

PUBLICATION OF THE SUPERIOR HEALTH COUNCIL No. 8698**Microbiological examination of human body material for human use to guarantee maximum microbiological safety: practical recommendations**

In this science-policy advisory report, the Superior Health Council issues practical recommendations on microbiological control for human body material intended for human use to ensure maximum microbiological safety

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1. INTRODUCTION

According to the Royal Decree (RD) laying down quality and safety standards for the donation, collection, procurement, testing, processing, storage and distribution of human body material (HBM) (RD, 2009), HBM establishments are responsible for the microbiological safety of HBM that they supply for potential recipients.

The working group “Cells, Tissues and Organs of Human and Animal Origin”, which is in charge of preparing quality and safety standards (Act of 19 December 2008) for HBM has found it necessary to provide additional information on some of the microbiological aspects related to the use of HBM such as testing, safety measures, etc. These microbiological aspects will be looked at both from a general perspective, as some of them are common to all tissues and cells, as well as with a focus on the specific characteristics of the various types of tissues and cells.

Recommendations will be made, based on advisory reports 8143, 8763 and 8785 of the Superior Health Council (SHC) (inactivation and safety of tissues and cells with respect to prions, bacteria and viruses, respectively) as well as on practices and the requirements of the new national regulations (SHC 8143, 2008; SHC 8763, 2014; SHC 8785, 2012).

The recommendations described in this advisory report supplement the specific quality standards for tissues and cells (SHC 8716, 2013) as regards microbiological standards.

This advisory report covers only aerobic and anaerobic bacteria (including mycoplasmas where cells are concerned), yeasts and moulds. It does not cover viruses and prions.

An ad hoc working group has been created to deal with issues related to the microbiological safety of HBM; it consists of experts in the following areas: cell and tissue banking, cell therapy, hospital hygiene, and clinical and pharmaceutical microbiology. This advisory report has been the subject of extensive discussions and was finally approved by the standing working group “Cells, Tissues and Organs of Human and Animal Origin”.

2. RECOMMENDATIONS

Any HBM intended for use in humans must be subjected to microbiological examination to detect any aerobic and anaerobic bacteria, yeasts and filamentous moulds. For such HBM to be considered microbiologically safe, these micro-organisms must not be detected in the final material. The aim of microbiological quality control is to provide the recipient with maximum assurance of the biological safety and quality of the HBM.

However, the manager of the HBM establishment may waive this general recommendation after thorough analysis of the risk posed by the contaminated HBM and its potential adverse effects, in particular:

- When the HBM is scarce and/or needed for major clinical indications or life-threatening conditions and/or where there are few therapeutic alternatives;
- For HBM naturally contaminated by commensal flora and/or when commensal flora is acceptable for human use: sperm, skin, etc.).

The decision to waive this rule is made in cooperation with the attending physician or transplant physician and is based on a procedure for exceptional release of the HBM.

The above recommendations are intended to provide specific information on the microbial sample (the material to be examined, the minimum quantity of this material), the culture methods as well as the specifications for detection and for reporting on the microbiological examination. They must be considered to be minimum tests. HBM establishments may carry out more extensive testing for the types of HBM they are authorised to handle. Any application of less restrictive standards must be validated by the establishment.

