

# Definitions

**Accreditation** The independent evaluation of conformity assessment bodies against recognised standards to carry out specific activities to ensure their impartiality and competence (ilac.org)

**Analytical sensitivity** The limit of detection, i.e. the smallest amount of the target marker that can be precisely detected (Official Journal of the European Union L 318/ Commission decision 2009/886/EC).

**Analytical specificity** The ability of the method to determine solely the target marker (Official Journal of the European Union L 318 /Commission decision 2009/886/EC).

**Bioburden** A measure of the numbers and variety of micro-organisms on a surface or volume (EDQM T&C guide 10.3.6.)

**Biological indicators** Test systems containing viable micro-organisms (usually spores of bacteria) that provide a defined challenge to verify the required effectiveness of a specified sterilisation process (Ph.Eur. 5.1.2.)

**CE-marked kit** Kitmarked by a manufacturer to indicate that a donor testing kit/assay is in conformity with the applicable requirements set out in Regulation (EU) 2017/746 on *in vitro* medical devices and other applicable Union harmonisation legislation providing for its affixing (modified from Regulation (EU) 2017/746).

**Closed system** A procurement system with equipment designed and operated such that the cells are not exposed to the environment. (EDQM T&C Guide)

**Critical process parameter (CPP)** A process parameter whose variability has an impact on a critical quality attribute and which therefore should be monitored and controlled to ensure the process produces the desired quality. (Directive (EU) 2016/1214 Art. 1, Good Practice Guidelines)

**Deceased donor** A person declared to be dead according to established medical criteria and from whom cells, tissues and organs have been recovered for the purpose of human application (EuroGTP II Guide)

**(Diagnostic) specificity** The probability that the donor testing kit/assay gives a negative result in the absence of the target marker (adapted from Official Journal of the European Union L 318/ Commission decision 2009/886/EC).

**(Diagnostic) sensitivity** The probability that the donor testing kit/assay gives a positive result in the presence of the target marker (adapted from Official Journal of the European Union L 318/ Commission decision 2009/886/EC).

**Donation** Donating human blood, tissues or cells intended for human applications (Adapted from Directive 2004/23/EC)

**Donor** Means every human source, whether living or deceased, of human cells or tissues intended for human application; and a person who voluntarily gives blood or blood components for therapeutic use (adapted from DIRECTIVE 2004/23/EC and the EDQM Blood Guide)

**F0** The time in minute for the specified temperature that causes the same lethality as one minute at 121 °C (Ph.Eur. 5.1.5).

**In-house** Manufactured and used within an organization (for example BE/TE/laboratory) and not distributed outside (adapted from Regulation (EU) 2017/746)

***In vitro* diagnostic medical device** Any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, piece of equipment, software or system, whether used alone or in combination, intended by the manufacturer to be used *in vitro* for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information on one or more of the following: (a) concerning a physiological or pathological process or state; (b) concerning congenital physical or mental impairments; (c) concerning the predisposition to a medical condition or a disease; (d) to determine the safety and compatibility with potential recipients (Regulation (EU) 2017/746).

**Kit** A set of components that are packaged together and intended to be used to perform a specific *in vitro* diagnostic examination, or a part thereof (Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices)

**Likelihood ratio** The likelihood of a given result arising in an individual with the target clinical condition or physiological state compared to the likelihood of the same result arising in an individual without that clinical condition or physiological state (Regulation (EU) 2017/746)

**Microbiological quality**

**Negative predictive value** The ability of a donor testing kit/assay to separate true negative results from false negative results for a given attribute in a given population (adopted from Regulation (EU) 2017/746)

**Open system** A procurement system that exposes the cells to the environment (EDQM T&C Guide)

**Partner donation** The donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship (Directive 2006/17/EC)

**Pathogen reduction technologies** Procedures that irreversibly impede proliferation of pathogens in BTC, either by removal or inactivation with physical and/or chemical methods. (EDQM Blood Guide)

**Performance evaluation** An assessment and analysis of data to establish or verify the scientific validity, the analytical and, where applicable, the clinical performance of a donor testing kit/assay (adapted from Regulation (EU) 2017/746)

**Positive predictive value** The ability of a donor testing kit/assay to separate true positive results from false positive results for a given attribute in a given population (adapted from Regulation (EU) 2017/746)

**Predictive value** The probability that a person with a positive test result has a given condition under investigation, or that a person with a negative test result does not have a given condition (adopted from Regulation (EU) 2017/746)

**Proficiency testing** The evaluation of participant performance against pre-established criteria by means of external quality assessment scheme, inter-laboratory comparisons by use of externally sourced samples or panels (EDQM Blood guide).

