

BARCELONA

2011 November, 9-10-11

# ORAL PRESENTATIONS

## ORAL - NOVEMBER 9TH - ETHICAL THEMES OF COMMON INTEREST AUDITORI - 12.00-12.30

### O-1

#### MODEL OF COEXISTENCE FOR PUBLIC AND FAMILY CORD BLOOD BANKS

ÁLVAREZ RAMOS, A.; LOSADA PESCADOR A. *VidaCord. Spain.*

##### Abstract/Introduction:

Cord blood from HLA-matched sibling is the best option to save the life of a child suffering from a subsidiary of transplant disease. Despite this clinical evidence, there is much controversy about family UCB banks: It is argued that storing UCB in a private bank for autologous use is useless (for UCB stem cells already carry the same genetic error that caused the disease) and that family UCB banks are a menace to key social values such as solidarity, altruism and fairness. To address these issues Spain published the Royal Decree 1301/2006, which places special emphasis on the risk of potential misleading information family CBB could spread about the real clinical applications UCB may have on what is defined as "potential autologous use," as well as the fact that any UCB unit stored in a family bank in Spain must be necessarily made universally available. There is no consideration in this RD about the potential allogeneic use of UCB within the family.

##### Material and Methods:

We analyzed the RD 1301/2006, the Spanish Umbilical Cord Blood National Plan (PNSCU), public statements made by the director of the Spanish National Transplant Organization, VidaCord (first UCBB licensed in Spain) Mission Statement, as well as studies on CBT done by Eliane Gluckman and the Spanish Constitution. DISCUSSION UCB of an HLA-matched sibling is the best option for hematopoietic transplant. Therefore, it is legitimate for parents to decide to store in private banks their children UCB. VidaCord objective, in its third point ("the consented transfer...") argues that individual and families' rights can be reconciled with the overall population's interests. The PNSCU states that the whole Spanish population CB transplant needs are met with 60,000 units stored in public banks, which increase their inventories from altruistic donations from part of the 450,000 annual births in Spain.

##### Conclusions:

1. Having stored the cord blood in a family bank is the best medical alternative in the event of illness subsidiary of allogeneic sibling transplant.
2. The right to health protection is prior to the likely incidence of a disease.
3. Public health authorities must balance the individual right to give your children the best clinical option with the whole population care.
4. It is possible to reconcile the two positions, favoring this way society interests as a whole. To achieve this, the value of solidarity must be subordinated to individual freedom and the principle of autonomy.

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**O-2**

**A FUNDAMENTAL RIGHT TO DECIDE WHETHER AND HOW OUR BODY WILL BE USED IN RESEARCH**

ZARDOYA, M.; VILARRODONA A.; RODRÍGUEZ C.; RUÍZ A.; PAREDES D.; TRÍAS E.  
*Transplant Services Foundation. Hospital Clinic of Barcelona. Spain.*

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**Abstract / Introduction:**

Human organs, tissues and cells can be used not only for clinical applications but also for teaching and research with or without commercial purposes. The supply of human tissue with this aim is increasingly demanded and sometimes indispensable. The main ethical and legal issues to be considered are related to confidentiality, data protection, secondary use of samples, return of results and informed consent. Taken into account the movement of tissues at European level and the International exchange we also face the problem of lack of harmonization and a common regulatory framework to protect autonomy. This study will mainly focus on consent requirements to guarantee and protect autonomy both from living and deceased donors. Aim The main objective is to analyze if current medical standards and legal regulations guarantee the autonomy of the will. A secondary aim is to examine the opinions and attitude of donors and donor families towards donation for teaching and research.

**Materials and Methods:**

A study of legal regulations and ethical requirements in different European countries has been done to find out differences in consent regulations and clinical practices to protect the donor. Not only legal regulations have been studied but also the kind of information given and methods used prior to request consent, as informed consent encourages difficulties when a broader consent is needed for a secondary use of the samples or for the involvement of private companies in the projects. It has also been analyzed the clinical practices used in different hospitals in our country to guarantee donor's autonomy when legislation does not differentiate between specific consent when the tissues, organs and cells are going to be used for research, for teaching or for research with commercial purposes. This information has been obtained from regional and hospitals transplant coordination units. The willingness to donate for teaching or investigation has been also studied, considering the organ and tissue donors from July 2009 to 30 June 2011 in our hospital.

**Results:**

Ethical, legal and social issues have not been regulated in a precise and uniform manner in the analyzed countries. Most of them are developing a binding legal document that regulates the distribution of samples for research. However, specific consent is not always required for using tissues and cells for research. A recent study conducted in our hospital has concluded that most of the families of potential donors are in favour of the use of their organs, cells and tissues in research (14.5% refused donation for other purposes than clinical)

**Conclusions:**

The recovery and distribution of tissue for research is a need in growing demand. It is a must to organize the activities and design the distribution procedure according to the current legal framework and respecting the ethical requirements. It will protect donor's autonomy concerning the use of the body or body parts for non clinical aims and it will contribute to creating a more favourable public opinion towards the donation of organs, tissues and cells for research and teaching aims. The following should be guarantee: - Donor must understand what the sample is to be used for - Donors are informed that their sample might be used in commercial research - Patients know that material left over following diagnosis might be used for research By protecting autonomy through the process of obtaining consent we will allow individuals to exercise the fundamental right to decide how their tissues, organs and cells will be used in research.

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**O-2 BIS**

**CORD BLOOD: PUBLIC AND PRIVATE**

BRANKA GOLIVICH

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**ORAL - NOVEMBER 9TH - INTERNATIONAL EXPERIENCE ON TB  
ROOM 1 - 11.30-13.30 H.**

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**O-3**

**TPM AS INTERNATIONAL EDUCATIONAL MODEL IN TISSUE BANKING**

NAVARRO, A.; DUQUE E.; PAEZ G.; MANYALICH M. *Banc de Sang Teixits. Spain.*

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**Abstract / Background:**

The tissue and cell donation have enabled the development of a health care field which has improved the quality of life of numerous patients. As its success relies on the staff professionalism, all personnel involved in the tissue and cell donation process should be appropriately qualified and able to access continuing knowledge. The Transplant Procurement Management (TPM) has been providing for nine years academic training courses on tissue banking in compliance with the agreed professional requirements, current practices and recognized standards of proficiency in the field. OBJECTIVE: To provide academic training courses on tissue banking in compliance with the agreed professional requirements, good current tissue practices and recognized standards of proficiency in the field.

**Method:**

The Tissue Banking Courses provided by TPM are meant to develop and reinforce the core skills and competences of professionals involved in the tissue and cell donation, recovery, processing and transplantation. They consist in a blended learning program (Face-to-Face and On-Line) and are structured in modules for a better assimilation of concepts. Participants perform practical activities throughout the whole course. General debates allow them to share opinions and draw on their practical knowledge and experience. Continuous and final assessment is performed.

**Results:**

A total of 376 professionals have been trained within the TPM courses on tissue banking as following: 265 participants from 41 countries (172 Europe, 51 America, 24 Oceania, 15 Asia and 3 Africa) attended the on-line courses and 111 participants (80 Europe, 12 Asia, 10 America, 6 Oceania and 3 Africa) the face-to-face ones. The overall assessment shows that the courses' objectives have been successfully accomplished with an average of: 4.0 in the on-line and 4.4 in the face-to-face course. The applicability to the daily job has also been evaluated positively with an average of 4.0 in the on-line and 4.1 in the face-to-face course (scoring performed on a 1-poor to 5-excellent scale).

**Conclusion:**

By providing specialized training with practical applicability in the tissue banking field, TPM allows practitioners to improve their core skills and competences, establish optimal policies and practices for human tissue and cell donation. Due to its international approach, specialists may become part of a wide network of colleagues who manage the same challenges worldwide.

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**O-4**

**BASIC CONDITIONS FOR A NETWORK IN TISSUE MEDICINE: QUALITY, TRANSPARENCY AND EFFICIENCY**

*BÖRGEL, M. DGFG - German Society for Tissue Transplantation. Germany.*

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**Abstract:**

The embedding of tissue medicine in the non-profit sector, primarily established at major university hospitals, is one goal of the German Society for Tissue Transplantation (DGFG). To meet the increasing regulatory requirements of the Human Tissue Act, the factors of success for a non-profit network in tissue medicine are quality, transparency and efficiency in many ways. Tissue donation and tissue processing in accordance with high uniform standards ensure high quality in all processes and tissue graft quality at the best. Uniform standards enable efficient and cost-saving banking processes and exploit synergies, as well. This continuously improves the supply of high-quality tissue grafts through DGFG. Transparency in all activities is necessary for an authentic and altruistic commitment in the sensitive issue of tissue donation. The annual report of DGFG unfolds transparently the non-profit character of all activities of the network.

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**O-5**

**TRANSFORMING TISSUE DONATION AND TRANSPLANTATION IN CANADA INTO A SINGLE INTEGRATED INTER-PROVINCIAL SYSTEM**

*HAUN, M.; MOHR J.; DERKSEN P.; PARSONS C.; SHER G. Canadian Blood Services. Canada.*

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**Abstract:**

Tissue banking in Canada consists of more than 20 independent tissue banks that recover, process, store and distribute tissue (In Quebec the provincial blood agency Hema-Quebec manages the tissue supply). Delivery of healthcare services is a provincial jurisdiction in Canada, which can present challenges with respect to standardization, equity of access for patients, and co-operation between programs. In August 2008, Canadian Blood Services was asked by the federal and provincial governments to leverage its unique role and expertise as the operator of the Canadian blood system (outside Quebec) to design an inter-provincial system for tissue (and organ) donation and transplantation. Canadian Blood Services has undertaken an exhaustive consultation with tissue donation and transplantation stakeholders, end users and experts across the country and internationally, documenting their concerns, advice and proposed solutions. A committee of tissue experts has developed a series of recommendations characterized by three key themes: safety and quality, equitable and timely access to tissue products, and efficiency. The plan recommends the existing independent and un-coordinated tissue banks transition to a system that will double tissue donation and recovery activity, consolidate tissue processing, standardize quality programs, maintain a single shared inter-provincial inventory, and be managed by one organization. The recommendations, a proposed implementation plan and cost estimates were delivered to governments in April 2011.

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## O-6

### **MINUTES FROM THE FIRST EDITION EXPERIENCE OF THE UNIVERSITY OF BARCELONA MASTER DEGREE IN DONATION & TRANSPLANTATION OF ORGANS, TISSUES AND CELLS**

*BALLESTÉ, C.; SEGUR J.M.; CASAROLI R.; POMAR LUIS J.; ALSINA M.; SUSO S.; MANYALICH M. Facultat de Medicina, Universitat de Barcelona. Spain.*

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#### **Abstract/Introduction:**

The first Master's degree in the field of Donation & Transplantation was offered by the University of Barcelona, 2010-2011. This official program, integrated within the EHEA, was designed to provide participants with the necessary knowledge, skills and competencies for their future clinical care activity as well as helping them to carry out any scientific research. The Master's degree was structured in 3 common modules: Research in donation, Research in organ transplantation, Research in tissues, cells & hematopoietic progenitors transplants and 2 specialized: Research & Professional Path.

#### **Objectives:**

It is with lot of interest to analyse and to define the feedback from this first edition. The results will allow knowing the strong and the weak points in several aspects.

#### **Methodology:**

The experience and the motivation of the candidates to continue their career in this field were evaluated. An evaluation questionnaire for the organizational aspects was used to know the participant's opinion for the following aspects: Content of the materials; the way of presenting; the way of running the question/answer minutes; personal benefits from the participation in the class. The percentage of the participation was also an indicator showing the interest of the students.

#### **Results:**

**Participant's profile** The total number of the participants was 25, with a heterogeneous cultural and professional profile background; 17 Medical Doctors, 4 Nurses, 2 Biologists and 1 Psychologist. 22 of 25 were graduated; 3 left the programme for personal or professional reasons. **Internal subjects evaluations** The evaluation showed the following scores: Content of the materials (4,29±0.26); presentation (4,22±0.32); Questions/answers minutes (4,32±0.29); Personal benefits (4,06±0.31) **On-line results** Online learning system was used. The participants were able to express their opinion, asking questions or raising discussions in the Open Forums. The evaluation was a summary of the student activities and the tasks fulfilled by them. **Research projects** Divided in 2 different fields: Research & Investigation (77% of the participants) and Clinical and/or Laboratory Practice (23% of the participants). 72.6% of the participants manage to fulfil the study in the scheduled time. All but 2 were considered as adequate by the ad-hoc tribunal and passed it.

#### **Conclusions:**

The great and heterogeneous participation showed the interest of the participants. This Master offers the possibility to enlarge the knowledge and skills of a large number of foreign professionals providing them with an official degree, known in the scientific and academic community. Analyzing the results, this programme achieved the expected results giving a great support to the following edition.

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**O-7**

**SPEED IS LIFE. APPLYING LEAN HEALTHCARE TO TISSUE BANKS**

*TORNOS JUAN, I.; SERIGO X.; GIRALT E. Auren. Spain.*

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**Abstract:**

Combat pilots have since the very beginning of military actions believed that "Speed is Life". In a Sense this principle is also applicable to Tissue Banks. - Processing tissues after extraction needs to be done at maximum speed to assure quality of the stored "product". - Service to Hospitals needs to be at maximum speed to assure reaching patients on time. Of course, speed is always subject to the Quality of the Processes involved. What is really needed is to perform the right steps, in the right sequence, with the right quality at the maximum speed possible. Being capable to achieve this goal is nothing else than getting as close to perfection as possible. Up till now, a high amount of effort has been placed on assuring that the correct steps are performed, and robust and strict Quality Systems have been developed and implemented in many Tissue Banks all over the world. This is in most cases not enough to guarantee the fastest process possible. Lean Healthcare is the correct tool to achieve this goal applying different methods and tools that start on a deep and solid analysis of the processes to look for anything not adding value to it. This is frequently called MUDA using the Japanese word. - Any time a tissue is waiting to be collected or queuing to be tested, MUDA is being generated. - Wrong extractions of stem cells generate in many cases storage of unusable tissues. This is also MUDA. - Errors in decisions about volumes of blood to be stored at hospitals generate stock breakdowns or excesses which are also MUDA. - Returns of unused blood from hospitals to Tissue Banks consume time and resources adding no value and are again a source of MUDA. - Etc. Systematic and exhaustive elimination of waste (MUDA) is the path to get closer to perfection and thus to be capable to perform processes at the "speed of light". In this talk we will propose a number of methods and tools inherited from the manufacturing world that will allow Tissue Banks to go a step forward in developing an Excellent process, providing exceptional service to hospitals and thus to patients. All of these methods are grouped today within the Lean Healthcare approach that is growing fast in its application in most of Health Services in the USA and Europe.

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**O-8**

**TISSUE RECOVERY TEAM: 8 YEARS OF EXPERIENCE**

*FARIÑAS, O.; VILARRODONA A.; SAVIO MANUEL A.; LUQUE S.; OLIVA R.; PÉREZ LUISA M.; TRÍAS E. Transplant Services Foundation - Hospital Clinic. Spain.*

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**Abstract/Introduction:**

Traditionally in Europe each tissue type from cadaveric donors is recovered by a different recovery team (orthopaedic surgeons, plastic surgeons, cardiovascular surgeons, ophthalmologists). In 2002 we developed a new model of tissue recovery team with the objective of improving our recovery system. This new model of tissue recovery team, that started its activity in 2003, was conceived from a combination of the European model where it is almost always present a medical doctor in the retrieval, and the American model where their members recover all the tissues (skin, cornias, cardiovascular and musculoskeletal tissue). Our new concept of tissue recovery team is composed by three members (medical doctor, nurse and technician) that are in charge of recover all the tissues. It is always mandatory that a medical doctor (team leader) is present in the team due to its medical knowledge, and the other two members can be combined in different ways (nurse-technician, nurse-nurse, technician-technician). Aim To evaluate the results and effectiveness of this new tissue recovery team model during the period between 2003 and 2010.

**Materials and Methods:**

This retrospective study evaluates the skin, cardiovascular and musculoskeletal retrievals performed by the new tissue recovery team during the period from 2003 to 2010. The analysis focused on different variables depending on the tissue recovered.

From skin tissue the total number of retrievals, total amount of skin obtained and the average skin surface obtained per donor were analyzed. From musculoskeletal tissue the total number of retrievals, % of recovery errors and the contamination rate were analyzed. From cardiovascular tissue the total number of heart-valves and arteries recovered were analyzed. Results The skin tissue recovery started in 2008. The number of skin retrievals increased from 91 (2008) to 139 (2010). It was observed the importance of the personnel learning curve because the average skin surface obtained per donor raised from 2489.67 cm<sup>2</sup> (2008) to 3257.01 cm<sup>2</sup> (2010). Regarding the musculoskeletal tissue recovery two main variables were analyzed: recovery error and contamination rate. The recovery error rate decreased drastically since the establishment of the new tissue recovery team (average from 2003 to 2010 was 1.04) comparing with the results obtained by the traditional team (average from 2000 to 2002 was 2.54%). The contamination rate was influenced by the incorporation of new personnel and the development of a new donor cleaning methodology (23.01% from 2003 to 2007 and 17.5% from 2008 to 2010).

#### Conclusions:

The new model of tissue recovery team provides many benefits to our tissue establishment. A more accurate control of the recovery procedures can be performed increasing its achievement as well as the possibility of personnel training. To conclude we obtained a more professional recovery team decreasing the recovery errors and the contamination rates during tissue retrieval.

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## O-9

### THE DONOR TISSUE BANK REPLACEMENT FACILITY – MEETING FUTURE DEMANDS IN TISSUE / CELL BANKING

HERSON ROMA, M.; PONIATOWSKI S.; ADAMAS F.; CORDNER S. Donor Tissue Bank of Victoria / Victorian Institute of Forensic Medicine. Australian.

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#### Abstract:

This presentation shares the considerations given to the design of a replacement facility for the Donor Tissue Bank of Victoria (DTBV), Melbourne, Australia. The DTBV was established in 1989, by the Victorian Institute of Forensic Medicine in awareness of the privileged access to human tissue for transplantation. The DTBV is the only multi-tissue tissue bank in Australia with all the relevant services under the one roof which include a team of transplant coordinators, processing scientists and technicians and a fully NATA/ TGA accredited microbiology and serology laboratory. As a multi-tissue banking facility (skin, cardiac and muscle-skeletal), the DTBV has provided increasing number of grafts in Victoria and Australia. DTBV's original purpose built 440m<sup>2</sup> tissue banking facility opened in 1992; by 2009, although still fully compliant with the requirements of the Therapeutic Goods Administration (TGA) weaknesses in the architectural design and structure of the processing core suite were becoming a liability. The facility could become non-compliant to the stricter incoming codes of manufacturing practice and inadequate to absorb growth of the DTBV's banking activity. Plans for future also acknowledged that current tissue grafts will be surpassed by biotechnology enhanced products incorporating cells and scaffolds. Tissue banks such as the DTBV are uniquely positioned to become translation platforms to convert successful bench-level results into quality compliant and cost effective transplantable products. In 2009, DTBV was successful in its submission to Commonwealth for a AUD\$13 million grant to build a state of the art replacement tissue and cell banking facility, at the Victorian Institute of Forensic Medicine (Coronial Services Centre) site. The new facility is currently under construction, with a design aimed to incorporate the elements of incoming increased tissue and cell manufacturing regulatory requirements, as well as attain the capacity to manufacture biotechnology enhanced tissue and cell products. The envisioned 1,600 m<sup>2</sup> gross built area, will be split into: ground level (Tissue Retrieval, Stores, Despatch); first floor (Offices, Micro Lab, R&D Lab) and second floor (processing core and CSSD). There have been quite a number of challenges in the design considering the need to ensure a versatile and long lasting plant, to allow for future changes in room use dictated by specific product requirements, and to incorporate the specific requirements of cell cultures within a GMP controlled environment. Whilst the new facility will ensure business continuity, only time and use will confirm if the goals have been met.