Keywords

Keywords	Mesh terms*	Sleutelwoorden	Mots clés	Stichwörter
Microbiology	Microbiology	Microbiologie	Microbiologie	Mikrobiologie
Bacteriology	Bacteriology	Bacteriologie	Bactériologie	Bakteriologie
Mycology	Mycology	Mycologie	Mycologie	Mykologie
Culture methods	Culture techniques	Cultuurmethoden	Méthodes de culture	Kulturverfahren
Human cells	Human, Cells	Menselijke cellen	Cellules humaines	Menschliche Zellen
Tissue allografts	Tissue transplantation	Weefselallogreffen	Allogreffes tissulaires	Allogene Gewebetransplantate
Human body material		Menselijk lichaamsmateriaal	Matériel corporel humain	Menschliches Körpermaterial
Culture medium	Culture media	Kweekmedium	Milieu de culture	Nährboden
Skin allografts	Homograft dressing	Huidallogreffen	Allogreffes de peau	Allogene Hauttransplantate
Cell culture	Cell culture techniques	Celcultuur	Culture cellulaire	Zellkultur
Musculoskeletal allografts	Bone-patellar tendon-bone allograft	Muskuloskeletale allogreffen	Allogreffes musculosquelettiques	Allogene musculoskeletale Transplantate
Reproductive cells	Germ cells	Reproductieve cellen	Cellules reproductrices	Geschlechtszellen
Tympano-ossicular allografts	Tympanic membrane	Tympano-ossiculaire allogreffen	Allogreffes tympano-ossiculaires	Allogene Gehörknöchelchen- und Trommelfelltransplantate
Amniotic membrane allografts	Amniotic membrane dressing	Amnionmembrana-allogreffen	Allogreffes de la membrane amniotique	Allogene Amniontransplantate
Corneal allografts	Corneal transplantation	Cornea-allogreffen	Allogreffes de cornées	Allogene Hornhauttransplantate
Scleral allografts	Sclera	Sclera-allogreffen	Allogreffes de sclérotiques	Allogene Skleratransplantate
Heart valve allografts	Heart valves/transplantation	Hartklepallogreffen	Allogreffes de valves cardiaques	Allogene Herzklappentransplantate
Vascular allografts	Vascular grafting	Vaatlogreffen	Allogreffes vasculaires	Allogene Gefäßtransplantate
Hematopoietic stem cells	Hematopoietic stem cells	Hematopoëtische stamcellen	Cellules souches hématopoïétiques	Hämatopoietische stammzellen
Hepatocytes	Hepatocytes	Hepatocyten	Hépatocytes	Hepatozyten
Keratinocytes	Keratinocytes	keratinocyten	Kératinocytes	Keratinocyten
Chondrocytes	Chondrocytes	Chondrocyten	Chondrocytes	Chondrozyten
Beta cells	Insulin-secreting cells	Bêtacellen	Cellules bêta	Betazellen
Cord blood	Cord blood	Navelstrengbloed	Sang de cordon ombilical	Nabelschnurblut

* MeSH (Medical Subject Headings) is the NLM controlled vocabulary thesaurus used for indexing articles for PubMed.

3. FURTHER DETAILS AND ARGUMENTATION

List of abbreviations

ABEF	<i>Association Belge des Embryologistes Francophones</i>
ATMPS	Advanced therapy medicinal products
CFU	Colony forming unit
IVF	In vitro fertilisation
ICSI	Intracytoplasmic Sperm Injection
HBM	Human body material
MAP	Medically assisted procreation
PCR	Polymerase chain reaction
QMS	Quality Management system
RD	Royal decree
SHC	Superior Health Council

3.1. Introduction and methodology

3.1.1. Introduction

The HBM from which the cells and tissues are prepared (source material) constitutes in itself the main source of microbiological contamination of human cells and tissues. The percentage of organs that are contaminated during removal varies between 2.2% and 84% (Carroll et al., 1992; Lakey et al., 1995; Lehec et al., 2009; Scharp et al., 1992; Zibari et al., 2000). The skin is constantly colonised by resident micro-organisms (Pirnay et al., 2012) and the level of contamination of tissues removed from the locomotor system varies between 8.6 and 50% (Barbour & King, 2003; Deijkers et al., 1997; Forsell & Liesman, 2000; Ibrahim et al., 2004). Similarly, over 90% of sperm samples are contaminated during collection (Nicholson et al., 2000).