**Qualification** As part of validation, means the action of verifying that any personnel, premises, equipment or material works correctly and delivers the expected results. (Directive 2005/62/EC)

**Quality system** The organisational structure, defined responsibilities, procedures, processes, and resources for implementing quality management and includes all activities which contribute to quality, directly or indirectly (Directives 2005/62/EC, 2006/17/EC).

**Rapid test** Qualitative or semi-quantitative *in vitro* diagnostic medical devices, used singly or in a small series, which involve non-automated procedures and have been designed to give a fast result (Official Journal of the European Union L 318/25).

**Reproductive cells** All tissues and cells intended to be used for the purpose of medically assisted reproduction (adapted from Directive 2006/17/EC)

**The sterility assurance level (SAL)** The probability of a micro-organism on one item within a batch or within a defined population. (Ph.Eur. 5.1.1)

**Spiking** The addition of a known amount of a compound to a standard, sample or placebo, typically for the purpose of confirming the performance of an analytical procedure. (adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7)

**Standard** The requirements that serve as the basis for comparison (Directive 2005/62/EC)

**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. (EDQM T&C Guide)

**Sterility** The absence of viable microorganisms, as defined by a sterility assurance level (SAL) equal to or less than 10-6. (Ph Eur 5.1.1.)

**Sterility assurance level (SAL)** 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, and grafts are then considered sterile. (EDQM T&C Guide)

**Terminal sterilisation** A process in which the product is sterilised in its final container (Ph.Eur. 5.1.1)

**Validation** Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes; a process is validated to evaluate the performance of a system with regard to its effectiveness based on intended use (modified from Directive 2006/17/EC)

**Validation plan** A document describing the activities to be performed in a validation, including the acceptance criteria for the approval of a process or method for routine use. (adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7)

**Validation report** A document in which the records, results and evaluation of a completed validation program are assembled and summarised. (adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7)

# Appendix I - Assessment checklist

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Product :** | |  | | | | | |
| **Laboratory**  **requirements** | | **Requirements** | **Criteria** | **Other** | **Specifications** | **Conform** |
| Donation/  donor screening laboratory | Laboratory | Quality system(s) of the laboratory | 2005/62/EC / Blood donations  2004/23/EC / T&C donors |  |  |  |
| Name of the laboratory | Standard that laboratory follows | Blood Directives  Good Practice Guideline/EDQM  T&C Directives | e.g. ISO15189,  ISO17025,  national standards |  |  |
| Name of the authority that has accredited, designated, authorised or licensed the laboratory | Name of the authority | e.g. Name of the external accreditation body (if applicable) |  |  |
| BTC graft  microbiological testing laboratory | Laboratory | Quality system(s) of the laboratory | 2004/23/EC |  |  |  |
| Name of the laboratory | Standard that laboratory follows | T&C Directives | e.g.  Good Practice Guideline/EDQM, ISO15189,  ISO17025 |  |  |
| Name of the authority that has accredited, designated, authorised or licensed the laboratory | Name of the authority | e.g. Name of the external accreditation body (if applicable) |  |  |
| Donor testing | |  | |  |  |  |
| Pathogen reduction | |  | |  |  |  |
| Sterilisation | | Bioburden (Ph.Eur. method, matrix validation)  Sterilisation process (Ph. Eur. method, validation, SAL) | |  |  |  |
| Microbiol. quality of final product | | Compliance with TC Guide Part A: 8.8, 10  Compliance with TC Guide Part B: specified tissue section  Sterility test (Ph.Eur. method, matrix validation)  AMM (compliance with Ph. Eur. 5.1.6) | |  |  |  |
| Specific issues | | Product specifications (e.g. TC Guide Part D)  Additional requirements (e.g. MS specific requirements) | |  | e.g. Guideline *abc*, National Medical Board:  additional testing requirement *xyz* |  |
| Expert board review  (e.g. CASoHO) | | Request for review (e.g. new processing methods, RMM)  Request for revision (e.g. EDQM Guide) | |  |  |  |

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|  |  |  |
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