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## O-10

### CURRENT STATUS OF TISSUE BANKING IN KOREA

KANG , Y.; CHUNG Y.; LIM J.; KIM Y. *St. Vincent's Hospital, Catholic University of Korea. Korea.*

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#### Abstract:

In 1971, first bone bank was established in Korea. The first clinical case of allograft transplantation was reported at the Journal of Korean Orthopaedic Association in 1973. After then, more than 60's hospital based surgical bone banks were established throughout the country. Since the law on safety and control for human tissues had been established in 2005, tissue transplantation was tremendously grown in Korean medical society. Currently, 57 hospital based bone banks, 5 processing tissue banks, three regional tissue banks and 70 tissue distributors are working in Korea. From 2005, every tissue banks have to report their activities to Korea Food & Drug Administration(KFDA) including number of donors, products and distribution of the tissues, and etc. KFDA with professional and academic associations annually has been performed quality assessment for the tissue banks within the country. Varieties of the quality failure were detected by KFDA inspection including inadequate facilities, equipments, manpower, documents and achieves etc. In every year, KFDA reported the activities of tissue banks. In 2005, 55,512 tissues were used in the country. At that time, only 10,158(18%) of tissues were produced by tissue banks in the country. Majority of the tissues (45,354 tissues; 82%) were imported from foreign countries due to lack of tissues produced by the country. The country had been paid to import tissues from abroad for approximately several million dollars every year. Those findings were due to shortage of tissue donors, lack of infrastructures of the tissue banks and shortage of tissue bank operators. In 2009, 223,158 tissues were used. However, 138,739 of tissues (62%) were produced in the country, and 84,419 (38%) tissues were imported from abroad. In 2003, Korea started the implementation of a national training program for tissue bank operators and the establishment of a National Training Centre(NTC) for tissue bank operators. Through past 7 years educational efforts a basic core of trained physicians and tissue bank operators has been established providing a professional training of tissue bank operators. These individuals and their respective banks have provided an increasing number of high quality grafts to the communities they serve at a cost far less than if they were acquired from abroad. The tissue bank operators trained so far in the NTC were 100s. These figures indicate the quality improvements of tissue banks and lots of cost saving of the country. Within 5 years, NTC will train another 100s tissue bank operators.

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## O-11

### OPTIMIZING THE PROCESS OF TISSUE BANKING IN A BLOOD BANK NETWORK

SAEZ, M., RODRÍGUEZ, L., GENIS, X., GRIFOLS, R., MASSUET, LL., PROFITOS, J., CASTELLA, D., CALLAO, V., MOYA, F., PANADES, M., SALINAS, R., BOSCH, A., NAVARRO, A. *Banc de Sang i Teixits. Spain.*

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#### Abstract:

Outcomes Banc de Sang i Teixits de Catalunya (BST) has developed a unique model which takes advantage of blood bank network to provide tissue banking services abroad in Catalonia. This approach is intended to bring tissue banking activities closer to donors, patients and specialists while reducing the structural cost of the services rendered. In our scheme, a central facility processes the tissues recovered by the local teams, stores and distributes them to the hospitals through the blood bank network. A close collaboration between BST staff and hospital specialists plays a central role in our model. The program of tissue services in our blood bank includes:

- Blood bank services assisting living donors for autologous eye drops and platelet rich plasma treatments.
- Specialized multitissue and cornea cadaveric recovery teams.
- Centralized processing and preservation facility of living and cadaveric tissues.

– BST is in charge of tissue availability in hospitals as well as delivery and biovigilance. Methods and materials From January 2006 to December 2010, BST engaged tissue banking activities in 14 hospital blood banks in a regular basis, including the assistance of living donors and patients for eye drops production, fertility preservation and platelet rich plasma processing. Also two cadaveric recovery teams for cornea, musculoskeletal, skin and cardiovascular tissues recovery have been trained and 12 autonomous cornea recovery centres established. Additionally to facilitate tissues availability in the territory, tissues are sent to transfusion services allowing a supply 24 hours 365 days a year. Results In 2006 BST managed 397 tissue donors (180 living and 217 cadaveric). After 5 years of implementing new tissue strategy activity increased 70% obtaining in 2010 a total of 674 donors (411 living and 263 cadaveric). Regarding tissue distribution we have observed an increase of 66 % of corneas transplanted (194 corneas transplanted in 2006 vs 322 in 2010), 61 % in musculoskeletal transplant (1152 tissues in 2006 vs 1858 in 2010) and 18% and 29% of skin and cardiovascular transplants respectively. Regarding living patients we provided in 2006 a total of 3762 eyedropper bottles compared to 6691 in 2010.

#### Conclusions:

Tissue services model in our blood bank has allowed:

- Better and closer care of living tissue donors through BST network services resources.
- Improving tissue quality before processing by our trained recovery teams.
- Overall control from tissue donation to transplantation assuring complete traceability.
- Responding specialist needs by increasing our human tissue portfolio.

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## O-12

### RETROSPECTIVE ANALYSIS OF 26 YEARS OF HOMOGRAFT HEART VALVE BANKING IN CENTRAL SOUTH AFRICA

VAN DEN HEEVER JACOBUS, J.; NEETHLING MORRIS LEONARD W.; SMIT EDWIN F.; BOTES L. *Dept of Cardiothoracic Surgery, University of the Free State, Bloemfontein, South Africa.*

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#### Abstract/Introduction:

The history of using homologous cardiac valves dates back more than 30 years. Through the years emphasis was placed on the optimization of graft retrieval, preservation techniques and clinical application. A cardiac homograft valve bank was established at the Department of Cardiothoracic Surgery, University of the Free State, Bloemfontein in 1982. Methods: A retrospective analysis was performed on all allograft data since 1984. Results: Since the first valve was successfully procured and transplanted in 1984, 2743 aortic and pulmonary homografts were harvested from 1927 donors, of which 1667 [1067 (64%) aortic and 600 (36%) pulmonary] were released for clinical use. Road accidents (36%) and violent deaths (56%) make up the majority of unnatural causes of donor deaths. 1076 (39.23%) of valves were discarded for various reasons, the main reasons being Human Immunodeficiency Virus (32%), Structural abnormalities (22%), Hepatitis B (7.5%), Positive cultures (10.9%) and venereal diseases (8.9%). The mean donor age was 26.34 years with a male predominance of 1459 males versus 468 females. The average ischemic time was 33 hours mainly due to medico-legal autopsies exceeding the desired 24 hour time limit. The valves were disinfected in an antibiotic cocktail of Mefoxin, Piperacillin, Amikacin and Amphotericin B prior to cryopreservation. The surgical procedures utilizing the majority of homografts were aortic valve replacements (42.9%), aortic root replacements (19.3%) and right ventricular-pulmonary artery conduits (33.3%). The bank also supplied 23 other centers with homografts (426 aortic and 301 pulmonary).

#### Conclusion:

The Bloemfontein bank has established itself over the years as a leading cardiac homograft bank in South Africa. However, availability of suitable donors and homografts of optimal quality still remains a major concern, and further research in this field to help alleviate the problem is constantly required.

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**O-13**

**CLEAN ROOMS AND TISSUE BANKING: HOW HAPPY I COULD BE WITH EITHER GMP OR GTP?**

KLYKENS, J.<sup>1</sup>, PIRNAY, J.<sup>2</sup>, VERBEKEN, G.<sup>2</sup>, GIET, O.<sup>3</sup>, BAUDOUX, E.<sup>3</sup>, JASHARI, R.<sup>4</sup>, VANDERKELEN, A.<sup>2</sup>, ECTORS, N.<sup>1</sup>  
1 - University Hospital Leuven, 2 - Queen Astrid Military Hospital, Brussels, 3 - University Hospital Liège, 4 - European Homograft Bank, Brussels. Belgium.

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**Abstract:**

The regulatory framework of tissue banking introduces a number of requirements for monitoring clean rooms for processing tissue or cell grafts. Although a number of requirements were clearly defined, some requirements are open for interpretation. This study aims to contribute to the interpretation of GMP or GTP guidelines for tissue banking. A multi center study was carried out in 4 centers: Cell and tissue banks from University Hospitals Leuven, (Leuven, Belgium), Cell and tissue banks from the Queen Astrid Military Hospital (Brussels, Belgium), Cell and tissue banks from University Hospital Liège (Liège, Belgium), European Homograft Bank (Brussels, Belgium). These centers contain a total of 17 cell and tissue banks. All centers use monitoring programs compliant to ISO 14698 and United States Pharmacopeia 29 (2005). Based on the experience of the participating centers, the results of the monitoring program were evaluated to determine the feasibility of a clean room in tissue banking and the monitoring program. Controlled environments of Grade A in B, Grade A in C and Grade A in D were evaluated. Grade A is obtained with laminar air flow cabinets. Also the microbial efficacy of an incubator in a clean room environment was evaluated. This study indicated that a monitoring program of a clean room at rest in combination with (final) product testing is a feasible approach. Although a limited amount of out of spec situations was recorded, the main contributor were measurements of 2 CFU in a laminar air flow cabinet. Although no statistical significance (p between 0.90 and 0.95) was found compared with a Grade A in B, further evaluation shows a strong indication that a Grade D environment is not the ideal background environment for a Grade A obtained through a laminar airflow cabinet, The microbial contamination of an incubator in a clean room were evaluated using air samples and contact plates. Results indicate a limited microbiological load. A controlled environment is mandatory for tissue and cell processing. A monitoring program based on at rest measurements is feasible for all cell and tissue banks and gives sufficient assurance, in combination with adequate standard operating procedures and (final) product testing, that safe allografts are delivered to the patients. A GMP Grade D environment does not seem to be ideal background for a Grade A environment obtained through a laminar air flow cabinet. Further measurements are required to evaluate the efficacy of a GMP Grade C environment as a background for a Grade A laminar airflow cabinet. The contamination level in incubators is limited in standard operations, although closed containers seem to be necessary to protect products where background environments don't qualify for open processing.

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**O-14**

**BANKING OF CRYOPRESERVED VASCULAR ALLOGRAFTS IN EUROPE: 20 YEARS OF ACTIVITY IN EUROPEAN HOMOGRAFT BANK (EHB) IN BRUSSELS**

JASHARI, R.; VAN HOECK B.; GOFFIN Y.; FAN Y.; ROUSSE N. European Homograft Bank (EHB), International Association. Belgium.

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**Abstract:**

EHB has been banking the Cryopreserved Human Heart Valves and Arteries since 1989 and 1991, respectively. The heart beating (MOD) and the non-heart beating cadaver donors (NHBD) of age 12 to 55 years, fulfilling the donor acceptance criteria, were the donors of arteries. The main procurement centers were in Belgium, France and Switzerland. The surgical preparation and morphological evaluation are performed by the trained surgeons that are assisted by experienced technical

assistants, in the cleanroom class A with B/C background. Incubation in antibiotic cocktail for 20-48 hours and cryopreservation are carried out by technical staff. Storage is carried out in the vapors of liquid nitrogen (LN) at  $\leq -150^{\circ}\text{C}$  and shipment in dry shipper in vapors of LN ( $\leq -150^{\circ}\text{C}$ ) or dry ice ( $-76^{\circ}\text{C}$ ). 3533 arterial allografts are evaluated during last 20 years: 2421 (68.5%) are accepted and cryopreserved whereas 1112 (31.5%) discarded for morphology (46%), contamination (35%), serology (6%), stock surplus (4.5%), histology (3.5%) and surgical damages at procurement (3%). 2297 arterial segments are implanted: 110 ascending and 396 descending thoracic aortas, 256 bifurcations, 138 iliac and 1176 femoral arteries for infected native and prosthetic arteries (80%) or critical limb ischemia without available autologous venous material (15%). In 5% of cases the arteries are used in congenital cardiac surgery. Post-thawing evaluation and implantation information are required from implanting surgeon and are received in almost 100% of cases. However the FU information is received only in about 50% of cases. Not all requests for arterial allografts are fulfilled due to shortage of available allografts. A multicentric FU study might give clarification on the durability of cryopreserved arterial allografts since there are some reports on aneurysm formation on long term.

## ORAL - NOVEMBER 10TH - DONATION I AUDITORI - 09.30-10.30

### O-15

#### DEVELOPING TISSUE DONATION IN NORTHERN GERMANY - ACTUAL STATUS, STRATEGIES AND CHALLENGES-

WULFF, B.; HEINEMANN A.; MONTENERO M.; PUESCHEL K. *Institute of Legal Medicine. Germany.*

#### Abstract:

For a long time post mortem donation of the cornea had been the only possibility to donate in Hamburg, but during the last four years the Hamburg Institute of Legal Medicine started an additional program for the retrieval of musculoskeletal and cardiovascular tissues. New contacts were tied to tissue banks, the University Medical Center's wards, hospitals in the city state of Hamburg and its surrounding, that means in the medical field. But tissue donation is nearly unknown by the inhabitants of our area, so we addressed ourselves to the task of "Spreading the News" in addition to the daily work of guiding the consenting process and improving surgical techniques. We present the different aspects, the actual status and also the problems of our resources, capacities and networking arising from our felt obligation to contribute to the supply of tissue transplants for the patients not only in our region.

### O-16

#### POSTMORTEM BLOOD ASSAYS FOR HIV, HBV AND HCV IN TISSUE DONORS. SYSTEMATIC LITERATURE REVIEW

MIETH, K.; MUÑOZ O.; SOTO C.; NAVAS J.; GONZÁLEZ J. *Fundación Cosme y Damián. Colombia.*

#### Abstract/Introduction:

The sensitivity, specificity and predictive values of the serologic assays for HIV, HBV and HCV in screening of living donors are well defined. The operative characteristics of these serologic tests are not well defined for cadaveric donors. There is a potential risk of disease transmission in association to false negative results. The frequency of discharging donors can be affected by the false positive results.

**Objective:**

Definition of the sensitivity, specificity and predictive values of the serological assays for HIV, HBV and HCV in the evaluation of cadaveric tissue donors. Evaluation of the quality of the blood samples in cadaveric donors and the association to the serological assay operative characteristics. Literature search A systematic review of the published literature in the last 25 years was done. The electronic data base Medline, Embase, Cochrane, LILACS and BIREME were used. Secondary manual search was added. Inclusion criteria Diagnostic test studies that evaluate serological assays for HIV, HBV and HCV in cadaveric tissue donors. Data extraction and analysis Two of the authors, in a standardized and independent way, revised the evidence, selected the papers for inclusion and evaluated the quality by using the QUADAS instrument. The findings were discussed and the final recommendations were produced using a consensus process.

**Results:**

The quality of the evidence is low. Most of the published evidence does not meet the standards to define sensitivity, specificity and predictive values for the serological assays in cadaveric tissue donors. There is a high variability in demographic characteristics, risk factors of the donors, time after dead and time elapsed for the process of the samples. These findings avoid definitive conclusions. The evidence suggests that the risk of having false negative results in these serological assays in cadaveric donors is low. The risk of false positive results is high, it is associated to serological assays for hepatitis B and C and when the time elapsed after dead is longer than 24 hours. More research is needed in this area.

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**O-17**

**TISSUE DONATION IN EMERGENCY MEDICINE DEPARTMENTS-  
THE SCOTTISH EXPERIENCE**

*GALEA, G.; GALEA G.; DONALDSON S. SNBTS Tissues and Cells Directorate. United Kingdom.*

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**Abstract:**

Since 2000 SNBTS Tissue and Cells Directorate (TCD) has been the preferred provider of tissues for Scottish patients. In order to ensure sufficiency, it was necessary to identify the best sources of donors for tissue donation. To this end a study was conducted to assess the number of potential tissue donors across Scotland using ICD code profiles over a 5 year period. The study identified 3 main hospital sites of potential tissue donors - Emergency medicine departments (EMD), Intensive Care Units and Coronary Care units- the former providing the highest potential numbers. Moreover the EMDs in the more densely populated areas were shown to provide the highest yields. Therefore the TCD team has focussed its activities in EMDs in Central Scotland and has worked in collaboration with clinical EMD teams to develop a programme for clinical staff to become Designated Requestors. Since this collaboration started the approach rate to families has increased significantly, the number of authorisations (consents) has increased from 38 to 48%. This level of consenting has fluctuated over the years and the reasons for this are being examined on an ongoing basis. A Potential Donor Audit shows that at least 20 tissue donors and 38 cornea donors are obtained via this route. This is a significant proportion of our tissue donor pool.

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**O-18**

**THE ROLE OF FORENSIC INSTITUTES IN TISSUE DONATION IN GERMANY – THE MUNICH EXAMPLE**

*BRAUN, C.; WULFF B.; GRAW M. Institute for Legal Medicine Munich. Germany.*

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**Abstract:**

In 2010 the existing tissue donation program concerning cornea and heart valves at the Institute of Legal Medicine in Munich was reorganized to better meet the conditions of the new German Tissue Law and to expand the Institute's activity to musculoskeletal tissues in cooperation with the German Institute of Cell and Tissue Replacement (DIZG). Experiences gained at the Institute of Legal Medicine in Hamburg with its 5 year old tissue donation program proved valuable for establishing a similar program in Munich. However, due to differences between the city state of Hamburg and the 'area' state of Bavaria there are many organisational and procedural problems, making it necessary to find unique solutions. Considering the different settings we will present the underlying problems as well as the resulting organisational structure of the Munich tissue donation program to date. Furthermore, an outlook and comparison is given on the part different forensic institutes in Germany can play in tissue donation.

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**O-19**

**Q FEVER IN TISSUE DONORS IN THE NETHERLANDS**

*VAN WIJK J, M.; MAAS E W.; HOGEMA B.; KOOT M.; RENDERS C N.; HERMANS H M.; BOKHORST G A. BISLIFE Foundation. The Netherlands.*

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**Abstract:**

In the Netherlands, large outbreaks of Q fever occurred between 2007 and 2009. After implementation of various measures the outbreak is currently decreasing. Although no *C. burnetii* transmission through tissue transplantation has been described in literature, there is some evidence that suggests this is possible. This study aimed to determine the seroprevalence of *C. burnetii* in Dutch tissue donors and to determine whether *C. burnetii* DNA can be present in tissues used for transplantation (cornea, skin, heart valves, tendons and bone). Methods Starting October 2010, 1000 consecutive Dutch tissue donors, of whom at least one tissue was approved at initial assessment, were tested for previous infection with *C. burnetii* with a commercially available IgG Phase 2 ELISA. During the study period donors with increased occupational hazard, signs suspect for acute Q fever or known previous Q-fever were excluded from donation. Of all donors who tested IgG positive, donated tissues were tested by PCR for the presence of *C. burnetii* DNA. Results Between October 2010 and June 2011, 1018 donors were tested for phase 2 IgG antibodies against *C. burnetii*. Of these donors 50 (4.9%) tested positive. Donated tissues were corneas (N=47), skin (N=9), cardiovascular tissues (N=7) and musculoskeletal tissues (N=8). Some tissues could not be tested by PCR, because there was no permission for research (N=3), corneas (N=4) or heart valves (N=1) were rejected for morphological reasons or because no bone marrow sample was available (N=1). In 39 of the 40 tested corneas (with rim) no *C. burnetii* DNA was detected. In one cornea the result of PCR was indeterminate. In 8 of the 9 tested skin donors, the only cardiovascular tissue donor tested thus far and in 6 of the 7 tested bone marrow samples of musculoskeletal tissue donors no *C. burnetii* DNA was detected. In 1 skin donor and 1 musculoskeletal tissue donor the PCR result was indeterminate.

**Conclusion:**

Almost 5% of the tissue donors in the Netherlands tested positive for anti-Coxiella IgG. In none of the donated tissues thus far tested *C. burnetii* DNA was detected. The results of this study could be used to optimize donor selection criteria.

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**O-20**

**TISSUE DONATION AND ORGAN DONATION: A COMPETITIVE OR A COOPERATIVE SYSTEM - EXPERIENCES AFTER THE TISSUE ACT FROM 2007 IN GERMANY**

*NITSCHKE, F.; MANECKE A.; WILLE D. DGFG - German Society for Tissue Transplantation. Germany.*

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**Abstract:**

The use of tissue transplants such as cornea, heart valves, blood vessels and musculoskeletal tissue is an important component in the treatment of tissue defects. The necessary tissues can be obtained according to standard procedures from organ donors also agreeing to tissue donation. In the German Society for Tissue Transplantation (DGFG) in the north east region (Mecklenburg-Vorpommern) such a donation program has been in place and under continuous development since the early nineties. By cooperation between transplant surgeons in hospitals, German Foundation for Organ Transplantation (DSO) transplant coordinators and local tissue banks, it was possible to obtain Cornea from non heart beating donors 98 % (1893/1925) and musculoskeletal tissues from 21% (402/1925). Additional were 71% (215/304) Cornea and 60% (181/304) musculoskeletal Tissues harvested from organ donors in the years 2004 to 2010. Agreement to donation of heart valves and vessels from non-usable donated hearts and vessels was 33% (101/304). According to the new German Tissue Law, tissue donation and procurement is subject to German Transplantation Law and German Drug Law. Appropriate standards that protect the priority of organ donation, but also enable tissue donation must therefore be established and put into practise.