The differences in the levels of contamination reported in the literature may be attributed to various factors:

1. The environmental conditions play a major role in the potential contamination of HBM. The level of contamination of tissues collected in operating rooms is 5 to 11% lower than in other collection sites such as hospital morgues (Forsell & Liesman, 2000). As regards instructions on optimising the environmental conditions during collection and processing, we refer to advisory report 8699 of the SHC (2012) on the validation and control of the environment in HBM establishments.
2. Blood cultures that are positive at the time of collection are much less frequent in multi-organ donors than in post-mortem donors (Barbour & King, 2003).
3. The size of the recovery team and whether or not an autopsy was performed before the tissues or cells were collected play a significant role (Deijkers et al., 1997; Forsell & Liesman, 2000).
4. The types of samples collected to detect microbiological contamination do not permit comparison between studies: cultures of donor blood or urine, microbiological samples from perfusion liquid, expectoration or transport media, use of swabs, tissue fragments or cell suspensions (Deijkers et al., 1997; Zibari et al., 2000).

5. The type of organ or tissue, the removal method and the duration of transport to the HBM establishment play an important role. The level of microbiological contamination of the transport media for pancreases removed in order to isolate beta cells ranges from 19 to 84% (Bucher et al., 2005; Carroll et al., 1992; Lakey et al., 1995; Scharp et al., 1992). When pancreases are removed in order to isolate beta cells, some of the small intestine is usually also removed, which is the cause of the high level of contamination of the transport medium (Bucher et al., 2005). The average level of contamination of the transport medium for livers removed to isolate hepatocytes is 37.5% upon arrival at the HBM establishment (Lehec et al., 2009).

Since the many steps from collection to allocation all involve a risk of contaminating the HBM, it is necessary to check the microbiological safety of the tissues and cells (in particular as regards aerobic and anaerobic bacteria, yeasts and filamentous moulds).

This advisory report provides HBM establishments with practical recommendations concerning sampling for culture preparation, microbiological culture methods and detection/reporting.

3.1.2. Methods

After examining the request, the Board and the chair of the working group identified the required types of expertise. The experts of the group filled in a general and an *ad hoc* declaration of interests and the Committee on Deontology assessed the potential risk of conflicts of interest.

These recommendations are based on the grey and scientific literature and the experience of consulted experts in the field.

After approval of the draft advisory report by the standing working group “Cells, Tissues and Organs of Human and Animal Origin”, the Board validated it as a final step.

3.2. Sampling

Macroscopic examination (odour, colour, turbidity, etc.) may reveal contamination, especially when standard culture media are used.

3.2.1. Sampling method for culture preparation

The following types of samples may be used:

- Swabs
 - Advantages:
This technique makes it possible to collect samples over large areas. More recent swabs using the flocked swab technique with greater capillary action yield a higher bacterial recovery than conventional swabs such as cotton swabs (Saegeman et al., 2011). A flocked swab has a tip covered in short nylon fibres perpendicularly attached to the swab; a highly absorbent layer with an open structure is thus created.
 - Disadvantages:
Sampling with swabs is not very sensitive (Martinez et al., 2003). In addition, the low recovery efficiency of the various types of swab introduces a factor of uncertainty (Winters, 2003).
- Fragments
 - Advantages:
Residual tissue fragments provide real microbiological information on a graft.
 - Disadvantages:
 - Accurate information requires taking a representative sample but such sampling concerns only a very small part of the HBM in question. Consequently, the information is not very representative and there is a real risk of false negative results.
 - Tissue taken with the graft is not always a good indicator of possible microbiological contamination of the graft itself.
 - For most tissues, contamination of the HBM during collection/transport or during preparation involves parts of the outer surface of the HBM rather than the inside. Microbiological examination of the surface of the tissue (supernatant medium / transport medium or swab) therefore complements the examination of the tissue fragments.
- Transport media/liquids, rinsing liquids, storage media/liquids, liquid samples.
These are liquids in or with which the tissues/cells have been transported, processed or stored. They may be subsequently inoculated into solid or liquid culture media (blood culture bottles may also be used), or filtered through a membrane filter which is then placed in culture media.
 - Advantages:
 - more homogeneous sampling;
 - volume concentration when using a membrane filter;
 - when using blood culture bottles, there are few additional handling steps.