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**ORAL - NOVEMBER 10TH - CARDIOVASCULAR I**  
**AUDITORI - 15.45-16.25**

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**O-21**

**ANTIBIOTIC DECONTAMINATION OF HEART VALVE ALLOGRAFTS: HISTORICAL TRENDS BEFORE AND AFTER THE IMPLEMENTATION OF A HIGHER TEMPERATURE OF INCUBATION**

*TREMBLAY, J.; PAQUET I.; BÉLIVEAU L.; GERMAIN M. Héma-Québec. Canada.*

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**Abstract/Background:**

We previously reported experimental data showing that antibiotic decontamination of heart valve allografts is inefficient at 4 °C and optimal at 37 °C. We hereby compared the efficacy of our decontamination protocol before and after our tissue bank increased the temperature of incubation of the antibiotic soak. Methods: Hearts are rinsed and put into cold, non-antibiotic containing transport solution. Prior to dissection, 1 ml of the transport solution is placed into a thioglycollate broth, another 1 ml in TSB, both incubated for 14 days at 35 °C and 22 °C, respectively. Two small pieces of dissected tissues are cultured in a similar fashion. Dissected valves and accompanying pieces of residual tissues are soaked for 18 to 26 hours into a solution containing cefoxitin, gentamicin and vancomycin and then rinsed three times with Ringer's lactate. Two pieces of control residual tissues and the filtered final rinse solution are cultured as above. The valves are then put into DMSO containing medium package and a 100 ml aliquot of this medium is filtered and cultured prior to sealing the package. Prior to the end of June 2010, heart valves were processed at 4 °C from procurement until final packaging, including the antibiotic decontamination soak. After that date, the procedure remained unchanged except for the 24 hour antibiotic decontamination step which is now done at 37 °C. In order to qualify for transplantation, a graft must have negative cultures on samples taken post decontamination and no high pathogenicity microbes on any of the cultures.

### Results:

Prior to June 2010, 272 valves were processed and decontaminated at 4 °C, of which 153 (56.3%) had positive cultures on samples taken prior to the decontamination step. Of those 153 valves, 77 had negative cultures after antibiotic decontamination. Since June 2010, 66 valves were decontaminated at 37 °C, of which 20 (30.3%) had positive cultures on samples taken prior to the decontamination step. Of those 20 valves, 17 had negative cultures after antibiotic decontamination. The efficacy of the decontamination procedure at 37 °C is therefore 85.0% (17/20), compared to 50.3% (77/153) at 4 °C ( $p=0.004$ , Fisher exact test). When taking into consideration the presence of high pathogenicity microbes, the proportion of valves that were acceptable for transplantation went from 61.0% (166/272) during the days of soaking at 4 °C, to 80.3% (53/66) after the temperature of incubation was raised to 37 °C ( $p=0.003$ , Chi-square test). The rate of positive cultures pre-decontamination also decreased significantly between both periods ( $p=0.0002$ , Chi-square test).

### Conclusions:

Compared with our historical results of placing heart valves in antibiotics at 4 °C, our new procedure of antibiotic soaking at 37 °C achieves a significantly higher rate of successful decontamination. That, in combination with a secular decrease in our rates of pre-processing contamination, has led to a major increase in the proportion of tissues deemed suitable for transplantation.

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## O-22

### COMPARISON OF THE EFFECTS OF ISCHAEMIC TIMES OF HARVESTED HOMOGRAFTS ON HISTOLOGICAL APPEARANCE AND TISSUE STRENGTH

VAN DEN HEEVER JACOBUS, J.; BESTER D.; SMIT EDWIN F.; BOTES L. *Dept of Cardiothoracic Surgery, University of the Free State, Bloemfontein, South Africa.*

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### Abstract/ Introduction:

Homografts in cardiac surgery are well established but availability remains the main limiting factor in their clinical application. Homografts are procured from beating heart donors and cadavers with a limited ischaemic time of 12-24 h post mortem. However, this time limitation has not been established scientifically. Several publications suggest that it would be acceptable to extend the harvesting times. Homograft availability is an international problem and the purpose of the study is to extend the harvesting time beyond 24 h, benefiting all cadaver donor based programs.

### Methods:

The study focused on three groups: (a) Group A (n=5) consisted of homograft valves subjected to < 6 h ischaemic time and stored at 4 °C prior to cryopreservation; (b) Group B (n=15), subjected to 24 h, 48 h and 72 h cold (4 °C) ischemic times prior to processing and cryopreservation; (c) Group C (n=15), subjected to 6 h room temperature warm (23 °C) ischaemic time, followed by 18 h, 42 h and 66 h cold (4 °C) ischaemia, after which the valves were processed and cryopreserved. Tissue strength was determined by thermal denaturation temperature (Td) and tensile strength. Tissue morphology was assessed by Scanning Electron Microscopy (SEM) and Haematoxylin and Eosin (H&E) stain.

### Results:

No statistically significant difference ( $p>0.05$ ) in tensile strength could be demonstrated between Group A, B and C with no differences between the three ischaemic time intervals. The results were confirmed by Td analysis. Tissue strength did not decrease as a result of prolonged ischaemic times or elevated temperatures. Autolysis were only observed in the 48 h (40%) and 72 h (100%) tissue of Group C but was not sufficient to affect tissue strength. The reduction of endothelial cells over time in both Group B and Group C did not influence tissue strength up to 72 h.

### Conclusion:

Based on scientific evidence regarding tissue strength it seems acceptable to extend harvesting time to 48 h. However, animal studies need to be performed to substantiate these results, as the in vivo graft host interactions might produce calcification and/or host rejection results that might still affect the safe clinical use of such tissue.

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**O-23**

**PULMONARY HOMOGRAFTS FOR RIGHT VENTRICULAR OUTFLOW TRACT RECONSTRUCTION DURING ROSS PROCEDURE: NINETEEN YEARS RESULTS**

*JUTHIER, F.; JASHARI R.; ROUSSE N.; VAN HOECK B.; BANFI C.; VINCENELLI A.; PRAT A. CHRU LILLE. France.*

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**Abstract/Background:**

Replacement of the aortic valve or aortic root with a pulmonary autograft (Ross procedure) is widely used for aortic valve disease in growing patients and young adults. In most cases, a cryopreserved pulmonary homograft is used for reconstruction of the right ventricular outflow tract (RVOT). Main drawbacks of this procedure are progressive dilatation of the pulmonary autograft and RVOT failure, the predominant indication for reoperation of the pulmonary conduit being stenosis. The aim of this study was to evaluate the long-term hemodynamic behaviour of pulmonary homografts in pulmonary position after Ross procedure.

**Methods:**

Three hundred seventy-one patients had a Ross procedure in our institution between March 1992 and May 2011. Among them, 307 patients received a pulmonary homograft (supplied by the European Homograft Bank) in pulmonary position and they represent the study population. Mean age was  $28 \pm 10.7$  years. Mean homograft diameter was  $25.8 \pm 2.2$  mm. A comprehensive echocardiography was performed at discharge, at 6 months and then on an annual basis. Pulmonary stenosis was defined as a mean transvalvular gradient of more than 20 mm Hg across the homograft. Median follow-up was 5.5 years (range, 7 days-18.9 years).

**Results:**

Perioperative mortality was 2.6% (8 patients). Late mortality was 2.6% (8 patients). During follow-up, 10 (3.3%) patients had reoperation on the RVOT with a mean time-interval of  $9.0 \pm 4.0$  years. Three of them received percutaneous implantation of transcatheter pulmonary valve prosthesis,  $12.2 \pm 1.7$  years after the Ross procedure. Causes of reoperation were homograft failure in 5 (1.6%) cases and pulmonary endocarditis in 5 (1.6%) cases. 5 (1.6%) other patients developed a pulmonary endocarditis medically treated. Mean transpulmonary gradients were respectively of  $4.1 \pm 2.5$  mm Hg;  $8.6 \pm 6.3$  mm Hg;  $11.9 \pm 10.9$  mm Hg and  $10.8 \pm 6.9$  mm Hg post-operatively and at 5, 10 and 15 years. Freedom from pulmonary stenosis was 99.0% (IC 95%; 98.3-99.7%); 97.5% (IC 95%; 96.2- 98.8%) and 88.6% (IC 95%; 88.2- 92.4%) at 5 and 10 and 15 years.

**Conclusion:**

Pulmonary homografts represent a safe valvular substitute for RVOT reconstruction during Ross procedure in our institution. This may be due to our policy of systematic oversizing of the allograft and the liberal use of anti-inflammatory drugs in the post-operative period. The gradual transvalvular gradient increase during follow-up however requires continued echocardiographic monitoring. Patients who meet criteria for isolated RVOT replacement can be successfully treated with catheter-based pulmonary valve implantation.

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**O-24**

**DECONTAMINATION OF CRYOPRESERVED CARDIO-VASCULAR ALLOGRAFTS:  
DETECTION AND ERADICATION OF THE SLOW GROWING SKIN GERMS**

*JASHARI, R.; FAN Y.; VAN HOECK B.; LE MERCIER N.; DE GELAS S. European Homograft Bank (EHB), International Association. Belgium.*

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**Abstract/Introduction:**

We have previously shown that up to 30% of raw material, retrieved for heart valve and vascular allograft preparation is initially contaminated. Although the majority of the allografts are sterilized by decontamination procedure, about 8-10% of the tissues remain germ- positive in the final step of the processing, decreasing importantly the yield of the allografts at the end of the processing. About 50% of contaminant germs are the slow growing skin anaerobes such as Propionibacterium. The aim of this study was to examine whether those contaminated tissues remain germ- positive after a long term period of cryopreservation.

**Methods:**

Total of 28 allografts (7 arteries and 21 valves), which were discarded for contamination with Propionibacterium after being incubated in the antibiotic cocktail, were cryopreserved and stored in the vapor phase of liquid nitrogen for 33 to 81 months (median 61 months). They were thawed and diluted according to the standard procedure of EHB. The tissue samples are cultured for aerobic and anaerobic germs (in the thyoglycolate and rezaurine medium) at 30-35°C and for the fungi and yeasts (in the tryptic soy bouillon) at 20-25°C following the European pharmacopeia. The results are presented at 7, 14 and 21 of incubation. Results. 32.1% of the allografts tested positive at 7 days and 64.3% at 14 day. No new samples tested positive at day 21. These preliminary data indicate that Propionibacterium is capable of resisting to a very low temperature of below -187°C as long as 81 months and remaining its "slow-grow" nature.

**Conclusion:**

Minimum 14 days' culture is essential for the accurate detection of this germ. Extension of incubation to 21 day is not necessary for its detection. Success in decontaminating this common bacterium is important for the safety of cardio-vascular allograft recipients.

## ORAL - NOVEMBER 10TH - CARDIOVASCULAR II - IMPROVING SAFETY AUDITORI - 17.30-18.20

### O-25

#### OPTIMIZATION OF CARDIOVASCULAR TISSUE DECONTAMINATION AND RINSING WITH BASE.128 AND BASE

TERZI, A.<sup>1</sup>, BUZZI, M.<sup>1</sup>, GUARINO, A.<sup>2</sup>, DAINESE, L.<sup>2</sup>, VASURI, F.<sup>3</sup>, TOTHOVA, J.<sup>4</sup>, GATTO, C.<sup>5</sup>

1 - Banca Dei Tessuti Cardiovascolari Regione Emilia Romagna, 2 - Lombardia Cardiovascular Tissue Bank, Milano, Italy, 3 - Pathology Unit, "f. Addarii" Institute Of Oncology And Pathology, S. Orsola-malpighi Hospital, Bologna, Italy, 4 - R&d Al.chi.mi.a Srl, Ponte San Nicolò (pd), Italy R&d Al.chi.mi.a Srl, Ponte San Nicolò (pd), Italy, 5 - R&d Al.chi.mi.a Srl, Ponte San Nicolò (pd), Italy R&d Al.chi.mi.a Srl, Ponte San Nicolò (pd), Italy.

#### Abstract:

**Purpose** To define the optimal time and temperature conditions for decontamination and rinsing of cardiovascular tissues using the BASE.128 and BASE medical devices in order to decontaminate efficiently the tissue and minimize the presence of antibiotic residues. **Methods** Ten cardiovascular tissues from heart beating and non heart beating donors, including valves and blood vessels, were retrieved by two different cardiovascular tissue banks. After transport to the bank, tissues were divided in three equivalent segments, which were decontaminated with BASE.128 (AL.CHI.MI.A., Italy) at 4°C for 24h, 22°C for 8h and 37°C for 6h, respectively. All tissues were rinsed with BASE (AL.CHI.MI.A., Italy) at 4°C overnight before freezing at -80°C. Before and after tissue processing, bacteriological tests were performed on tissues and transport and rinsing liquids using BACT-ALERT and Thioglycollate and TSB mediums. After thawing, the presence of antibiotic residues was evaluated on tissue homogenates by disk diffusion method, and sterility test performed according to the European Pharmacopeia after removing potential interfering antibiotics with a specific resin mixture. Each tissue was sampled before and after processing and fixed in formalin for subsequent histological examination (Hematoxylin-Eosin and Weigert's stain). **Results** Almost all investigated tissues were initially contaminated with one or more bacterial spp. (Staphylococcus spp., Streptococcus spp., Propionibacterium spp.). Contamination was more often detected in the transport media rather than tissue. Post-processing microbiological tests of all investigated tissues and liquids were negative in all investigated time and temperature conditions. Despite the overnight rinsing, disk diffusion analysis showed that some antibiotic residues were present, mainly depending on the decontamination condition. The highest residue content was found after decontamination at 4° C for 24h. Residues were lower after decontamination at 22°C for 8h and almost absent after decontamination at 37°C for 6h. Inhibition zones were detected in Staphylococcus aureus seeded plates, indicating the possible presence of Vancomycin residues. Significantly smaller inhibition zones were found on Candida albicans seeded plates, indicating the presences of traces of antimycotic. No inhibition zones were found in Pseudomonas aeruginosa seeded plates, indicating the absence of any antibiotic active against Gram negative – bacteria (Gentamicin and Cefotaxime). Histological analysis showed no differences among tissues in terms of integrity. **Conclusions** Tissue decontamination with BASE.128 allowed to eliminate efficiently all contaminants from human cardiovascular tissues in all investigated conditions, without affecting the tissue integrity. Decontamination at 37°C for 6h followed by an overnight rinsing allowed to eliminate antibiotic residues, thus minimizing both antibiotic transmission to recipient and interference with microbiology tests.

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**O-26**

**IMPACT OF ANTIBIOTIC RESIDUES ON MICROBIOLOGICAL ANALYSIS: IMPLICATION OF TISSUE BANKING**

GATTO, C.; GIURGOLA L.; BECCARO M.; LIPARTITI M.; D'AMATO TOTHOVA J. R&D Alchimia SRL. Italy.

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**Abstract:**

Decontamination of tissue allografts in antibiotic cocktails can lead to the antibiotic carry-over effect, which in turn can result in the false negative microbiological analysis. Purpose Investigate impact of antibiotic residues induced by tissue decontamination on microbiological analysis. Methods Cryopreserved human cardiovascular tissues and skin or corneas were retrieved, processed and cryopreserved by four different tissue banks. Cardiovascular tissue and skin were decontaminated at 4°C for 24h/72h (cardiovascular) or at 22°C for 90 min. (skin) either with bank solution or BASE.128 (AL.Chi.MI.A.SRL) and cryopreserved in RPMI 1640 with addition of 10% DMSO. Corneas were processed and stored in organ culture conditions. Microbiological analysis on tissue and processing liquids were performed according to bank standard procedures using BACT-ALERT, BACTEC and Thioglycollate/ TSB mediums. After thawing, the presence of antibiotic residues was evaluated on tissue homogenates by disk diffusion and sterility test performed according to the European Pharmacopeia after removing potential interfering antibiotics with a specific resin mixture. Contaminants were genetically identified by ribosomal RNA sequencing.

**Results:**

Bacteriological analysis reports on cardiovascular tissues, before processing, showed tissue contamination by one or more microorganisms. Except for one cardiovascular tissue, the bacteriological test reports after the decontamination showed negative results for all tissues and liquids. Bacteriological analysis reports on skin samples showed negative results before and after processing. Microbiological analysis performed on corneal storage liquids reported negative results. Disk diffusion test of thawed cardiovascular tissue and skin or cornea homogenates showed inhibition zones mainly on *S. Aureus* and on *P. Aeruginosa* seeded plates, indicating the presence of antibiotic residues active mainly against gram-positive rather than gram-negative bacteria. Inhibition zones were significantly reduced (*S. Aureus* plates) or completely eliminated (*P. Aeruginosa* plates) after the treatment of tissues and liquids with a resin mixture for elimination of antibiotic residues. Sterility test on the tissue and liquid samples after elimination of the residual antibiotics, showed significant positive (turbid) results for cardiovascular tissues treated with the bank solution. Turbidity was not detected on cardiovascular tissues decontaminated with BASE.128RED. Significant number of skin samples was found positive in sterility test, independently on used antibiotic cocktails, indicating inappropriate decontamination conditions. Sterility test on corneal storage liquids after removing antibiotic residues, showed significant number of positive (turbid) samples not always detected on tissue sample. Ribosomal DNA sequencing identification demonstrated the presence of clinical and environmental contaminants.

**Conclusions:**

The presence of the residual antibiotics in tissue and liquid samples submitted to bacteriological analysis can result in significant number of false negative results. Validation of decontamination processes and of microbiological methods are necessary in order to guarantee the safety of tissue allografts.

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**O-27**

**NEW INSIGHTS OF ANTIBIOTIC SOLUTION: ANTIBACTERIAL SUSCEPTIBILITY IN THE 4 DEGREE MEDIUM CONDITION**

*MOTOMURA, N.; SAITO A.; TAMURA K.; NOGUCHI N.; HATTORI O.; ODA N.; SEKI M. University of Tokyo. Japan.*

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**Abstract/Background:**

In many tissue banks, disinfection of retrieved tissues by antibiotics cocktail has been done under the 4 degree condition in a refrigerator. Antibiotics have been designed to use at the body temperature per se, and some combination of the cocktail may not exert the best performance as an antibacterial agent. We examined the susceptibility of several commonly used antibiotics at the 4 degree condition in order to find out the better combination under the refrigerator atmosphere.

**Methods:**

Number of survival cells after treatment of antibiotic agents at 4 degree for 24 hours was compared. Following bacterias were used; *P. aeruginosa*, MRSA, *En. faecalis*, *Propionebacterium acnes*, *E. coli*, and *S. epidermidis*. Used antibiotic cocktails (concentration, microgram/ml) were as follows. Solution A

**We use this solution at present:**

cefmetazole (240), lincomycin (120), vancomycin (50), polymixin B (1000 u/ml). Solution B: gentamicin (50), sitafloxacin (50), vancomycin (50), polymixin B (1000 u/ml). Solution C: gentamicin (50), sitafloxacin (50), clindamycin (100), polymixin B (1000 u/ml). Solution D: gentamicin (50), sitafloxacin (50), vancomycin (50), clindamycin (100), polymixin B (1000 u/ml).

**Results:**

In *P. aeruginosa* and *E. coli*, all the 4 solutions showed complete remission. In *P. acnes* and *S. epidermidis*, Solution A did not show any effectiveness. In MRSA and *E. faecalis*, Solution B, C, and D worked well when the amount of bacterias was less.

**Conclusion:**

Cefmetazole which is used in our Tissue Bank at present did not show better performance than other new generations under the 4 degree condition. This result may encourage revising the antibiotic cocktails even in other tissue banks.