- Disadvantages:
 - it cannot be assumed that 100% of the micro-organisms that might be present will be eliminated by simple rinsing or soaking of the tissues. The recovery efficiency of the method is variable (Winters et al, 2003);
 - dilution effects if a membrane filter is not used;
 - when using a membrane filter, further handling steps are necessary which entail an additional risk of secondary contamination of the sample to be analysed (false positives);
 - obstruction of the membrane filters;
 - membrane filtration is not a technique that is used routinely in most microbiological laboratories;
 - risk of false negatives for commensal contaminations inside certain tissues.

3.2.2. Quantity and number of samples

Specific recommendations are given later in this document concerning the quantity of samples to be collected as part of the microbiological examination of the various tissues/cells.

A summary of the general instructions is given below.

- Samples of tissues packed into containers

Table 1 shows the required number of containers described in the European Pharmacopoeia 7.1., 2.6.1 (2011 and subsequent editions) in the test for sterility. The number of containers to be tested for the microbiological examination depends on the total number of containers prepared from the same source material.

Table 1. Minimum number of items to be tested
(European Pharmacopoeia, table 2.6.1.3.)

Number of containers	Minimum number of items to be tested (unless otherwise justified and authorised)
≤ 4 containers	Each container
4 < containers ≤ 50	20% , not less than 4 containers
> 50 containers	2% , not less than 10 containers

Owing to the limited availability of HBM, the SHC proposes that the minimum number of samples of the final HBM product to be collected after processing is the following:

- a sample of each graft in a separate container;
- for material prepared in batches, we have chosen to modify the procedure prescribed in the European Pharmacopoeia 7.1, 2.6.1 (2011 and subsequent editions); i.e., for a batch of:
 - > 50 containers: 5% of the packed samples / containers of residual tissue fragment;
 - ≤ 50 containers: 2 packed samples / containers of residual tissue fragment.

- Liquid samples: transport media, rinsing liquids, storage media, liquid samples

Table 2 shows the required volume for the sample described in the European Pharmacopoeia 7.1., 2.6.1. (2011 and subsequent editions) in the test for sterility. The quantity of samples for

the microbiological examination by inoculation depends on the total volume of the liquid sample.

Table 2. Volumes of liquid samples to be tested
(European Pharmacopoeia, table 2.6.1.2.)

Total volume of the liquid sample (mL)	Inoculum volume
$V > 40$	10% of the contents of the container but not less than 20 mL
$1 \leq V < 40$	Half the contents of each container but not less than 1 mL
$V < 1$	The whole contents of each container

The test for sterility as described in the European Pharmacopoeia 7.1, 2.6.1 (2011 and subsequent editions) for human cells is not always applicable owing to the volume required for the sample. The samples to be collected for the microbiological examination of cell products are described in Table 3.

Table 3. Volumes to be tested for samples with limited volumes
(European Pharmacopoeia. 2.6.27 (2011 and subsequent editions))

Total volume of the liquid sample (mL)	Inoculum volume
$V \geq 10$	At least 1% of the total volume
$1 \leq V < 10$	At least 100 μ L

Depending on the technique used for microbiological analysis, the type of HBM, its use, the volume collected and other specific aspects to be justified in the Quality Management System (QMS) of the HBM establishment concerned, the above sampling method and/or quantities can be adjusted provided that the method is then validated in consultation with the microbiologist.

3.2.3. Materials used for sample collection

Samples (residual tissue material or [rinsing] liquids) are stored in a closed sterile container until further processing is carried out by the laboratory.

Samples of cell products are inoculated into blood culture bottles when automated detection systems are used.

3.2.4. Conclusions

Samples can be collected for microbiological examination as follows:

- on the source material;
- during processing;
- on the final product;
- when used in the patient.