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**O-28**

**QUANTIFICATION OF RESIDUAL ANTIBIOTICS IN CARDIOVASCULAR TISSUE PREPARATIONS**

*BROECKER, S.; HARTWIG S.; MEYER R. Charité – University Hospital Berlin. Germany.*

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**Abstract/Keywords:**

Cardiovascular tissue preparations, liquid chromatography mass spectrometry (LC-MS), antibiotics, residual quantification  
Aims: In the preparation of cardiovascular tissues for transplantation purposes an antibiotics step with a mixture of different antibiotics is included. In order to determine the degree of possible effects of the antibiotics in the recipient organism by transplantation, the presence and concentrations of the antibiotic residues in the cardiovascular tissue preparations were examined. Furthermore the systemic effect on the organism by the detected antibiotics was estimated.

### Methods:

The investigated samples (vascular walls or myocardial tissue) were collected in the Deutsches Herzzentrum Berlin from post mortem cases during autopsy. The preparation of the samples was performed in the same way the homografts were treated. They were incubated in 100 ml of a "medium 199" and 2 ml of an antibiotic solution consisting of amikacin, ciprofloxacin, flucytosine, metronidazole and vancomycin at 5 ° C for 24 hours. After that, they were washed three times with 0.9% NaCl and blot dried. An aliquot of approximately 20 mg vascular wall or myocardial tissue were accurately weighed and 500 µl acetonitrile + 1 % formic acid were added. Then the sample was homogenized using a vibrating ball mill. After centrifugation, 400 µl of the supernatant were removed and evaporated to dryness. The residue was dissolved in 100 µl water + 1 % formic acid. 1 µl was directly injected for analysis.

### Results:

The determination of concentrations was performed by external calibration and by standard addition. The results were in good agreement. The limit of detections (LOD) were: amikacin (10 µg/g), ciprofloxacin (0.1 µg/g), flucytosine (0.1 µg/g), metronidazole (0.1 µg/g) and vancomycin (20 µg/g). The ten investigated samples from five homografts showed that the residues lead to concentrations in the recipient organism in concentrations that are three to four orders of magnitude below the therapeutic level of the individual antibiotics.

### Conclusions:

The detected residual amounts of antibiotics have no specific risk for the transplantation recipients. Allergic reactions cannot be excluded. For this reason, the user has to be informed about the composition of the antibiotic mixture and the risk of possible allergic reactions. According to this study, a change of the composition or method of application of the antibiotic mixture in the procedure of cardiovascular tissue preparation is not necessary.

### References:

[1] M. Schulz, A. Schmoldt, Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics, Pharmazie 58 (2003), 447-474.

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## O-29

### USE OF ANTIBIOTICS/ANTIMYCOTICS IN THE NEW TISSUE PRESERVATION SOLUTION TIPROTEC®

RAUEN, U.; EBNER A.; DEUSSEN A. *Institut für Physiologische Chemie, Universitätsklinikum Essen. Germany.*

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### Abstract:

We have previously developed the tissue preservation solution TiProtec®, which has proved to provide largely superior protection of porcine aortic segments, rat mesenteric arteries, human internal mammary arteries and human saphenous veins than diverse current cold storage solutions, suggesting its use in the tissue banking of vascular grafts. However, in many countries antibiotic/antimycotic treatment of allografts is required by the regulatory authorities. Therefore, we here assessed whether antibiotics/antimycotics can safely be added to TiProtec® without compromising its protective potential. To this end, an antibiotic/antimycotic cocktail widely used in tissue banking in Germany, consisting of gentamicin (40 µg/mL), piperacillin (1 mg/mL), flucloxacillin (1 mg/mL), metronidazole (200 µg/mL) and amphotericin B (100 µg/mL), was added to TiProtec® solution and assessed in cold storage of cultured porcine aortic endothelial cells and of the A. saphena of the rat. During 7 days of cold storage, TiProtec® solution without the antibiotic/antimycotic cocktail strongly protected the endothelial cells against cold-induced injury (LDH release 8 ± 4%) compared to cold storage in Krebs-Henseleit buffer or HTK solution (LDH release > 70%). The addition of the antibiotic/antimycotic cocktail, however, eliminated this protective effect (LDH release 87 ± 6%). Further experiments revealed that the toxicity of the cocktail was due to its antimycotic component amphotericin B, while the four antibiotics did not have any effect on endothelial cell survival. The antimycotic's toxicity proved to be due to both toxicity of the amphotericin B itself and toxicity of the additives in the galenic preparation

used. Reduction of the antimycotic's concentration to 10 µg/mL did not avoid the toxicity. Functional studies in the rat A. saphena confirmed these results: A. saphena cold-stored in TiProtec® for 7 days showed norepinephrine-induced vessel tone development and endothelium-dependent relaxation equivalent to fresh controls. When the antibiotic/antimycotic cocktail was added during cold storage no vasoreactivity at all could be detected, whereas omission of amphotericin B from the cocktail gave almost identical results to TiProtec® without antibiotic/antimycotics. Taken together, these results show that – with regard to cell/tissue protection – the antibiotics gentamicin, piperacillin, flucloxacillin and metronidazole can safely be added to TiProtec® solution whereas the antimycotic amphotericin B should be avoided. Testing of alternative antimycotics and of other currently used antibiotic cocktails is under way and the preservation of the antibiotic/antimycotic activities in TiProtec® solution will be assessed thereafter.

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## O-29BIS

### **ASSESSMENT OF BIOBURDEN ON SAMPLES OF HUMAN AND ANIMAL TISSUES: PART 1- RESULTS OF METHOD DEVELOPMENT AND VALIDATION STUDIES**

OSBORNE, J.; KOWALSKI, J.; MOSLEY, G.; MERRITT, K., MTF. US.

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#### **Abstract:**

Recovered human and animal tissues are used extensively in surgery for wound repair and reconstruction of various anatomical sites. In preparation for the validation of chemical disinfection and radiation sterilization processes, studies were performed on the development of bioburden recovery methods and validation of recovery efficiency factors for human bone and soft tissue and also for porcine dermis. The "inoculated product" approach was used in combination with four repetitive extractions. Although each of the three recovery methods tested appeared to reach "exhaustion", less than 10% recovery when compared to the first rinse, sonication plus mechanical shaking gave the highest recovery efficiency in most cases when compared to the inoculation control. The highest recovery efficiency was generally observed with Fluid D as the rinse medium. The results demonstrated the importance of performing bioburden method development and validation studies. The method validation strategy described here, using a combination of tissue inoculation and repetitive treatment, showed the superiority of sonication plus mechanical shaking using Fluid D as the rinse medium. In addition, the use of only the exhaustive extraction approach could have resulted in the development of a methodology that consistently underestimated the bioburden present on/in recovered tissue.

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## **ORAL - NOVEMBER 10TH - MUSCULOSKELETAL I ROOM 1 - 16.00-16.30**

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## O-30

### **THE INACTIVATION EFFECT OF STANDARD AND FRACTIONATED ELECTRON BEAM IRRADIATION ON ENVELOPED AND NON-ENVELOPED VIRUSES**

SCHMIDT, T.; GOHS U.; HOBURG A.; SCHUMANN W.; SCHEFFLER S.; NITSCHKE A.; PRUSS A. Charité University Medicine Berlin, Julius Wolff Institut, Germany.

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#### **Abstract/Introduction:**

As a basic safety measure, donated tendon grafts, to be used in anterior cruciate ligament replacement, currently undergo serological screening for markers of virus infections to avoid the transmission of pathogens. However, current terminal sterilization methods which inactivate all pathogens also impair the biomechanical properties of the grafts. Gamma

irradiation shows dose dependent detrimental effects. As an additional safety tool we investigated Electron beam (Ebeam) radiation in vitro and found favourable biomechanical results which could be further improved if the required absorbed dose of 34 kGy was applied in 10 fractions of 3.4 kGy. In this study, we aimed to investigate the virus inactivation kinetics of standard and fractionated Ebeam irradiation to evaluate its impact on the virus safety of transplants.

**Methods:**

We investigated the following viruses: the enveloped human immunodeficiency type 2 (HIV-2) and pseudorabies virus (PRV, a model for human herpesviruses) and the non-enveloped hepatitis A (HAV) and porcine parvovirus (PPV, a model for parvovirus B19). All virus stocks were prepared from the supernatant of cultured infected cells. The Ebeam treatment was performed in eppendorf plastic vials in CO<sub>2</sub> gas atmosphere at  $-70 \pm 5^\circ\text{C}$ . For evaluation of standard Ebeam (SEbeam) treatment, virus stocks were irradiated with 3.4; 6.8; 13.6-34 kGy within one step. In the case of fractionated Ebeam (FEbeam) process, virus stocks were irradiated with 1 x 3.4 kGy; 2 x 3.4 kGy; 3 x 3.4 kGy; -10 x 3.4kGy. The log (10) reduction was measured by cytopathogenic effects after virus titration (TCID<sub>50</sub>/ml) and the D10 values (kGy) were calculated for the different viruses.

**Results:**

We determined the following D10 values: HIV-2: SEbeam  $9.0 \pm 0.5$  kGy; FEbeam:  $8.0 \pm 0.5$  kGy, PRV: SEbeam:  $5.6 \pm 0.4$  kGy; FEbeam:  $5.8 \pm 0.4$  kGy, HAV: SEbeam:  $6.5 \pm 0.2$  kGy; FEbeam:  $5.9 \pm 0.2$  kGy, PPV: SEbeam  $8.6 \pm 0.6$  kGy; FEbeam:  $7.5 \pm 0.6$  kGy. A dose of at least 36.0 kGy at  $-70^\circ\text{C}$  for SEbeam treatment and a dose of 32.0 kGy for FEbeam treatment were necessary to achieve a sufficient reduction of 4 log steps in the case of HIV-2, which was the most resistant of all viruses investigated in this study.

**Discussion:**

For both Ebeam processes comparable virus inactivation kinetics and D10 values were determined. The superior biomechanical in vitro results using the fractionated Ebeam process compared to standard Ebeam or gamma treatment suggest that this novel procedure is a safe and effective option for a terminal sterilization method which achieves full pathogen inactivation without impairing the biomechanical properties of the grafts. However, the biological effects must be confirmed in an animal model before it can be used for human graft sterilization.

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**O-31**

**EFFECT OF GAMMA IRRADIATION ON MECHANICAL PROPERTIES OF HUMAN CORTICAL BONE: INFLUENCE OF DIFFERENT PROCESSING METHODS**

JASTRZEBSKA, A.; GRAZKA E.; GUT G.; UHRYNOWSKA-TYSZKIEWICZ I.; MAROWSKA J.; KAMINSKI A. *National Centre for Tissue and Cell Banking, Warsaw, Poland.*

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**Abstract/Objectives:**

Gamma irradiation of cortical bone grafts utilized in reconstructive medicine is used to prevent infective disease transmission. However, gamma irradiation usually performed at low dose rate in combination with a high exposure period, has been supposed to impair mechanical properties of bone tissue, essential for bone graft clinical functionality. To address this issue, we evaluated mechanical effects in human cortical bone grafts, induced by irradiation with two doses of gamma rays (25 and 35 kGy) in different processing conditions\*.

**Material and methods:**

Left and right femoral shafts procured from six male cadaveric donors (mean age  $51 \pm 3$  yrs) were cleaned of soft tissues, transversely cut into slices of 10 mm height from which bone marrow was removed, and assigned to eight experimental groups of twelve specimens each, according to different processing methods (defatted or non-defatted), gamma irradiation dose (25 or 35 kGy, Co-60 Gamma Irradiator ISOGAMMA-LLCo, Hungary), as well as temperature conditions of irradiation (ambient temperature or dry ice). Specimens from control groups (defatted or non-defatted) were not irradiated. Prior to mechanical testing for compression, cross-sectional area of each bone slice was measured using computer tomography

(Toshiba Aquilion CT scan system TSX-101A). The compressive strength of bone rings was measured in the wet state with the speed of compression of 1mm/s, using Material Testing Machine Z250 (Zwick/Roell, Germany), and mechanical properties calculated from the load-deformation curve. Results We did not observe any statistically significant differences in strength at fracture and Young's modulus of gamma-irradiated cortical bone rings as compared to control ones (non-irradiated), irrespective of gamma dose applied (25 or 35 kGy), temperature of irradiation (ambient temperature or dry ice), as well as processing procedure (defatting or non-defatting) prior to irradiation.

**Conclusion:**

Gamma irradiation of compact bone graft at the doses applied seems not to impair their mechanical competence under compression within elastic strain region, irrespective of irradiation temperature and processing method. \* The study was partially supported by International programme „Safety and Optimisation of Radiation Sterilization in Tissue Banking: Studies on Functional Properties of Irradiated Tissue Grafts (CRP E3.10.06), IAEA Research Contract No.16114/RO.

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**O-32**

**EFFECT OF ELECTRON BEAM IRRADIATION ON MECHANICAL PROPERTIES OF HUMAN CORTICAL BONE: INFLUENCE OF DIFFERENT PROCESSING METHODS**

GRAZKA, E.; JASTRZEBSKA A.; GUT G.; UHRYNOWSKA-TYSZKIEWICZ I.; MAROWSKA J.; KAMINSKI A.  
National Centre for Tissue and Cell Banking, Warsaw, Poland.

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**Abstract/Objectives:**

Electron beam (EB) irradiation of bone grafts is an alternative method of sterilization to gamma irradiation, with advantages including high dose rate and short exposure time on the expense of the approximately ten-times lower penetration into materials, requiring, in some cases, two-sided irradiation. Due to its characteristics, EB sterilization might result in low radiation damage of biological tissues, and, in consequence, low impairment of mechanical function. To adress this issue, we evaluated mechanical effects in human cortical bone grafts, induced by irradiation with two doses of EB irradiation (25 and 35 kGy) in different processing conditions\*.

**Material and methods:**

Left and right femoral shafts procured from six male cadaveric donors (mean age 51 +/-3 yrs) were cleaned of soft tissues, transversely cut into slices of 10 mm height from which bone marrow was removed, and assigned to eight experimental groups of twelve specimens each, according to different processing methods (defatted or non-defatted), EB irradiation dose (25 or 35 kGy, Electron Beam Accelerator LAE-10, 10,2 MeV, Institute of Nuclear Chemistry and Technology, Warsaw), as well as temperature conditions of irradiation (ambient temperature or dry ice). Specimens from control groups (defatted or non-defatted) were not irradiated. Prior to mechanical testing for compression, cross-sectional area of each bone slice was measured using computer tomography (Toshiba Aquilion CT scan system TSX-101A). The compressive strength of bone rings was measured in the wet state with the speed of compression of 1mm/s, using Material Testing Machine Z250 (Zwick/Roell, Germany), and mechanical properties calculated from the load-deformation curve.

**Results:**

We did not find any statistically significant differences in strength at fracture and Young's modulus of EB-irradiated cortical bone rings as compared to control ones (non-irradiated), irrespective of EB dose applied (25 or 35 kGy), temperature of irradiation (ambient temperature or dry ice), as well as processing procedure (defatting or non-defatting) prior to irradiation.

**Conclusion:**

Electron beam sterilization of compact bone graft at the doses applied seems not to impair their mechanical competence under compression within elastic strain region, irrespective of irradiation temperature and processing method. \* The study was partially supported by International programme „Safety and Optimisation of Radiation Sterilization in Tissue Banking: Studies on Functional Properties of Irradiated Tissue Grafts (CRP E3.10.06), IAEA Research Contract No.16114/RO.

## ORAL - NOVEMBER 10TH - MUSCULOSKELETAL II ROOM 1 - 17.00-18-40

### O-33

#### **BENEFITS OF USING A DECONTAMINATION METHOD IN BONE PROCESSING**

FARIÑAS, O.<sup>1</sup>, VILARRODONA, A.<sup>1</sup>, VITO, S.<sup>1</sup>, TABERA, J.<sup>1</sup>, HINOJOSA, M. A.<sup>1</sup>, SAVIO, A.<sup>1</sup>, SEGUR, J. M.<sup>2</sup>, SUSO, S.<sup>2</sup>, TRÍAS, E.<sup>1</sup>

1 - Transplant Services Foundation - Hospital Clínic. Barcelona. Spain., 2 - Hospital Clínic. Barcelona. Spain.

#### **Abstract/Introduction:**

The main objective of a tissue establishment is to minimize the most disease transmission through allografts transplantation. This aim is achieved establishing a strict donor selection, a protocolized recovery system and a controlled tissue processing. In addition to bone processing into monitored cleanrooms areas, the use of a decontamination method on the grafts decreases significantly the risk of infectious disease transmission. It has been published that bacteria and fungi are mainly located inside the musculoskeletal tissue grafts. The function of some decontamination methods is to remove the blood, fat and bone marrow remains from inside the grafts affecting also the presence of microorganisms. Aim To evaluate the influence of using a decontamination method in musculoskeletal tissue processing from 2007 to 2010, comparing the contamination rates with the results obtained without using it from 2004 to 2006.

#### **Materials and Methods:**

This retrospective study evaluates the musculoskeletal tissue processings performed in our tissue establishment from 2004 to 2010. Two different time periods have been differentiated: 2004 to 2006 when no decontamination method was used, and 2007 to 2010 when we started using it. The analysis focused on the number of grafts obtained after processing, the positive cultures obtained, and the rate of grafts discarded because of microbiological contamination. The results obtained were compared between the two different periods with and without using decontamination method.

#### **Results:**

The number of musculoskeletal tissue donors have been increasing from last seven years (31 to 145 donors per year). In a parallel way the number of allografts obtained after processing have increased too (541 grafts in 2004, 4413 grafts in 2009). During the period from 2004 to 2006 the musculoskeletal allografts were only subjected to a superficial clean process with sterile water. The rate of positive cultures after processing ranged 10.53-16.46%. The average rate of allografts discarded due to positive microbiological culture ranged 7.21-8.13%. In 2007 we started using a decontamination method that consisted in a mix of mechanical and chemical procedures to eliminate the remains of blood, fat and bone marrow from inside the grafts. The rate of positive cultures after processing ranged 0.45-3.16%. The average rate of allografts discarded due to positive microbiological culture ranged 0.23-1.58%.

#### **Conclusions:**

The use of a decontamination method in musculoskeletal tissue processing (independently from a terminal sterilization method) decreases the risk of infectious disease transmission. Other benefit obtained is to increase the availability of allograft to transplant due to its effect on the number of tissues discarded because of a positive microbiological culture.

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## O-34

### EVIDENCE BASED BONE AND TISSUE BANK PROCESSES STANDARDIZATION

NAVAS, J.; MIETH K.; SOTO C.; GONZÁLEZ J. *Fundación Cosme y Damián. Colombia.*

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#### Abstract/Introduction:

Standardization is essential to improve quality in every step of the bone and tissue chain of action, from donor selection to graft distribution. Objective Using the Fundación Cosme y Damián bone bank experience we show a state of the art evidence based view of how, who, when and why should do a standardize process including the whole spectrum of activities of a bone and tissue bank. Material and methods There are 7 steps in any standardization process: To establish and entrance and an exit point, to build the process, define the desired outcomes, determine the key questions to obtain the outcomes, answer the questions based in current evidence, standardize and measure the results.

#### Conclusions:

Standardization improves quality in every level of the organization, it is a way of detection and correction of process errors and a form of allow to repeat the right steps in the same way every time. However, it is of paramount importance that before an activity is standardizing it must be validated. It would not make sense to standardize processes that are not legitimate.