Microbiological examination must be performed **on the source material** unless other considerations are mentioned in the specific chapter on the HBM. The HBM manager is the only person responsible for evaluating the results of the microbiological examination of the source material and their implications on the process and the final validation of the HBM concerned.

During processing, microbiological examination is recommended to check that the various steps of the preparation are aseptic.

On the final product, microbiological examination is the obligatory test that can guarantee the microbiological safety of the released HBM.

Collecting samples **during the clinical use of the HBM** may be considered (SHC 8716, 2013). Cultures of these are used for monitoring and may provide information in the event of subsequent problems with infection due to the implanted/used/infused HBM. The physician in charge of the use of this HBM in humans is responsible for this microbiological examination rather than the manager of the HBM establishment.

3.3. Microbiological culture methods

3.3.1. Culture media

The main components of culture media are a source of proteins or protein hydrolysate and a specific salt concentration.

3.3.1.1. Aerobic and anaerobic bacteria

Conventional solid culture media such as blood agar or chocolate agar allow not only the culture of rapidly growing aerobic and anaerobic micro-organisms but also of micro-organisms that are difficult to culture.

Examples of conventional liquid culture media are Trypticase Soy Broth and thioglycolate broth. This latter medium is also suitable for the culture of anaerobic bacteria owing to the reducing properties of thioglycolate (Greenwood et al., 2007; Verhaegen et al., 2007).

If a risk of mycobacterial infection (*Mycobacterium tuberculosis* complex and/or atypical mycobacteria) is associated with certain tissues/cells or the medical history of the donor, specific culture media are used for detection (for example, Löwenstein-Jensen, MGIT, Middlebrook 7H10, incubation at 37°C for 6 to 8 weeks, at 30°C for *M. Marinum*) (Warwick et al., 2008).

3.3.1.2. Yeasts and filamentous moulds

Specific culture media are recommended to grow yeasts and filamentous moulds selectively, for example, Sabouraud dextrose agar. Indeed, this medium is specific for yeasts and filamentous moulds owing to its high sugar concentration and low pH. The growth of bacteria and filamentous moulds/saprophytic yeasts is countered by adding antibiotics and cycloheximide, respectively.

3.3.2. Recommendations of the European Pharmacopoeia

As regards microbiological tests for sterility, the European Pharmacopoeia proposes fluid thioglycolate medium, mainly for the culture of anaerobic micro-organisms. Soya bean casein digest medium is suitable for the detection of yeasts, filamentous moulds and aerobic bacteria (European Pharmacopoeia 7.7, 2.6.1., 2011 and subsequent editions). The test for sterility may be carried out either by membrane filtration or by direct inoculation of the sample to be tested into the storage medium. Negative controls are always included. The inoculated medium is incubated for at least 14 days and is checked regularly for turbidity and/or macroscopic evidence of microbial growth.

Other microbiological techniques may be used provided that they are compared with a reference method during a parallel validation study. This should be done with the collaboration of the microbiologist. If the storage media for the cells/tissues contain antibiotics or antimycotics, these potential growth inhibitors must be eliminated by filtering the medium to be examined unless inhibitors or absorbents such as activated charcoal have been added to the microbiological culture medium.

3.3.3. Manual versus automated microbiological incubation systems

To verify the sterility of cell products, the European Pharmacopoeia mentions the use of microbiological culture methods that may be either manual or **automated** (European Pharmacopoeia, 7.0, 2.6.27., 2011 and subsequent editions).

Owing to the long duration of incubation (14 days) and the sample volume required, the test for sterility as described in the European Pharmacopoeia 7.1, 2.6.1 (2011 and subsequent editions) is not always applicable to human cells.

Various studies have shown that automated microbiological culture methods are better suited for the detection of micro-organisms in cell products. These automated culture methods are characterised by greater sensitivity, a shorter detection time, a very small number of false-positive results and a reduced workload compared with the conventional test for sterility (Khuu et al., 2006; Kielpinski et al., 2005; Plantamura et al., 2011; Vigano et al., 2002). Since 2011, these automated microbiological culture methods have not only been accepted in Europe as alternatives to the test for sterility, but have actually been preferred for the microbiological testing of cell products (European Pharmacopoeia 7.1, 2.6.27., 2011 and subsequent editions).

Provided that the automated detection method has been validated in accordance with the European Pharmacopoeia 7.1, 2.6.27 (2011 and subsequent editions), it is sufficient to incubate the samples for 7 days at 35°C-37°C under aerobic and anaerobic conditions in order to detect aerobic and anaerobic bacteria, yeasts and filamentous moulds (Beres et al., 2013; Horvath et al., 2004).

Taking account of the specificity of the HBM, an automated system may be used as a detection method to check the absence of micro-organisms, not only for HBM consisting of cells but also for HBM consisting of tissues.

3.3.4. Duration of incubation

As regards media used for the growth and storage of cells, inoculated media are incubated for 7 days when automated detection systems are used and for 14 days when microbial growth is to be detected visually (European Pharmacopoeia 7.1, 2.6.27, 2011 and subsequent editions).

Similarly, cultures of non-cellular HBM are incubated for 14 days (European Pharmacopoeia 7.1, 2.6.1, 2011 and subsequent editions).

In clinical practice, incubation for at least 7 days is usually sufficient to ensure the clinical safety of HBM. This allows most of the virulent micro-organisms implicated in infectious diseases to grow, with the exception of some filamentous moulds that require longer incubation periods (up to 21 days) (McGowan, 2011; Murray & Witebsky, 2010; Sutton, 2007).

If durations of incubation other than those specified in the European Pharmacopoeia are used, they must be validated in consultation with the microbiologist.

3.3.5. Incubation temperature

For the conventional test for sterility, samples are incubated at 30°C-35°C in thioglycolate media and at 20°C-25°C in soya bean casein digest media (European Pharmacopoeia 7.1, 2.6.1., 2011 and subsequent editions). The European Pharmacopoeia mentions an incubation temperature of 35°C-37°C for inoculated culture media when an automated detection method is used.

If other incubation temperatures are used, they must be validated in cooperation with the microbiologist.

3.3.6. Inhibitory substances

Solutions of antibiotics/antiseptics/chemicals used during the processing of tissues and cells or media that have some bacteriostatic/fungistatic or bactericidal/fungicidal activity (for example, 4% formaldehyde, 70% ethanol, 85% glycerol, DMSO) are whenever possible treated with neutralising agents (such as activated charcoal, resins, dilution and rinsing) before they are inoculated into microbiological culture media. The request form given to the laboratory should therefore mention whether there may be residual quantities of inhibitory substances in the culture samples and should provide the names of these substances.

3.4 Micro-organism detection/reporting

3.4.1. Detection / reporting of the absence of micro-organisms

Microbiological examination of HBM must include testing for aerobic bacteria, anaerobic bacteria, yeasts and filamentous moulds. A specific test for mycobacteria must also be carried out before allografts can be released if the medical history of the donor suggests there is a risk of mycobacterial infection.

The results for aerobic bacteria, anaerobic bacteria, yeasts and filamentous moulds are communicated separately by the laboratory.

The laboratory is expected to provide a clear qualitative result (positive/negative; detected/not detected) concerning the presence of micro-organisms (aerobic and anaerobic bacteria, yeasts and filamentous moulds) and to identify at least the detected micro-organisms.

3.4.2. Bioburden detection / reporting

3.4.2.1. Concept of bioburden applicable to HBM

In the context of the microbiological examination of medicines and medical products, bioburden is defined as the microbial load (the number of live bacteria) in the source material; this known value is used to demonstrate that the preparation process is capable of eliminating these bacteria through the decontamination/sterilisation process described in the procedure (GMP, 2008).

Given that there are few specific references in the literature, this concept cannot be applied directly to HBM; it must be adapted, taking the specific characteristics of HBM into account.