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## O-35

### COMPARISON OF FROZEN AND FREEZE-DRIED CANCELLOUS BONE GRAFT IN CAVITARY DEFECTS

CAMACHO CARRASCO, P.; SEGUR VILALTA J.M.; GARCÍA OLTRA E.; GARCÍA ELVIRA R.; TORNER PIFARRÉ P.; FARIÑAS BARBERÁ Ó.; SUSO VERGARA S. *Hospital Clínic of Barcelona. Spain.*

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#### Abstract:

Bone graft is the second most common transplanted tissue just behind blood. Autogenous grafts harvested from iliac crest remain the gold standard. However, their use is associated to rates of morbidity that range from 8.5 to 20%. Bone allografts are the most frequently chosen bone substitute and are mainly processed by freezing and freeze-drying. Few studies are reported in the literature that compared biological behaviour of frozen and freeze-dried bone allografts. We performed a histological and histomorphometric study to evaluate and compare the incorporation of cancellous grafts in cavitory defects. Forty-eight New Zealand rabbits weighing 3.5-4.5 kg were used in this experiment as it involved skeletal maturity. According to the method described by Katthagen in 1984, which is a modification of Maatz's, a 6 mm defect was created in medial femoral condyle. This diameter restricts the spontaneous regenerative capacity as it represents more than a half of the condyle. Four treatment groups were established, control (without any implant), autologous, frozen allograft and freeze-dried allograft, and each of them was subdivided attending to the sacrifice time, at 4 or 12 weeks. After sacrifice bone specimens were processed for the study of non-decalcified bone. Sections were stained with von Kossa's stain, which offered a great contrast that eased histomorphometric analysis. Histomorphometric measurements, such as implant surface, trabecular area, osteoid surface and osteoblast surface, were obtained directly from the digitalized microscopic images. Histomorphometric parameters derived from histomorphometric measurements and were specific trabecular bone surface (Sv), relative bone formation surface (Sf), osteoblastic-osteoid surface (OBOID) and mean osteoid seam thickness (MOST). These parameters informed about structure (Sv) and osteoblastic activity (Sf, OBOID, MOST). The histological analysis revealed minimum bone formation in periphery without spontaneous regeneration in the majority of the defect in control group. Autologous group

showed bone trabeculae surrounded by abundant osteoid seams homogeneously distributed and osteoblasts. The frozen allograft group presented a more heterogenous distribution of bone trabeculae and a smaller quantity of osteoid and osteoblasts. The freeze-dried allograft group also exhibited the histological findings of frozen allograft group, but the most striking feature was the large number of osteoclasts. In global histomorphometric analysis related to treatment groups autologous graft showed best results, with statistically significant differences in OBOID. Overall frozen and freeze-dried allografts were comparable. One hand there was not statistically significant differences in Sf and MOST and on other hand there was found statistically significant differences in Sv and OBOID (greater values of Sv in freeze-dried group and of OBOID in frozen group).

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## O-36

### **VALIDITY OF AN AUTOMATIC MEASURE PROTOCOL IN DISTAL FEMUR FOR ALLOGRAFT SELECTION FROM A THREEDIMENSIONAL VIRTUAL BONE BANK SYSTEM**

APONTE-TINAO ALBERTO, L.; MILANO EDGARDO F.; FARFALLI LUIS G.; RITACCO EDUARDO L.; SCHWINT O.; SEILER C.; REYES M. *Italian Hospital of Buenos Aires. Argentina.*

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#### **Abstract/Background:**

Ostearticular allograft is one of the possible treatments after wide surgical resections in large bone defects. Performing best allograft selection is of great relevance for optimal exploitation of the bone databank, good surgery outcome and patient's recovery. Current approaches are, however, very time consuming hindering these points in practice. We present a validation study of a software able to perform automatic bone measurements used to automatically assess the distal femur sizes across a databank, from six pre-defined anatomical landmarks.

#### **Methods:**

170 distal femur surfaces were reconstructed from CT data and measured manually using a measure protocol taking into account the transepicondyle distance (A), anterior-posterior distance in medial condyle (B) and lateral condyle (C). Intra-, and inter-observer studies were conducted and regarded as ground truth measurements. Manual and automatic measures were compared using, a statistic description (means, maximal and minimal differences), intraclass correlation coefficient.

#### **Results:**

A single operator was tested for intraobserver repeatability while using the above-mentioned A-B-C protocol twice on the bone surfaces, obtaining an intraclass correlation coefficient of 0.99 for all measures. Interobserver consistency of two separate observers was quantified as well for the same cohort, leading to an intraclass correlation coefficient of 0.99 for A measure, and of 0.98 for B and C measures. For the automatic measurements, the correlation coefficients between observer one and automatic method, were of 0.99 for A measure and 0.96 for B and C measures. The average time needed to perform the measurements was of 16 hrs for both manual measurements, and of 3 minutes for the automatic method.

#### **Conclusion:**

The proposed methodology is presented as a key element towards effective and fast allograft selection, advancing the state of the art over current time-consuming and labor-intensive solutions. The results demonstrate the high reliability and, most importantly, high repeatability of the proposed approach, and considerable speed-up on the planning.

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**O-37**

**WHOLE ACETABULUM ALLOGRAFTS : A TWENTY YEARS EXPERIENCE**

RIBAS, M.; VILARRUBIAS J.; GINEBRED A I.; DE LA TORRE B.; BELLOTTI V.; DE MEO F.; CAVALIERE P.  
*University Hospital Dexeus. Spain.*

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**Abstract/Introduction:**

Even today there is still no absolute consensus in the reconstruction of the severe columnar defects in hip revision. It is precisely in these cases where bone reconstruction provides by far lower survival, and therefore the trend is the early revision at the first sign of any eventual osteolysis induced loosening, that the patient may present. But historically this was not the case. During the seventies and eighties many cases have been associated to massive bone loss. In our case this condition led us in 1988 to use massive acetabular allografts that reproduced faithfully the bone stock and bony anatomy so far. This study presents a historical series that allows us to keep in mind how these allografts behave in the medium and long term.

**Material and methods:**

We present a series of 44 transplants acetabulum with a mean of 16.2 years (range 9-22). The mean patient age was 58.6 years (range 19-83). According to the Classification of Gross 26 cases had type III acetabular defect, while 18 had type IV. The evaluation included Merle D'Aubigné score and radiological evaluation of the graft and the acetabular implant was performed according to radiological Engh criteria (JBJS, 1994).

**Results:**

The homogenization of the trabecular radiological pattern was observed in 42 of 44 cases (95.4%). There were 3 infections and 8 cases of aseptic loosening (18.1%), which were revised with only a new cup implantation. To date none of these eight cases have shown more signs of loosening. According to the Kaplan-Meier the overall survivorship rate, - endpoint hip revision for any given reason -, was 76.4% at 15 years in the type III cases, but in cases of pelvic discontinuity (type IV), survival was significantly higher ( 85.7%, p = 0.018). There was a remarkable improvement in Merle d'Aubigné score up (preoperative 2.2 to 4.9 during follow-up, p = 0.021) and pain (2.5 preoperatively - 5.4 during follow-up, p = 0032).

**Conclusions:**

In spite of some articles published by different authors with high incidence of failures in the midterm with massive structural allografts in this series clearly it has been shown, that an original acetabular allograft can provide an acceptable result along the time and allows in the most severe cases restoration of acetabular bone stock, making possible that further reconstruction is possible in these patients.

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**O-38**

**ONLAY CORTICAL STRUT ALLOGRAFTING IN TOTAL HIP ARTHROPLASTY (THA) REVISION**

MEDRANO, C.; BORI G.; GALLART X.; SEGUR J. M.; FERNÁNDEZ-VALENCIA J.; RIBA J.; GARCÍA S.  
*Hospital Clínic Barcelona. Spain.*

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**Abstract/Objective:**

To report our experience in onlay cortical strut allografting in revision Total Hip Arthroplasty (THA). Material and methods: Retrospective, observational study on 13 patients treated with revision THA that were performed in our center and used a cortical strut allograft, from January 2001 to April 2009. For all the patients we determine before the surgery the following data: age, sex, comorbidities, age of the prosthesis, protein C reactive (PCR) and globular sedimentation velocity (VSG),

corporal mass index (IMC), bony scintigraphy with  $^{99m}\text{Tc}$ -HMPAO leukocytes and TC hip scan in some cases. In all the cases samples from the periprosthetic material were taken for microbiological studies and polymorphonuclear count. We classify the type of femoral defects according to Paprosky, the type of allograft used and the prosthesis. We also evaluate complications and the functional evaluation by Merle d'Aubigné and Harris Hip Score.

**Results:**

14 cases were identified (13 patients: a case was bilateral); 12 cases were aseptic revisions and 2 cases were the second stage of a septic revision HA; 11 women and 2 men, the average age was 72.5 (rank 60-77) and the average prosthesis age was about 4 years (rank 2-11). The scintigraphy with  $^{99m}\text{Tc}$ -HMPAO leukocytes was negative in the 12 aseptic cases and it was not performed in the 2 cases of the second stage of a septic procedure. The femoral defects were all type IIIA Paprosky. Two cases presented positive microbiological studies for ECN but the PMN count was not more than 5pmn/pc. In these 2 cases the antibiotic treatment was maintained 6 weeks postoperatively. The radiological results are: 9 cases with total integration of allograft, 4 cases of total resorption and 1 case of nonunion. No cases of implant failure was observed. The average follow-up has been of 31.57 months (rank 12-97). The functional evaluation Merle d'Aubigné score was about 14, 53 (rank 11-17) and the Harris Hip Score was 66.21 (rank 43-91,80).

**Discussion:**

The use of onlay cortical strut allograft in revision hip surgery can be appropriate in those cases with radiological criteria of femoral defects and bone loss to prevent intraoperative periprosthetic fractures and to increase bone stock for successive surgeries if needed.

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**O-39**

**TREATMENT OF BENIGN BONE TUMORS WITH DEMINERALIZED BONE MATRIX AFTER CURETTAGE**

*SOTO, C.; SOTO C.; NAVAS J.; MIETH K.; GONZÁLEZ J. Fundación Cosme y Damián. Colombia.*

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**Abstract/Introduction:**

Curettage has been the gold standard for treatment of benign bone tumors stages 2 and 2-3. It is important to fill the residual bone defect in order to recover the bone structure and mechanical properties. Therefore, a number of materials like autografts, demineralized bone matrix, and synthetic composites have been used to fill such defects.

**Objectives:**

To determine the feasibility and effectiveness of demineralized bone matrix (DMO) to fill bone defects after intralesional treatment of benign bone tumours.

**Methods:**

From December 2002 to March 2010, eighty nine (n=89) consecutive patients with benign bone tumours were treated with curettage and high speed burring. We use demineralized bone matrix to fill residual bone defects. The patients were followed every four months with simple X rays for an average of 12 months, time when radiographic signs of healing were evaluated.

**Results:**

The average of healing and allograft incorporation was 6.2 months. We report tumoral recurrence in four patients. No patient had a pathologic fracture during the early bone healing stage. We obtained 96% rate of success, measured as no tumor recurrence and restoration of the normal intramedullar pattern using the Neer radiologic assessment tool.

**Conclusion:**

The use of demineralized bone matrix to fill residual bone defects is an effective osteogenic material in the intralesional treatment of benign bone tumors.

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**O-40**

**AN OVERVIEW OF THE OPERATIONS, TRACEABILITY AND SAFETY ASPECTS OF HUMAN BONE ALLOGRAFTS AT THE CENTRE FOR TISSUE ENGINEERING – BONE BANK IN SOUTH AFRICA**

*KARAKATSANIS, E. Centre for Tissue Engineering - Bone Bank (Tshwane University of Technology). South Africa.*

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**Abstract:**

Purpose of study The Bone Bank provides more than 18 000 allografts to the medical fraternity across South Africa annually. Therefore, the purpose of the study is to familiarise the end user of the various stringent critical quality control (QC) points that have been established in the processing of human bone allografts. The QC points are initiated prior to the procurement of tissue right through to the processing of the tissue.

**Description of methods:**

Musculoskeletal allografts provide the best solution to particular surgical procedures including revision surgery where an autograft is unavailable or as a supplement when an ample supply of autograft tissue is not available. Safety of tissue allografts remains priority, hence the thorough pre-donor selection criteria questionnaire and serology and microbiology (pre-procurement, in-processing and post-sterilisation) testing and verification. Aseptic processing is a common method of allograft processing and aims to minimise contamination of the allograft tissue from the environment, processing personnel and equipment. Hence, the implementation of strict audited operating protocols and the maintenance of clean room facilities that minimise contamination. Traceability of tissue from procurement to the utilizable allograft is ensured and recorded at all critical control points of the process. Gamma irradiation (terminal sterilization) is utilised for the sterilisation of allografts.

**Summary of results:**

The quality and safety of our products is achieved through effective planning and monitoring which governs every step of all the required processes to minimise any possibility of product failure. Donor statistics, risk factors and trend analysis have been identified and understanding these results allows for more effective donor consent rates and the improvement of operational protocols.

**Conclusion:**

Implementing and maintaining ISO 9001 (Quality Management System) and ISO 13485 (Medical Device) allows us to measure the effectiveness and consequences of our work, which is continually monitored against defined process and quality objectives, which are seen as the foundation to high-quality process management.

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**O-41**

**TEST SYSTEM FOR OSTEOINDUCTIVE ACTIVITY OF MATERIALS CONTAINING RECOMBINANT MORPHOGENETIC PROTEIN RHBMP-2**

*AKATOV V.; CHEKANOV A.; LEKISHVILI M.; FADEEVA I.; SKLYANCHUK E.; GURIEV V.; RYABOV, A.  
Institute of theoretical and experimental biophysics of RAS, Pouchchico. Russian Federation.*

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**Abstract:**

Development of materials including recombinant morphogenetic proteins(BMPs) is one of the promising areas of traumatology, orthopedics and maxillofacial surgery. BMP proteins are key factors in the remodeling and regeneration of bone tissue. These proteins have a powerful osteoinductive effect and can stimulate new bone formation through

differentiation of mesenchymal cells to osteoblasts. This circumstance serves as the basis for application of recombinant human morphogenetic proteins (rhBMPs) in tissue-engineered materials to enhance their osteoinductive properties. Since 2002, the material under the trade mark Infuse containing rhBMP-2 is used widely. Now the engineering of new materials including rhBMPs, in particular rhBMP-2, is active developing field. To evaluate the osteoinductive activity of the materials, different test systems are applied including cell assays in vitro, ectopic implantation models, and models of orthotopic implantation in experimental animals. This report presents the results of a comparative evaluation of osteoinductive properties of the material produced using recombinant morphogenetic protein rhBMP-2 or without rhBMP-2 in the model of ectopic implantation in experimental animals. We used in our study a composite material including demineralized bone matrices, which was filled with alginate gel containing rhBMP-2 or without rhBMP-2. Osteoinductive activity of materials was evaluated in a model of subcutaneous implantation in Wistar rats for a period of 1.5 months. Inclusion of rhBMP in material drastically increased the mineralization of the implants, stimulated the formation of a structured collagen. In particular, after the implantation, the content of mineralized calcium in fragments, which were previously treated with guanidine and then loaded with rhBMP-2 containing gel, was of  $150 \pm 30$  mg / g of dry weight of the fragment, whereas in the same material, which were loaded without rhBMP-2, the calcium content was of  $1 \pm 1$  mg / g of dry weight. Demineralized bone matrix without guanidine treatment (without removal of bone BMPs) and without the inclusion of rhBMP-2 was detected of  $15 \pm 5$  mg / g of dry weight of explanted material. Histological analysis showed destruction of collagen and its replacement by disorganized collagen (scar) in explanted materials containing no rhBMP-2, whereas the formation of a structured collagen was detected in the implants containing rhBMP-2. The results obtained show the effectiveness of subcutaneous implantation model as test system of osteoinductive activity of materials containing rhBMP-2, and point to the prospects of the material tested for practical applications. This work was supported by the Ministry of Education and Science of Russian Federation, contracts № 2.1.1/11708, № 02.740.11.0710 and № P609 and was made using devices of the Regional Center for Collective Use at the Pushchino Institute of Theoretical and Experimental Biophysics.

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## O-42

### **FREEZE-DRIED HUMAN SERUM ALBUMIN IMPROVES THE ADHERENCE AND PROLIFERATION OF MESENCHYMAL STEM CELLS ON MINERALIZED HUMAN BONE ALLOGRAFTS**

CSÖNGE, L.; SKALICZKY G.; KLARA T.; WESZL M.; SCHANDL K.; SZENDROI M.; LACZA Z.  
*West Hungarian Regional Tissue Bank. Hungary.*

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#### **Abstract:**

Mineralized scaffolds are widely used as bone grafts with the assumption that bone marrow derived cells colonize and remodel them. This process is slow and often unreliable so we aimed to improve the biocompatibility of bone grafts by pre-seeding them with human mesenchymal stem cells from either bone marrow or dental pulp. Under standard cell culture conditions very low number of seeded cells remained on the surface of freeze-dried human or bovine bone graft or hydroxyapatite. Coating the scaffolds with fibronectin or collagen improved seeding efficiency but the cells failed to grow on the surface until the 18th day. In contrast, human albumin was a very potent facilitator of both seeding and proliferation on allografts which was further improved by culturing in a rotating bioreactor. Electron microscopy revealed that cells do not form a monolayer but span the pores, emphasizing the importance of pore size and microstructure. Albumin coated bone chips were able to unite a rat femoral segmental defect, while uncoated ones did not. Micro-hardness measurements confirmed that albumin coating does not influence the physical characteristics of the scaffold, so it is possible to introduce albumin coating into the manufacturing process of lyophilized bone allografts.

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## ORAL - NOVEMBER 10TH - SKIN & AMNIOTIC MEMBRANE I ROOM 3 - 15.30-17.30

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### O-43

#### SKIN DECONTAMINATION AND IMPACT OF ANTIBIOTIC RESIDUES ON MICROBIOLOGICAL TESTS

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*Tissue Bank of Verona. Italy.*

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#### Abstract/Introduction:

The aim of the present study was to evaluate the performances of the BASE.128 medical in comparison with the antibiotic solution currently used by the Tissue bank of Verona and validate the time and temperature conditions of skin decontamination. Methods. Skin samples from five different donors were retrieved by the Skin Bank of Verona. Samples were divided in two halves of 100 cm<sup>2</sup> each and processed in parallel using an antibiotic cocktail containing Penicillin, Streptomycin, Amphotericin B, Bactrim and Gentamicin prepared by the bank or BASE.128 containing Cefotaxime, Gentamicin, Vancomycin, Amphotericin B (AL.CHI.MI.A., Italy). Samples were decontaminated with the bank cocktail or BASE.128 at 22°C for 90 min. followed by three consecutive rinsing of 5 min. each with NaCl 0,9% or BASE (AL.CHI.MI.A., Italy), respectively. All samples were cryopreserved in BASE containing 10% CRYO.ON (AL.CHI.MI.A., Italy) and stored at -80°C. Bacteriological tests were performed on donor skin swabs, tissue samples and transport and rinsing liquids using thioglycollate medium incubated at 37°C for 7 days. After thawing, the presence of antibiotic residues was evaluated on tissue homogenates by disk diffusion and sterility test performed according to the European Pharmacopeia after removing potential interfering antibiotics with a specific resin mixture. Contaminants were genetically identified by ribosomal RNA sequencing. Vitality assay with MTT colorimetric method was performed on each tissue before processing and 10 days after thawing.

#### Results:

No bacterial or fungal contamination was detected in the microbiological tests performed on donor skin swabs, tissue samples before and after processing, and transport and rinsing liquids. The sterility test performed on thawed tissues after removal of the antibiotic showed bacterial (*Staphylococcus* spp.) and fungal (*Penicillium* spp., *Candida* spp.) contamination in all investigated tissues. The sterility test performed without removing the residual antibiotics showed the absence of contaminants, thus indicating a possible interference of residual antibiotics with bacterial growth during test. 6- and 2-fold greater *S. Aureus* and *P. Aeruginosa* inhibition zones were observed in the disk diffusion test of tissues treated with the bank cocktail as compared to tissues decontaminated with BASE.128. No inhibition of *Candida Albicans* was observed in tissues treated with both solutions, thus indicating the absence of antifungal residues. The vitality assay showed an average of 40% metabolically active cells in cryopreserved tissues decontaminated with both solutions as compared to fresh donor skin.

#### Conclusions:

The presence of antibiotic residues after tissue decontamination can interfere with the microorganism growth during sterility test and result in false negative. The amount of antibiotic residues can vary significantly with the antibiotic cocktail. Consequently, microbiological analysis shall only be performed after an accurate removal of the antibiotic residues. The time and temperature conditions, used for both decontamination solutions were not effective eliminating contaminants from donor skin. Further studies evaluating different decontamination time and temperature conditions are in progress.

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**O-44**

**INACTIVATION OF BORRELIA BURGdorFERI BY GLYCEROL**

*RICHTER, C.; OEI A.; DE WEVER B.; HOVIUS J. Euro Skin Bank. The Netherlands.*

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**Abstract/Introduction:**

Lyme disease is caused by the spirochete *Borrelia Burgdorferi* and is transmitted by infected ticks to humans. The first clinical sign of Lyme disease is an erythematous expanding cutaneous lesion, designated erythema migrans, caused by an inflammatory reaction directed against the spirochetes residing in the skin. A significant increase in the number of patients is observed the last decade. Therefore, a recent tick bite is a contra-indication for skin donation. It has been shown that several bacteria species can be inactivated after incubation in glycerol at high concentration (85%). Storage of donor skin in 85% glycerol is used by skin banks as a method to decontaminate donor skin. In this study, we investigated the effect of glycerol on the survival of the *Borrelia Burgdorferi*.

**Method:**

Full thickness sheets of skin were injected subcutaneously with different concentrations (10<sup>1</sup> till 10<sup>5</sup> as enumerated by dark-field microscopy and a Petroff-Hausser counting chamber) of viable *Borrelia Burgdorferi* strain B31 (50 µl per biopsy). The skin was cultured for 16h at 33°C in RPMI medium. Thereafter, part of the biopsies were directly put in an assay to detect viable *Borrelia* spirochetes (culture method using modified BSK medium), part were used for DNA isolation and detection by semi-quantitative PCR. The other biopsies were first treated with glycerol according to the normal procedure used in the skin bank for donor skin. Briefly, the skin is put first for 24h in 50% glycerol with pen/strep and then for 3 weeks in 85% glycerol. After the glycerol treatment again part of the biopsies were tested in the culture assay to detect viable spirochetes and part was used for DNA extraction and PCR. Biopsies of the skin without any treatment and skin injected with only medium served as controls. In addition, non-viable *Borrelia* (incubated at 56°C for 30 min) were injected.

**Results:**

The lowest concentration of injected spirochetes that could be detected using this human skin model was 100, both in the culture test and the semi-quantitative PCR. All biopsies injected with spirochetes were negative after the glycerol treatment in the culture assay, no viable bacteria could be detected. The PCR results showed the presence of *Borrelia* DNA in the biopsies where spirochetes were injected, also in the biopsies treated with glycerol. Positive PCR results were obtained also in the biopsies injected with the heat inactivated non viable *Borrelia*.

**Conclusion:**

And discussion In biopsies of skin that were injected with viable spirochetes and treated with glycerol 85%, no bacteria could be detected using the culture method. *Borrelia* DNA could be detected using the PCR. Most probably, the bacteria are non viable but the DNA is still intact. In earlier studies, it has been shown glycerol at high concentration results in cell death with intact morphology. In addition, the biopsies injected with heat inactivated spirocytes were also positive in the PCR. The results with the culture assay indicated glycerol 85% inactivates for at least 3 weeks inactivates the *Borrelia* spirochetes. Since the sensitivity of this model is 100 bacteria, further risk assessment must be used to decide if donor skin from a donor with a tick bite without an erythema migrans can be accepted after treatment with glycerol.

**O-45**

**ESTABLISHMENT OF A RINSING PROCEDURE TO ELIMINATE ANTIBIOTIC AND GLYCEROL RESIDUES FROM CRYOPRESERVED SKIN DECONTAMINATED WITH BASE.128**

*PIANIGIANI, E.; GATTO C.; GIURGOLA L.; IERARDI F.; D'AMATO TOTHOVA J. Siena Skin Bank. Italy.*

**Abstract:**

Skin decontamination and cryopreservation can result in the presence of antibiotic and glycerol residues that shall be removed before transplantation in order to ensure tissue safety. Purpose To establish a rinsing procedure, to be performed before skin transplantation, with the aim of removing at least 80% of the antibiotic and glycerol residues from the cryopreserved skin. Methods Human skin samples from five donors were processed by the Tissue Bank of Siena. For each donor, 100 cm<sup>2</sup> of skin were decontaminated and cryopreserved in a single processing phase either with solution prepared at the bank (15% glycerol, Penicillin, Streptomycin, Gentamicin sulphate, Amphotericin B, and DMEM) or with the medical device BASE.128 (AL.CHI.MI.A s.r.l.) containing Vancomycin, Gentamicin, Cefotaxim and Amphotericin B with the addition of 15% glycerol. After thawing, all tissues were divided in three segments for different rinsing treatments. In the first group, the skin samples were not rinsed. The second group was rinsed twice with BASE (AL.CHI.MI.A s.r.l.) for 10 min. and the third group was rinsed three times with BASE for 10 min. After rinsing, skin homogenates were prepared and assessed semi-quantitatively for antibiotic residue content by agar diffusion test using agar plates seeded with *C. Albicans* (CA), *P. Aeruginosa* (PA), *S. Aureus* (SA). For determination of glycerol concentration in the skin samples, tissue homogenates were centrifuged and supernatant was derivatized with periodate and acetylacetone solution before injection in U-HPLC system (Dionex) equipped with reversed phase C18 column. Results Agar diffusion test showed inhibition areas on SA, PA and CA seeded plates in all skin samples not undergoing rinsing, thus indicating the presence of different antibiotic residues. Inhibition zones were significantly higher in bank solution treated tissues not undergoing rinsing as compared to tissues treated with BASE.128. Two rinses of 10 min. each with BASE allowed to reduce the inhibition areas on SA, PA and CA seeded plates of 32 %, 48% and 100% in tissue treated with bank prepared solutions and of 65%, 96% and 100% in tissues treated with BASE.128, respectively. In addition, a third rinse of 10 min. in BASE allowed to further reduce the inhibition zone on SA seeded plates in BASE.128 treated tissues, thus achieving an 80% reduction of antibiotic residual concentration. Conversely, the third rinse did not affect the tissues treated with bank prepared solution, indicating possible binding of the antibiotic to the tissue. HPLC analysis of tissues homogenates showed similar glycerol content (98,3 mg/g of tissue, on average) in tissues not undergoing washing, both treated with bank prepared solution and BASE.128. Three rinses of 10 min. each in BASE allowed removing, on average, 89% of the glycerol from the tissue.

**Conclusions:**

The efficacy of elimination of antibiotic residues from cryopreserved skin depends on the type of antibiotics. Specific rinsing procedures should be established and validated for each solution used for tissue processing. The present study proposes a rinsing procedure, consisting of 3 rinses of 10 min. each with BASE, resulting in effective removal of all antibiotic and glycerol residues from cryopreserved skin after decontamination in BASE.128.

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**O-46**

**THE INFLUENCE OF RADIATION STERILIZATION AND PRESERVATION ON CELL MORPHOLOGY OF HUMAN**

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2 - Nuclear Malaysia Agency, Bangi, 43000 Kajang, Selangor, Malaysia. Indonesia.

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**Abstract:**

The application of gamma doses lower than 25 kGy for terminal sterilization is a new approach in tissue banking for minimising the radiation effect. This is due to gamma radiation in combination with tissue preservation may have a significant effect on the morphology of human amniotic membrane (HAM) hence physiological condition. The aim of this study was to investigate the influence of different doses of gamma irradiation after different preservation techniques on cell morphology of HAM under Scanning Electron Microscope (SEM). HAM was processed and then preserved by either air drying (ADHAM) or submerged in glycerol (GPHAM). The sterilization of HAM was carried out using gamma irradiation from Cobalt-60 source at 0 kGy, 15 kGy, 25 kGy and 35 kGy. In the control group (fresh HAM) no preservation and sterilisation were performed. The samples were fixed in 2.5% glutaraldehyde and the surface morphology was analysed under the SEM. The SEM examinations revealed some alteration in cell morphology of ADHAM and GPHAM as compared to fresh HAM. In addition, the cell morphology of ADHAM was more affected than GPHAM. The structure of HAM was not distinctively clear at low magnification ( $\times 250$ ). However, at higher magnifications (more than  $\times 1000$ ) the intercellular channels and the cell surface covered with microvilli were clearly observed in both ADHAM and GPHAM. The cell structure was more preserved when stored in glycerol, the cells were beautifully arranged, homogenous, tended to round up at 25 kGy and started to have a gap between intercellular channels at 35 kGy. As for ADHAM, the cells seemed to form flat sheet of polygonal cells and the cytoplasmic strands were flattened and condensed at all of the sterilization doses. While in the fresh HAM the membrane was composed of polygonal cells forming mosaic pattern. At  $\times 10000$  magnifications, the SEM showed the microvilli covered the whole surface, causing intercellular channels in fresh HAM less clear. There were significant changes in the HAM surface after processing/preservation and sterilization. GPHAM was found to cause less morphological changes than ADHAM at doses lower than 25 kGy due to glycerol as a radioprotectant. The intercellular channels of ADHAM were not visible because the cells collapsed during air-drying and likely further damaged when irradiated. High dose of gamma irradiation at 35 kGy led to marked changes in the cell morphology and the intercellular channels of HAM, fortunately the influences were less pronounced in GPHAM. In conclusion, this study revealed that the different preservation methods followed by sterilization by gamma irradiation caused changes in cell morphology of HAM. The findings recommended the use of glycerol preservation in combination with irradiation dose lower than 25 kGy for processing of HAM.

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**O-47**

**EFFECT OF INCUBATION TEMPERATURE ON VIABLE SKIN DECONTAMINATION**

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Banc de Sang i Teixits. Spain.

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**Abstract/Background:**

Viable skin decontamination by grafts incubation in antibiotics is routinely performed in Tissue Establishments (TE). Major parameters defining effective decontamination protocols include, but not limited to; initial graft bioburden, type and concentration of antibiotic/antimicrobial cocktails, incubation times and working temperature during decontamination process.

**Purpose:**

The effect of antibiotic incubation temperature was evaluated for skin grafts arrived to our TE processing facility after procurement. Several grafts were incubated at 4°C (n=36) or 37°C (n=70) using the next decontamination cocktail composition (CPLVA): 240 µg/mL Cefoxitin; 123 µg/mL PolimixinB; 102 µg/mL Lincomycin; 50 µg/mL Vancomycin and 5 µg/mL AmphotericinB. Final concentrations in culture medium M199 described.

**Materials and Methods:**

Biopsy (3-4 small pieces), antibiotic free transport and cryopreservation medium samples (5mL/each) were used to determine microbiological contamination status before and after decontamination. Tissues microbiological status acceptable for clinical transplantation, including those grafts positive for non pathogenic skin contamination, served as the evaluation criteria regarding the effect of temperature on decontamination.

**Results:**

Microbiological species isolated in skin grafts under study were mainly; Staphilococci sp including S.Aureus, Enterococci, E.Coli and Bacillus sp. Before decontamination samples taken resulted microbiologically out of specifications for 42% and 54.3% of the grafts included in the 4°C and 37°C study groups respectively. In the 4°C group, after decontamination, 61% and 44% of the evaluated grafts passed the clinical criteria for transplantation when biopsy or supernatant samples microbiological results were evaluated. Those proportions raised up to 94% and 74% for the above mentioned kind of samples when decontamination was performed at 37°C. Incubation times ranged 19-23 hours and 11-24 hours in the 4°C and 37°C groups respectively.

**Conclusions:**

Incubation temperature is an important parameter for viable skin decontamination effectiveness. Rising temperature from 4°C to 37°C during incubation resulted in a 30% increase on medical accepted for transplantation skin grafts for both biopsy and supernatant samples. Microbiology test results varied regarding the kind of analyzed sample (biopsy vs supernatant) in terms of microbial detection (sensitivity). As expected, medium samples showed a major proportion of positive results (up to 20%) when compared to biopsy samples, irrespective the incubation temperature during decontamination, suggesting major representation of those samples.

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**O-48**

**GAMMA RADIATION STERILIZED AMNIOS: RECENT CLINICAL APPLICATIONS IN MEXICO**

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**Abstract:**

The use of radiosterilized amnios in recent different clinical applications in Mexico is presented. The Banco de Tejidos Radioesterilizados del Instituto Nacional de Investigaciones Nucleares (Radiosterilized Tissue Bank at the National Institute of Nuclear Research BTR-ININ) have been collaborating with public health institutions, mainly with Ophthalmology Service, to treat affected patients with amnios esterilized with gamma radiation. The results have proven to be excellent as much for cosmetic purposes as for functional ones. Radiosterilized amnios do not provoke rejection, adverse reaction or transmit an infective disease and improve the quality of life of the patients.

**Introduction:**

In Mexico, the Banco de Tejidos Radioesterilizados del Instituto Nacional de Investigaciones Nucleares (Radiosterilized Tissue Bank at the National Institute of Nuclear Research BTR-ININ) was established in 1999, thanks to strong support of

the International Atomic Energy Agency (IAEA). Since that time, the BTR, which is a nonprofit tissue bank, processes and sterilizes with  $^{60}\text{Co}$  gamma radiation tissues such as amnion and pig skin. These tissues have been used mainly as biological wound dressings in patients with first, second and third degree burns, ulcers, epidermolysis bullosa, bloody areas, and in wounds difficult to heal. The Secretaría de Salud (Mexican Ministry of Health) issued the sanitary license No. 1062000001 to the BTR-ININ on July 7, 1999 [1]. The Quality Management System QMS of the BTR-ININ was certified by ISO 9001:2000 on August 1, 2003 [2]. At present, the ISO 9001:2008 certification is kept. To offer more and diverse sterilized tissues to the medical community, the bank is now validating the process of human skin and musculoskeletal tissues, both from cadaveric donors. For that, the ININ has signed an agreement with the Instituto de Salud del Estado de México ISEM (Health Institute of Mexico State) since June, 2007, to obtain amnion, human skin and musculoskeletal tissues. Amnios Processing: Amnios from healthy mothers, with written consent and rigorous screening for HIV 1, 2, Hepatitis B and C, Syphilis and Chagas and clinical history, among others, were procured at an authorized ISEM hospital. The selected hospital is also responsible of preliminary cleaning of the tissue and temporary storage at refrigeration temperature, preserved in saline solution. Then the tissue and its documents were sent to the BTR-ININ. After first quality control inspection (QCI), the amnios are processed at the bank. The following activities are performed at the BTR in its facilities at the Nuclear Centre, according to its QMS: Tissue Reception, Checking of documentation, Washing, Drying, Cutting at desired size, Packaging (primary and secondary package) under laminar flow conditions, Labeling to identify the tissue bank, Microbiological control performed at the Departamento de Biología (Biology Dept.), to determine average initial bioburden, verification dose and sterilization dose. After second QCI, the processed tissues are sent to the Departamento del Irradiador Gamma (Gamma Irradiator Dept.). Sterilization is done at room temperature with cobalt-60 gamma radiation at a minimum dose of 25 kGy. Then the irradiated product is sent to the Departamento de Materiales Radiactivos (Radioactive Mtls. Dept.) for Final product sterility test. Again at BTR facilities, the Storage of irradiated tissues at room temperature, waiting for negative second serology results and Distribution of high quality tissues for clinical application are performed. Irradiated tissues are distributed mainly to public hospitals, after the corresponding quality control approval and the tissues are delivered with an instruction sheet and a follow-up form [1].

### **Clinical applications:**

Radiosterilized amnios processed at the BTR have been used in Mexico to treat patients with burns, damaged ocular surface and dystrophic epidermolysis bullosa DEB (dominant form), among others[3]. Written and informed consent was obtained from all patients that were treated with the irradiated tissue. The Epidermolysis Bullosa is a rare hereditary and genetic illness due to a failure of connection between the epidermis and the dermis. The skin is very fragile and to blister easily. DEB is one of the major forms of epidermolysis bullosa. Mutations in the COL7A1 gene cause all three major forms of DEB. This gene provides instructions for making a protein that is used to assemble type VII collagen. Type VII collagen plays an important role in strengthening and stabilizing the skin. When type VII collagen is abnormal or missing, friction or other minor trauma can cause the two skin layers, epidermis and dermis, to separate. This separation leads to the formation of blisters, which can cause extensive scarring as they heal[4]. One-month old patient suffering Dystrophic Epidermolysis Bullosa (DEB) was treated with radiosterilized amnios in a private hospital at Pachuca city. At the patient's birth, April 2010, the DEB affected his lower extremities, feet and thorax. One month later, the majority of his injuries were in remission, except the feet. That is why on May 19, 2010, the amnios were placed in both to prevent anemia and to improve the lesions. The use of this irradiated amnios in Ophthalmology started in the country in 2005, either as a graft to replace the damaged ocular surface, or as a patch to prevent unwanted inflammatory reactions. Patients from the public Hospital General de México (HGM, Mexico City), suffering diverse pathologies such as keratoconjunctivitis, recurrent pterygium associated with symblepharon, corneal neurotrophic ulcers, chemical and thermal burns, and corneal thinnings, had been successfully treated with irradiated amnion. In the HGM, a clinical prospective study on lesions of the ocular surface of 17 eyes from 15 patients, affected with the mentioned above pathologies, was successful in 88.2%[5]. In the Instituto Mexicano del Seguro Social in Metepec, Méx. (IMSS-UMAA 231), a controlled clinical randomized trial with 108 eyes from 100 patients, affected with primary nasal pterygium, was performed in 2009. The degree for the pterygium was classified in I for less than 1 mm, II for the range of 1 to 3 mm, and III for more than 3 mm. Fifty four eyes (Group A) were treated with radiosterilized amnion and intraoperative Mitomycin C to prevent recurrence after excision of the primary pterygium. Group B consisted of 54 eyes from 49 patients, was subjected to conjunctival autologous graft with intraoperative mitomycin C. Topical mitomycin C at 0.05% concentration was used in this research [6]. In the IMSS-Centro Médico del Bajío, Unidad Médica de Alta Especialidad in León City, in the center of the country, a two year-old boy patient was admitted for management of bilateral chemical burn with alkali on January 7, 2011. After initial exploration, the diagnosis was bilateral chemical burn grade II

in right eye and grade III in left eye, according Hughes- Roper- Hall Clasiffication of chemical burns. Five days after treatment with antibiotics, analgesic, antiinflammatory and lubricant, pain and inflammation were getting better, and corneal epithelialization was started. Right eye with epithelial abrasion, 20% of total corneal suface (tcs), and a paracental leucoma. Left eye had epithelial abrasion, 60% of tcs, with epithelial blebs and stroma still hazy, affecting visual axis. Radiosterilized amnios were applied in both eyes covering cornea wound and fixed on clear cornea and conjunctiva, under general anesthesia and autolougus platelet rich plasma injected subconjunctival. Finally both eyes were covered with a therapeutic bandage contact lens. Results.-The results have proven to be excellent as much for cosmetic purposes as for functional ones. Gamma radiation sterilized amnion (A) represents an effective and secure treatment of the damaged ocular surface, with the advantage of total absence of bacteria, due to a SAL of 10<sup>-6</sup>. The tissue does not provoke rejection or transmit an infective disease from donor to recipient. Irradiated (A) does not require refrigeration, it can be stored at room temperature without any deterioration of its properties. The tissue has easy handling & low cost. The use of irradiated amnion in the paediatric patient affected with DEB gave him a better quality of life. Four months after (A) treatment, the appearance of his feet skin was dramaticalley improved. Unfortunately, this rare disease has a bad prognosis. The outlook depends on the severity of the illness. In the case of the paediatric patient affected with chemical burn in both eyes, four months lather, right eye cornea was completely clear. Left eye cornea only with a small and mild leucoma in the nasal edge of the pupil, which not affects visual axis. Due to the lack of corneal tissue suitable for transplantation (Tx), the irradiated (A) gives an extra time for those eyes waiting for cornea or before Tx, to improve the prognosis.

#### Conclusions:

Irradiated amnios can be satisfactory used in the treatment of the affections mentioned above. Without the treatment, the patients could have suffered a healing after-effect or loss of sight. The inflammation and pain were significantly reduced. Radiosterilized amnios do not provoke rejection, adverse reaction or transmit an infective disease and improve the quality of life of the patients.

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**O-49**

**MICROBIOLOGICAL CONTROLS IN SKIN TISSUE BANK**

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1 - Transplant Services Foundation-Hospital Clínic. Spain.

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**Abstract/Introduction:**

Skin grafts from cadaveric donor have become one of the best options treating great burns, traumatism, surgeries and diseases entailing the loss of cutaneous tissue. To ensure the viability of these grafts the tissue banks facilitate low temperature storage between procurement and delivery, this storage period providing the time necessary for microbiological assessment of the skin prior to grafting. In our tissue bank two basic preservation methods are carried out: glycerol preservation and cryopreservation. After processing the skin is maintained in quarantine, waiting for the blood culture results and the processing culture results. Looking for the highest quality of the grafts a new microbiological culture test was added to the protocol of skin procurement and processing. Thus, since middle 2009 a sample of skin is taken during retrieval for microbiological testing, as well as blood culture and culture during processing. Moreover in some cases we were able to collect a sample before the last preservation presentation, a post processing culture, in the case of glycerolized skin. Aim To evaluate the contamination rate of blood cultures and skin cultures from donors banked in the period between May 2009 and December 2010, assess the influence of any contamination in the viability of the tissues processed in the bank, and compare the numbers with the data obtained with the donors banked during 2009, with or without the extra microbiological information.

**Methodology:**

The study was conducted on 220 skin donors available to the skin bank from May 2009 to December 2010. Blood cultures were carried out to each donor, one (217), two (185) or three (68), as well as cultures of cutaneous tissue from each preserved fragment, both cryopreserved (426) and glycerolized (216). Moreover, a skin sample was taken during retrieval of all of these donors (220). Results The viability rate obtained with the 140 donors processed in 2009 was 85%, whereas the one obtained with the 220 donors with the extra information of the result culture of the skin taken during retrieval was almost the same, 84%. Thirty-five out of the total 220 donors were discarded as viable donors, 18 of them due to microbiological contamination (positive blood culture, skin culture or both of them). Focus on the results obtained with the new culture of the skin taken during retrieval, there are 19 donors whose cryopreserved skin was discarded but yet it was possible to distribute the glycerolized skin, due to the virucidal and bactericidal potential of glycerol.

**Conclusions:**

The accomplishment of several cultures pre and post processing contributes to the safer skin grafts generation. Our decontamination method during processing has demonstrated to be highly effective. Both cryopreservation and glycerolization are suitable methods to assure the viability of the skin grafts distributed.

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**O-50**

**AMNIOTIC MEMBRANE IN USE FOR VARIOUS PRECLINICAL AND CLINICAL INDICATIONS**

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Red Cross Blood Transfusion Service of Upper Austria, Linz/ Austrian Cluster for Tissue Regeneration. Austria.*

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**Abstract:**

Preserved amniotic membrane is used in the field of ophthalmology and wound care due to its supporting properties. The Multi-Tissue-Bank of the Red Cross Blood Transfusion Service of Upper Austria supplies local and international hospitals with cryopreserved amniotic membranes for both indications. A less-known possible field of application could be hernia repair, as we could demonstrate in an experimental intraperitoneal onlay mesh technique in rats that cryopreserved, but still viable amniotic membrane as antiadhesive mesh coating reduces adhesions to the used mesh and suture materials. Furthermore, data of a clinical study applying amniotic membrane in oral surgery to cover split-skin donor sites will be presented. Within this study amniotic membrane was compared to conventionally used materials, particularly with regard to exudation, change of wound dressing, pain, pruritus, comfort and healing progress.

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**ORAL - NOVEMBER 10TH - LEARNING FROM BIOVIGILANCE  
AUDITORI - 09.55-10.05**

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**O-51**

**CATALONIAN SURVEILLANCE REGISTER**

*BARRIO, R.; FÉLIX JESÚS M.; ARAN B.; DEULOFEU R. - OCATT. Barcelona. Spain.*

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**Abstract:**

Surveillance was inserted in the current Spanish legislation in 2006 by the RD 1301/2006, based on the European Law 2004/23/CE. This law settles the requirements of quality and security in every tissue and cell procedure. Catalanian Surveillance Register was set up in June 2008. Before that, a manual containing all the reporting system and the different facts to notify, was published and distributed among all the professionals involved. In Catalonia, there are almost 230 authorized activities related to tissues and cells. There are nearly 150 teams authorized to graft different types of tissues and cells. Forty centers are authorized to obtain cord blood, 26 to retrieve different types of tissues and cells and 6 tissue banks. Two of them are multi-tissue and 4 are monographic (cord blood, hepatocytes, ocular and ovarian). Due to the large number of authorized centers, in 2010 more than 5800 tissue and cell implants were performed. Although the current law recognizes two different types of notifications, Serious Adverse Effects (SAEs) and Serious Adverse Reactions (SARs), we have differentiated three events. On one hand there are the Serious Adverse Effects (SAEs). Although the law does not differentiate, we have split SAEs into two sections. The first takes into account tissues grafted prior to detection, this is known as clear SAEs. The second section refers to detection before grafting. In this case we speak of incidents. On the other hand, there are the Serious Adverse Reactions (SARs). It was agreed to notify every fact detected at one stage of the process, but which had been overlooked at the previous one. From June 2008 till December 2010, the Catalanian Surveillance Register has received 63 notifications. 22 were clear Serious Adverse Effects, 22 incidents, 7 Serious Adverse Reactions and 2 medical alerts. 3 of the Serious Adverse Reactions were reported in living donors and the other 4 in recipients. All of them recovered without further complication.

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**O-52**

**NATIONAL HISTOVIGILANCE EDUCATION SYSTEM IMPROVES PROFESSIONAL UNDERSTANDING OF SEVERE ADVERSE REACTIONS AND EVENTS**

*CEBULC, G.; AVSEC D. Slovenija-transplant. Slovenia.*

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**Abstract/Introduction:**

To review the implementation of notification of severe adverse events and reactions (SARE) under Slovenian Act and Rules but Directives 2004/23/EC and 2006/86/EC as well. A national histovigilance education model is suggested. Background: After adoption of the Directives 2004/23/EC, 2006/17EC and 2006/86/EC, next step forward was to prepare national Rules for histovigilance (HV). First national report on histovigilance was done in 2009 but no reports from tissue establishments (TEs) were received to Competent Authority (CA) Slovenija Transplant (ST). In next year were collected 5 cases only. Despite ST delivered EUSTITE vigilance and surveillance (V&S) tools to all accredited TEs within country. At the end of 2010 ST decided to organize professional meeting with well defined timetable for professionals from tissue and cells field. Second annual national meeting for histovigilance in May 2011 was performed.

**Objective:**

Despite Directives' provisions related to HV were placed nothing happened in practice. As mentioned there were no expected reports of SARE in 2008. Following year some deficient reports were received by ST. EUSTITE V&S tools were translated and delivered by ST within all responsible persons of TEs in Slovenia. The need of education was huge and by decision of ST first national meeting for HV was performed in November 2010. It was measured that annual meeting including workshops and high skilled tutors are necessary.

**Methods:**

ST prepared one day education meeting model. Target groups were TEs responsible persons, transplant coordinators, clinicians and other professionals working in tissue and cells field. In Slovenia are registered 6 donor hospital and 16 TEs at the moment. Lectures included legislation V&S tools basis. Second part was consisted of interactive group workshop, whereas 5 to 7 participants from different background. Evaluation of the model was performed by questionnaire covered major aspects of the model including content relevance of lectures and workshop, presentation and sufficiency of lecturers' answers, organization and accessibility of the event as well. Presented were 55 participants in 2010 and 27 in 2011. Every participant had to fulfill test with 10 questions related to the presented topics. The average score of the test was 8,76 of 10 in 2010 and 7,81 of 10 in 2011.

**Conclusions:**

National HV education system was established. International tutor from EUSTITE project was presented and guided practical part of the meeting. The huge necessity for HV in practice was confirmed by number of participants and feedback where the first meeting was scored with 4,28 of 5 (85,6%) comparing 4,06 of 5 (81,2%) in 2011. Additionally the average score of importance of HV education was 3,94 of 5 in 2010 comparing 3,76 of 5 in 2011. It was suggested one day professional meeting with HV workshop annually.

## ORAL - NOVEMBER 10TH - GLIMPSE INTO THE FUTURE; ADVANCED THERAPIES - AUDITORI AND ROOM 3 - 14.10-15.30

### O-53

#### MICROBIOLOGICAL CONTAMINATION MONITORING OF CLEANROOM FACILITIES IN A TISSUE AND CELL ESTABLISHMENT

VILARRODONA, A.

*Transplant Services Foundation-Hospital Clínic. Barcelona, Spain.*

##### Abstract/Introduction:

Routine control of environmental microbiological contamination in cleanroom facilities is a critical part of tissue and cell establishments. Security of tissues and cells which are processed in cleanrooms may be compromised by the presence of microorganisms in the environment or surfaces of these facilities. Tissues and cells are processed in grade A area surrounded by grade B with adjacent grade C areas.

##### Aim:

The aim of this study is to evaluate the bioburden load in the controlled areas of our tissue and cell establishment (5 cleanrooms, 1 corridor, 2 changing rooms and 1 material air-lock).

##### Methods:

According to the guidelines of Good Manufacturing Practices (GMP) our tissue and cell establishment has developed a monitoring program to control microbiological contamination of cleanroom areas. Between December 2009 and June 2011 with a variable periodicity of 14 or 21 days, defined sampling points and airborne particles were collected using Tryptone Soy Agar (TSA) and Sabouraud Chloramphenicol (SC) contact and settle plates respectively. The plates were incubated at 30-35°C for bacteria and 20-25°C for fungi during 72 h and subsequently 4 days at room temperature. Sampling was conducted in an at rest state (which is the condition where the facility is installed and operating complete with production equipment but with no operating personnel), the day before a radical sanitation and after the routine and daily cleaning procedures of the cleanrooms because it was considered the worst situation.

##### Results:

The obtained results are summarized in the following table (including working and non working areas):  
Negative results In of specs Out of specs Surface areas 2507 278 247 31 Air control 128 32 30 2 Total 2635 310 277 33  
From the total amount of microbiological cultures (2945 samples) the 10.53% resulted positives and the 10.65% from them were out of specifications. The most common microorganisms identified were *Micrococcus* sp (55.10%), *Bacillus* sp (28.23%) and Gram positive cocci (12.92%). The following table shows the distribution of the positive results on the different working areas:  
CR1 CR2 CR3 CR4 CR5 Air Class B B B B B In specs Air 8 5 7 6 4 Surface 5 5 1 12 3 Out specs Air 1 0 0 1 0 Surface 0 1 0 0 5  
None of the out of specifications results were obtained from working surfaces, and most of them correspond to door handles and floor surfaces. In reference to the accomplishment of GMP guidelines we observed a 99.88% of results within the specifications.

##### Conclusions:

In terms of traceability, it is important to highlight the bioburden knowledge to determine traceability from donor to final recipient considering the possibility of cleanroom facility influence. Despite the effectiveness of the decontamination process coming out from the corresponding validation study, the necessity of developing a microbial monitoring program and a periodical review of the validation study is concluded as highly advisable to guarantee the quality and safety of the tissues and cells with clinical purposes. Due to the major part of the microorganisms detected become from normal skin flora, the measures taken to achieved lower contamination rates must be focused on personnel hygienic practices.

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**O-54**

**PRESENTATION OF THE PROJECT OF THE SUPPORT OFFICE TO THE CLINICAL RESEARCH IN ADVANCED THERAPIES**

ARAN, B.; FELIX M.; BARRIO R.; DEULOFEU R. *Organització Catalana de Trasplantaments. Spain.*

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**Abstract:**

Advanced Therapies (AT) involve cell therapy, gene therapy and tissue engineering. All of these therapies comprise regenerative medicine and try to restore or replace cells and tissues when cell function has been lost. AT are moving forward and lots of researchers are working to know how to improve the results. However, most of the treatments are still in an experimental phase. The AT are regulated guided by the Regulation 1394/2007. According to this regulation, cells and tissues used in AT must be considered medicinal products with all the requirements and conditions involved (GMP conditions, quality controls, etc). Cells and tissues are considered medicinal products if they have suffered a substantial manipulation before transplant, if the initial and final place in the human body is not the same or if the cell function changes. In Spain, the Medicament Spanish Agency (AEMPS) is in charge of approving the clinical trials with AT and also the industrial production of AT medicinal products. The requirements are the same in both conditions; although the situation is very different. Nevertheless, sometimes it is difficult to know if a therapy is an AT or a transplant, and the law is different in each case. Furthermore, researchers sometimes ignore technical and administrative prerequisites before a clinical trial is put in place. For these reasons, setting up of a Support Office to the Clinical Research in Advanced Therapies is proposed. It will be created by the Catalan Transplant Organization (OCATT) through an instruction from the Health Department and an agreement between the Catalunya-OCATT and the Center of Regenerative Medicine in Barcelona (CMRB). The office's principal aims would be to create a registry including all the AT clinical trials taking place in Catalonia and to offer help and support to all researchers who need some advice before putting an AT clinical trial in place. The office will provide information about the requirements needed to perform the assay (available facilities, paperwork management, etc) proceeding as a link between researchers and the AEMPS. Other tasks of this office will be to approach basic and clinical research facilitating initiatives and creating relationships between both of them, as well as, to promote the diffusion of the results and to search calls and grants to fund the projects. An Advisory Commission will counsel and support the office by working groups. The Support Office to the Clinical Research in Advanced Therapies wishes to be a useful tool to help and support clinical researchers' task. The office wants to establish relationships with them to know their needs and pushing up the research towards the clinic avoiding duplications.

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**O-55**

**BIOMECHANICAL PROPERTIES CAMPARATION BETWEEN FREEZE DRIED FLEXOR TENDON AND COMPOSITE OF FREEZE DRIED FLEXOR TENDON – BONE MARROW MESENCHYMAL STEM CELL IN RABBIT MODEL**

SUROTO, H.; FERDIANSYAH F.; RANTAM ABDUL F.; ROESHADI D. *Biomaterial Center and Tissue Bank - Dr Soetomo General Hospital. Indonesia.*

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**Objective:**

To evaluate biomechanical properties of freeze dried flexor tendon and reconstitution of freeze dried flexor tendon seeded with bone marrow mesenchymal stem cell in rabbit model.

**Design:**

The post test only control group design of rabbit freeze dried flexor tendon and rabbit bone marrow mesenchymal stem cell.

**Subject:**

We studied New Zealand White rabbit bone marrow mesenchymal stem cell cultured and expanded. 5 fresh and 5 freeze dried flexor tendon of NZW rabbit.

**Methods:**

Five flexor carpi ulnaris tendon was harvested from adult, male New Zealand White Rabbit. They were divided into three groups according to processing: group1, fresh flexor tendon as control group; group2, freeze dried flexor tendon; group3, composite freeze dried flexor tendon – bone marrow mesenchymal stem cell. All processing was performed at Biomaterial installation and tissue bank of dr Soetomo Hospital. Each specimen was tested by tension load using autograft shimatzu machine. The parameters of maximal load, tensile strength, tensile strain and modulus elasticity were obtained from the load-elongation curves and the stress-strain curves. Stastical analyses were performed using one-way analysis of variance (ANOVA) to compared maximal load, tensile strength and modulus elasticity. And to analyse tensile strain comparison we performed non parametric Kruskall-wallis test. Subsequent post-hoc comparisons were performed to detect significant differences ( $p < 0.05$ ).

**Result:**

Freeze dried processing of flexor tendon decrease tensile strength till 51,14%, but after seeded by bone marrow mesenchymal stem cell the decreasing just until 77,65% and insignificant statistically ( $p = 0,151$ ). This happening similar with the other parameter of biomechanical properties, except in tensile strain that the decreasing is significant statistically ( $p = 0,004$ ).

**Conclusion:**

Composite freeze dried flexor tendon with bone marrow mesenchymal stem cell can improve biomechanical properties.

**Keywords:**

freeze dried flexor tendon, bone marrow mesenchymal stem cell

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**O-56**

**IMPROVEMENT OF LIMBAL STEM CELLS AND MUCOSAL EPITHELIAL CELLS ISOLATION AND CULTIVATION METHOD**

DRAGUNOVA, J. *Central Tissue Bank, University Hospital Bratislava. Slovakia.*

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**Abstract:**

Dragúňová J1., Černák A2., Cucorová V1., Koller J1. 1. Central Tissue Bank, University Hospital Bratislava, Slovak Republic 2. Department of Ophthalmology, University Hospital Antolská, Bratislava, Slovak Republic Aim: To establish the standard method which allows improvement of the procedure of limbal stem cells (LSC) and the mucosal epithelial cells (MSC) cultivation. Introduction: In Central Tissue Bank Bratislava (CTB) cultivation of autologous limbal cells for corneal reconstruction has been used routinely since 2004. Insufficient size of the biopsy is usual practice. Small biopsy usually results to prolonged time of cultivation. Reduction of cultivation time is also important because of the change of phenotype from limbal to corneal after 2nd-3rd. passage. To solve this problem we improved our method how to obtain maximal number of the cells in minimal cultivation time. The principal of this method is parallel cultivation of isolated cells and cultivation of the rests of the biopsy after isolation as an explant culture. Material and Methods: The cells from the biopsy were separated by trypsinisation (37 °C 1 hour), the reaction was stopped by the few drops of autologous patient serum. The cells were isolated by the gently scraping with the forceps and subsequently cultivated. The remnants of the biopsy after trypsinisation were seeded onto a Petri dish as an explant culture and cultivated parallel.

**Results:**

1. After the enzymatic isolation the cells started to grow within 24 hours and the confluent layer was reached within 7-14 days.
2. The cells from the explant culture started to grow after 2- 3 days and the monolayer was formed after 10- 14 days The sufficient number of the cells from both parallel cultivations was obtained within 10- 14 days without any passages of the cells. To obtain  $2, 5 \times 10^6 - 4 \times 10^6$  cells using standard method take more than 28 day and higher number of passages is needed. This number of the cells is adequate for seeding on the amniotic membrane for transplantation to the patient.

**Conclusions:**

The utilization of the remnants of the biopsy allows reduce cultivation time to the half without passages and risk of changing of the limbal phenotype. Reduced cultivation time is more profitable for the patients.

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**O-57**

**TESTING ON CELL CULTURES OF BIOGENIC AND SYNTHETIC MATERIALS APPLIED IN DENTISTRY**

VOLOVA L.T., ROSSINSKAYA V.V., SHAROVATOVA A.U., MILYUKOVA M.N., KUPRYAHIN S.G. *Russia, Samara State Medical University, Samara Tissue Bank. Russian Federation.*

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**Abstract/Introduction:**

Hydroxyapatite-containing materials are used in dental surgery and implantation. But their application in clinical practice doesn't always lead to the required results. The Objective. To conduct the testing on cell cultures of such materials for cytotoxicity and biocompatibility. Materials and methods. The investigations were conducted on human dermal fibroblasts cultures. Russian synthetic osteoconductive preparatuions KollapAn-G, Kollapol, as well as allogenic hydroxyapatite (HAP) and spongiosa of "Lioplast®" series and samples of titanium rods with and without synthetic HAP spraying have been used as target test-objects. Investigations have been carried out using morphological, morphometrical, histochemical, biochemical methods of analysis as well as scanning electron (raster) microscopy.(SEM).Results. KollapAn-G and Kollapol preparations , containing xenogenic collagen and synthetic HAP, possess weak cytotoxicity (Kollapol cytotoxicity is more). Monomolecular layer cells proliferation processes inhibition, cells and nuclear form changes, cytoplasm vacuolization have been notes since the 3rd day of the experiment. The death of  $67.3 \pm 4.2$  % of monomolecular layer cells have been registered on the 7th day of Kollapol testing, the majority of the survived cells contain fat inclusions in their cytoplasm, which are normally absent in fibroblasts. Biochemical methods of cultural growth assessment confirm the damaging effect of these materials; by the 9th day of the experiment the number of living cells has reduced in 2.8 times in KollapAn-G testing and in 4.8 times in Kollapol testing. Living cells in these cases have been observed only up to the 7th day of experiment. Allogenic HAP and mineralized spongiosa of "Lioplast®" series testing did not reveal any cytotoxic effect and demonstrated their evident biological effect ( cells proliferation stimulation in the culture). The presence of titanium sample with spraying results in adhesion decrease and the appearance of cells with vacuole dystrophy. Titanium samples without spraying are not toxic. Conclusion. Allogenic HAP and spongiosa of "Lioplast®" series as well as titanium sample without spraying are biologically compatible, not cytotoxic, stimulate the cells proliferation in the culture. KollapAn-G, Kollapol and titanium sample with synthetic HAP spraying are slightly cytotoxic: they cause vacuole and fatty fibroblasts dystrophy, reduce their proliferation activity and adhesion.

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**O-58**

**BMP-2 AND BMP-7 CONTENT IN DBM PUTTY PRODUCED BY TISSUELAB IN A GMP PHARMACEUTICAL PLANT**

RAMELLI, M.; DELLA VALLE E.; MARINIELLO R. *Tissuelab S.P.A. Italy.*

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**Abstract/Introduction:**

BMP-2 and BMP-7 are bone morphogenetic proteins, that have a putative role as osteogenic factors in vivo in the healing of bone defects. They have been implicated as a bone-stimulating agent for spinal fusion therapy and the treatment of non-union fractures. This study investigates the capacity of processing method developed by Tissuelab to safeguard the presence of these proteins. Material: The cortical bone is obtained from 50 bone donations processed for Florence and Treviso Tissue Banks (Italy) and BISLIFE (The Netherlands). Quantikine immunoassay kits for BMP-2 and BMP-7 were purchased (R&D Systems) Methods: DBM putty is produced by mixing DBM powder with a carrier composed by PEG and glycerol. The paste is successively sterilised with gamma radiation at low temperatures (-80°C). On every batch Tissuelab carries out an ELISA assay to quantify the content of BMP-2 and 7. The BMPs were extracted from DBM powder with 4 M guanidine-HCl in 0,5 M sodium acetate and then dialysed with an ipotonic solution. The samples were analyzed with the immunoassay kits and spectrophotometric detection (450 nm).

**Results:**

The ELISA analysis detected BMP-2 and BMP-7 in all 50 samples of DBM product. The average content of BMP-2 was 36,2 ng/g of tissue with a coefficient of variation of 79,4%; the average concentration for BMP-7 was 99,6 ng/g of tissue with CV%= 56,4% Discussion and conclusion: The bone morphogenetic proteins (BMPs) make up a subgroup of the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily. They were originally identified as regulators of cartilage and bone formation. They have also been implicated in embryogenesis and morphogenesis of various tissues and organs. According to literature there is an high variability related to characteristic of donors, such as gender, age and presence of metabolic bone disease. The obtained values are in compliance with the BMPs content calculated for similar products by Hyun W. Bae et al., (2006). The BMP values are measured by ELISA assay before and after Tissuelab processing and BMP content is preserved for about 80%, both for BMP-2 and BMP-7 The Tissuelab process minimally affects BMP values in putty and their content is closely related to the donor characteristic. References: 1) Hyun W. Bae et al., Spine (2006); 12:1299-1306. 2) Blum N. et al., Orthopedics 2004; 2: 161-5 3) Alanay A. et al., Spine J. 2008 Sep-Oct;8(5):789-95.

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**O-59**

**DIFFERENTIATION OF PORCINE DERMAL CELLS INSIDE AUTOLOGOUS FIBRIN SCAFFOLDS**

PUENTE, P.; LUDEÑA D.; LÓPEZ M.; RAMOS J.; ARANDA LUIS J.; VARELA G.; IGLESIAS J.  
*Tissue Bank San Francisco Clinic Foundation. US.*

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**Abstract:**

Mesenchymal stem cells (MSCs) are an ideal candidate cell type for tissue engineering and regenerative medicine. Our aim has been to evaluate multilineage potential of porcine dermal cells to differentiate inside autologous fibrin scaffolds. Fibrin scaffold provide a 3D structure for the cells to adhere, proliferate and differentiate. Porcine Dermal Cells have showed the ability of differentiation in vitro towards adipocytes, osteoblasts, and chondrocytes inside autologous fibrin scaffolds. Oil Red O staining confirmed the presence of intracellular red lipid droplets in adipogenic differentiated cells, Von-Kossa staining showed an evident mineralization of the matrix with calcium deposits in osteoblasts differentiated cells and osteogenic

marker osteopontin was detected, and Alcian Blue staining revealed mucopolysaccharides synthesized and immunohistochemistry of collagen type II evidenced the production of collagen fibers by chondrogenic differentiated cells. In approximately a month we have been able to obtain differentiated cells in a 3D scaffold, which is totally autologous, biocompatible and implantable. Autologous fibrin scaffolds represent a suitable structure for cell growth and tri-lineage mesenchymal differentiation, suggesting that fibrin scaffolds may be useful for tissue engineering.

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## O-60

### **COMPARISON OF DIFFERENT SEEDING STRATEGIES TO ENHANCE FIBROBLAST PENETRATION WITHIN A HUMAN ACELLULAR DERMIS FOR SOFT TISSUE AUGMENTATION**

VITACOLONNA, M.; SMITH M.; HOHENBERGER P.; ROESSNER E. *Universitätsmedizin Mannheim ; Chirurgische Klinik. Germany.*

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#### **Abstract/Introduction:**

Effective cell seeding often determines the success of tissue-engineering products. To create a stable soft tissue replacement, it would be desirable to achieve a maximum seeding efficiency, but also a homogenous cell distribution throughout the ADM. Natural matrices such as acellular dermis have the disadvantage of low permeability, due to their dense network, compared to synthetic materials with larger pore size. The purpose of this investigation was to compare different cell seeding methods regarding their seeding efficiency, homogeneity, infiltration depth and proliferation within an acellular dermis. Methods The examined methods can be divided into static, dynamic seeding techniques and a combination of both optional with PDGF as mitogen. Static seeding techniques include surface seeding, pretreatment of ADM with collagenase, direct injection of cell suspension by a syringe, cutting the matrix to increase the surface and diffusion and application of low-pressure and ultrasonic bath to remove trapped air. Dynamic seeding methods include an orbital shaker and the use of centrifugal force with different rotational speed and duration. After seeding, ADMs were incubated for up to 12 days and analyzed at day 0, 4, 8 and 12. At each corresponding time point, seeded ADMs were fixed, embedded vertically in paraffin, histologically sectioned and stained with propidium-iodide to analyze the cell distribution and penetration depth. Furthermore, cell proliferation, seeding efficiency and survival was evaluated by a MTT assay.

#### **Results:**

When using static methods without low-pressure pretreatment, cells were deposited on the surface as a single layer and no penetration into the matrix could be detected. However, after degassing the matrix, we were able to detect a significant improvement in penetration and proliferation. Dynamic seeding using a centrifuge increases the initial number of entrapped cells into the ADM; nevertheless we could neither demonstrate a high proliferation nor find any cells in the central areas. Whereas centrifugal force combined with low-pressure forces significantly more cells inside the ADM and increases the cell mass and homogeneity within 12 days than compared to the other methods.

#### **Discussion:**

As we have shown, the air in the pores significantly impeded the proliferation and therefore the penetration. Thus, the use of a single conventional method results in relatively inefficient colonization results when trying to colonize a dense matrix. We could archive the highest seeding efficiency, homogeneity, infiltration depth and proliferation by using low-pressure and centrifugation at 300g for 3x 1Min in addition with PDGF. Thus, we conclude that this combination is the most effective to repopulate dense natural matrices for soft-tissue augmentation.

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**ORAL - NOVEMBER 10TH - CORNEA**  
**ROOM 1 - 15.15-16-55**

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**O-61**

**CORNEAL ASSESSMENT USING LIGHT MICROSCOPY: THE DIFFERENCE BETWEEN HEALTHY AND PATHOLOGICAL TISSUE**

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Organ culture is the method of choice for the preservation of a human donor cornea for transplantation throughout Europe. This method is often closely connected with the assessment of the cornea using light microscopy.

Photographs from light microscopy (both phase contrast and bright field) will be presented. The individual corneal layers (the corneal epithelium, the stroma and the endothelium) obtained from both normal and pathological corneas will be shown.

The presentation will be focused particularly on qualitative and quantitative changes of the corneal endothelium, such as endothelial cell density, polymegathism, pleomorphism, and other abnormal endothelial morphologies, including nuclear abnormalities. Epithelial and stromal opacities will be presented.

Photographs taken before and after corneal storage in organ culture will be compared and discussed in relation to the changes in endothelial morphology and the swelling of the intercellular spaces. Bacterial and fungal contamination of the corneas will be shown.

Among corneal pathologies, cornea guttata, corneal dystrophies (Fuchs endothelial corneal dystrophy, posterior polymorphous corneal dystrophy) and crystalline keratopathy will be shown using pathological explants. Moreover, the corneal pathologies will be correlated with histological findings. Finally, bacterial and fungal contamination of the cornea during organ culture will be shown.

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**O-62**

**CORNEAL PROCUREMENT, BANKING AND TRANSPLANTATION IN FRANCE**

*MARTINEACHE, I. France.*

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**O-63**

**PARAMETERS INFLUENCING MICROBIOLOGICAL SAFETY OF CORNEAS**

*VAN GEYT, C. Belgium.*

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## O-64

### QUALITY MANAGEMENT WITHIN THE EYE BANK

*GAREISS-LOK, A. CEBT CEO, Hornhautbank Muenchen GmbH, Munich. Germany.*

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#### **Purpose:**

Establishment of a reproducible and traceable quality management system within the Eye Bank to release corneal tissues for all purpose of surgery.

#### **Methods:**

Standardized evaluation of corneal tissues with slitlamp- and endothelium microscopy within a closed system.

Corneal tissues are retrieved by enucleation of whole eye (preparation of corneal button is performed in eye bank under sterile conditions) or in-situ-excision of corneal button. Every corneal tissue is stored within corneal viewing chambers – no matter if organ culture or hypothermic storage is performed which allows every necessary examination within a 'closed system'. All examinations are documented and filed within the database system of eye bank. Special programmed authorization and lodged quality criteria will avoid wrong usage of evaluated tissues.

#### **Results:**

The developed and established way of evaluation in combination with an exclusively programmed data base system reduced the failure rate of wrong documentation which may lead to wrong usage of corneal tissues tremendously (failure rate < 1%).

#### **Conclusion:**

The establishment of a unique customized database system in combination with evaluation of corneal tissue leads to a reproducible and traceable documentation of the tissue evaluation within the eye bank.

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## O-65

### PRE-CUTTING OF GRAFTS FOR POSTERIOR LAMELLAR KERATOPLASTY

*JESPER HJORTDAL, MD, PhD, The Danish Eye Bank, Department of Ophthalmology, Aarhus University Hospital, Nørrebrogade 44, 8000 Aarhus C, Denmark.*

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Today, the preferred treatment of primary and secondary corneal endothelial failure is posterior lamellar keratoplasty. In this technique, a human donor cornea is split in two parts. The inner part consisting of endothelial cells, Descemets membrane, and a thin part of stromal lamellae is inserted into the anterior chamber of the patient's eye and pressed towards the inner side of the patient's cornea by air.

Preparation of the posterior lamellar graft is typically done in the operating theatre by the surgeon. This process is time-consuming and failure in the preparation may result in cancellation of surgery.

Preparation of the posterior lamellar graft in the eye bank one or more days before surgery is possible. In the United States such pre-cutting of corneal donor tissue before cold storage in Optisol is very common. In Europe, organ culture storage is the most common storage method and there is very little published experience with pre-cutting of organ cultivated donor corneas.

In the presentation, a summary of studies on the use of pre-cut corneal donor tissue will be given, along with the authors experience of using pre-cut organ cultivated tissue for posterior lamellar keratoplasty.

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**O-66**

**SEROLOGY AND MICROBIOLOGICAL TESTING IN EYE BANKS:  
LEGAL ASPECTS IN EUROPE**

*MAIER, P. University Eye Hospital Freiburg. Germany.*

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When the EU Directives 200423EC, 200617EC and 200686EC were transformed into the national legislation of the EU member states many procedures including the serological testing of the donor and the microbiological testing of the culture media were regulated in a new way.

To minimize the risk of transmitting an infectious disease of the donor testing for HIV antibodies, hepatitis B antigen, hepatitis C antibodies, and Lues antibodies have already been standard procedures in most European eye banks. According to the EU directives tests for Hepatitis B core antibodies were added for donor screening and some countries even integrated NAT testing for HIV, HBC and HCV in their national test algorithm. However, it is not clear, whether these additional blood tests might help to improve patients' safety as there are only two suspected transmissions of Hepatitis B by corneal grafts that happened when no routine testing was done at all. The major problem of the EU directives however was the decision of the European commission to limit the time frame for taking blood samples from deceased donors for serological and NAT testing to 24 hours whereas the cornea can be procured up to 48 or even 72 hours after death. This so called 24 hour rule lead to a significant reduction in corneal donation in many European countries although the reason for this limitation remains unclear as there is no scientific evidence that the 24 rule leads to increased safety of the tissue. Besides the problem of the 24 hour rule there are no validated serological and NAT tests for postmortal blood samples so that in some regions blood from deceased donors is not accepted at all. To overcome these problems a clinical trial has been performed that proved that serological and NAT test values regarding HIV, HBV and HCV remain stable for at least 48 hours after death of the donor and a validation for the necessary tests was also done. Therefore, we hope that it will be possible to change the European directive regarding the 24 hour rule in the future.

Besides testing of the donor for infectious diseases the media of the tissue need to be tested microbiologically in order to detect contaminations of the tissue to avoid transmissions of bacterial infections by the graft. In this context many different testing methods exist ranging from simple agar plates to membrane filtration techniques. The European Directives do not give any detailed information on how the sterility tests should be performed so that each member state has to define its own regulations. In Germany for example there is no specific tissue law, but all tissues are included under the pharmaceutical law. This leads to various problems where one major issue is the sterile testing of the culture media. According the European Pharmacopoeia membrane filtration is the only suitable method for sterility testing of solutions like the organ culture media containing antibiotics and antimycotics. However, this method is very time and cost consuming compared to the currently most common method using blood culture bottles for sterility testing. Furthermore, as for serological tests there are no validated methods for sterility testing of culture media from corneal organ culture. Besides the cost factors one has to keep in mind that the cornea itself is never sterile, so if testing becomes too sensible many tests may give positive results leading to an even more reduced availability of donor tissue.

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**O-67**

**IMPLEMENTATION OF EUROPEAN LEGISLATION IN EYE BANKING: THE CZECH EXPERIENCE**

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**Purpose:**

To draw attention to problems arising from the implementation of European legislation in the Czech Republic.

**Introduction:**

**Act No 296/2008 Coll.** (Act on Human Tissues and Cells) and **Decree No 422/2008 Coll.** (concerning the detailed requirements for the safeguarding of the quality and safety of human tissues and cells) have been enacted in the Czech Republic implementing the European legislation: **Commission Directives 2004/23/EC, 2006/17/EC and 2006/86/EC.**

Czech eye banks need accreditation from the State Institute for Drug Control (SIDC) as the competent authority. During inspections and evaluations with regards to legislation, these directives have started to be strictly enforced.

We would like to draw attention to difficulties with the observance of the rules. Some of the critical material routinely used for tissue culturing, defined as In Vitro Diagnostic Medical devices (Directive 98/79/EC), is no longer allowed to be used and must be replaced by material based on Directive 93/42/EEC (Medical Devices). Thus, materials such as minimal essential medium, phosphate buffer and antibiotic solution (AppliChem, Germany or Gibco/Invitrogen, UK) have to be replaced by commercial solutions (Eurobio, France or Alchimia, Italy). This situation especially affects the storage of corneas in organ culture. No commercial solutions are offered for the storage of human amniotic membranes. Similar problems arise with the use of storage plastics.

The resulting increase in the cost of the tissue is not matched by an increase in the reimbursement offered by insurance companies. The changes required by Czech legislation can not be introduced without appropriate clinical studies, and their impact on tissue quality and safety has yet to be assessed.

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**O-68**

**PROSPECTIVE DEVELOPMENT OF EYE BANKING IN THE RUSSIAN FEDERATION**

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**O-69**

**ORGANOTIPIC CULTURE PRESERVATION AS STRATEGY TO INCREASE THE NUMBER OF CORNEAS FOR TRANSPLANTS**

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**Abstract/Introduction:**

Our Tissue Bank is using simultaneously two methods for preserving corneas from retrieval to transplant: Preservation in Optisol® at 4-8°C with a maximum expiration date of 8 days (fresh corneas) and organotypic culture preservation during 14-28 days. Corneas in organotypic culture method from January 2010 till April 2011 were included in this study. In that period 33.2% of the retrieved corneas were organ-cultured. The final suitability for transplantation was 76% for fresh corneas and 62% for cultured ones.

**Purpose:**

To evaluate the quality and microbiological status of organ cultured corneal grafts from donors who have died mainly from septic multi-organ failure. Methods: 390 corneal grafts (205 donors) from septic and non-septic donors were stored in organ culture method at 31°C for 14-28 days. Evaluation of cultured corneas was carried out by specular microscope, slit lamp microscope and optic microscope examination. From each cornea donor one blood culture for aerobic and aerobic microorganisms was taken at the moment of clinical screening and a second blood culture during the tissue retrieval. Three microbiological tests have been performed at different preservation stages, the first microbiological sample was taken from the medium after 5 days of culture, the second one corresponded to the moment that tissue is transferred to the deturgescence medium and the final one 24 hours after. Corneal grafts with cell density values above 2000 cells/mm<sup>2</sup> were transplanted. Follow-up and traceability of transplanted corneas were analyzed taking into special consideration on satisfactory clinic evolution complications and microbiological controls.

**Results:**

In the period of study 390 corneas were included in the organotypic culture group in which 323 (82.8%) corneal grafts were finally put in organ-culture from 205 donors and 67 (15.9%) were discarded due to don't achieve corneal quality criteria or donor history. The average of donor age was 67.8 years ( $\pm$  13.9). From the group of cultured corneas 244 (75.5%) were suitable for transplantation and 79 (24.4%) were non-suitable from which 11 (3.4%) were discarded due to contamination medium. In contrast, 158 corneal grafts (40.51%) had positive blood cultures. In the follow-up forms analyzed 97.8% had a satisfactory evolution versus 2.2% that had not a good evolution because of persistent epithelial defect with overinfection and corneal oedema during 2 months.

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**O-70**

**MICROBIAL RISK ASSESSMENT IN EYE BANKING**

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**Abstract:**

The manufacture of human cornea transplants is regulated by comprehensive rules to ensure that sound, high quality practices are followed to reduce the risk of tissue contamination and of communicable disease transmission to recipients. A central part of the manufacturing process in a GMP facility is a thorough risk assessment process. Even the healthy human eye as part of our body is steadily colonized by microorganisms. Therefore, the preparation of corneal tissues, even under rigorous conditions of good manufacturing practice (GMP), inevitably harbors a risk of pathogen transmission into the manufacturing plant. The main objectives of this study were (1) to determine the extent and specificity of the initial contamination of donated eyes upon arrival in the eye bank, (2) to examine the specificity and extent of pathogen transfer to the surrounding environment during corneoscleral disc preparation, (3) to develop a rational approach for microbial monitoring of corneoscleral disc processing. GMP-compliant manufacturing of cornea transplants was done in the grade A area (laminar air flow cabinet (LAF) of the clean room with the background environment of grade B. The work space of the LAF was divided in three zones - a tissue decontamination zone, an in-between zone and a zone for corneoscleral disc processing. Airborne microbial contaminations of these zones during the manufacturing process within the LAF were analyzed by use of settle and contact plates. The initial microbial flora of donated eyes was investigated through swab approaches. Subsequently, eyes were decontaminated in 5% povidone iodine solution for 5 min. Prepared corneoscleral discs were stored in MEM supplemented with 2% FBS, antibiotics (penicillin, streptomycin) and antimycotics (amphotericin) at 37° C in a closed culture system without exposure to carbon dioxide. At day 4 to 5 the presence of contaminants in the organ culture medium was studied via BACTEC Plus blood culture systems. The results of this study show that (1) the PVP decontamination treatment during the eye procurement process, moistening the eye surface with antibiotics (gentamicin) and adherence to temperatures between 2 and 10 during transport to the eye bank result in a significant reduction in microbial contaminations; (2) the manufacturing process does not notably increase the risk of pathogen transmission; (3) the enrichment of organ culture medium with common antibiotics and antimycotics effectively eliminate most of the remaining contaminants. However, a strict separation of work space under the LAF during corneoscleral disc processing and a continuous microbial monitoring are strongly recommended for a safe manufacturing process.