Bioburden in terms of the microbiological examination of HBM may be defined as the number and type of live bacteria in the source material before this material is subjected to sterilisation, inactivation or decontamination. For HBM, bioburden mainly concerns the source materials, sometimes after certain initial treatment steps.

Determining the bioburden of the HBM is used primarily to redirect the process (destruction or additional steps to ensure safety, etc.) and/or to reinforce control measures (identification using Polymerase Chain Reaction (PCR), etc.) to ensure that the microbiological testing of the final product is carried out according to the accepted process. Determining the bioburden also provides opportunities for improvement of the process and control measures.

3.4.2.2. Quantitative approach to the bioburden: microbial load

According to the literature, it has been proven that the time required for (blood)cultures to become positive is correlated with the initial bioburden (expressed as Colony-forming units (CFU)/mL) (Kassis et al., 2009; Mermel et al., 2001).

However, this correlation has been studied well only in the case of blood cultures for which automated detection systems record electronically and very precisely the time required for the culture to become positive.

The same approach can nevertheless be transposed to other culture methods.

Semi-quantitative culture is possible by inoculating solid agar and enriched liquid medium using swabs. If growth is observed only in the liquid medium but not in the solid agar this indicates a rather low microbial load. If growth is observed in both the liquid medium and the solid agar this means that the tissue is significantly contaminated or has a high microbial load (Pirnay et al., 2012).

The European Pharmacopoeia describes different methods to determine the microbial load quantitatively (European Pharmacopoeia. 7.1. 7.1., 2.6.12, 2011 and subsequent editions).

As part of the quantitative approach to the bioburden of the source HBM, it may be acceptable to only test whether or not micro-organisms are present 3-5 days before the re-inoculation to identify the micro-organisms.

The person responsible for the HBM, in collaboration with the microbiologist of the analytical laboratory, may choose and document other methods, incubation times and criteria depending on the type of HBM, the preparation process or the clinical indications.

3.4.2.3. Qualitative approach to bioburden: classification of micro-organisms according to their degree of virulence

It is important to identify the micro-organisms that have been isolated during the microbiological examination of HBM.

The micro-organisms found on the HBM when collected and before processing can be classified according to their degree of virulence (Table 4).

The degree of virulence of bacteria depends on many factors: virulence factors related to the micro-organism, the immune status of the recipient of the specific tissue (namely, patients with severe burns, patients with haematological conditions, etc.), the tissue type (external application or implantation), how and where the micro-organism has entered the body (associated with the type of tissue to be implanted) and antibiotic resistance.

In this context, Casadevall & Pirofski's damage-response model is interesting (SHC 8763, 2014). This classification scheme for microbial pathogens takes account of the contributions of both the host and the micro-organism (pathogen) (Casadevall & Pirofski, 2003). This model is based on the ability of micro-organisms to cause damage depending on the immune response of the host (Casadevall & Pirofski, 2000).

"High virulence" indicates a high potential pathogenic risk to the recipient. In principle, tissue with such risk must be destroyed.

For certain types of HBM whose source may be naturally contaminated before collection (sperm, skin, etc) or for tissues that are scarce and/or used in vital indications (skin, amniotic membranes, cardio-vascular allografts, etc), the physician responsible for the HBM, when faced with a bioburden consisting of micro-organisms listed in Table 4, may legitimately take, after a risk/benefit analysis, documented and validated measures or additional steps in the

process and/or test to ensure the microbiological safety of the allografts supplied. In such cases he or she must always inform the physician implanting the material.

"Low virulence" means in principle that the pathogenic risk to the recipient is low and that this low virulence is acceptable in the microbiological samples collected before the decontamination/sterilisation procedure is carried out.

Table 4. Micro-organisms considered to be highly virulent if detected in the collected HBM before processing (Martinez, 2003; Pellet, 1996; AATB, 2002) (non-exhaustive list).

Staphylococcus aureus

Beta-haemolytic *Streptococcus*, *Enterococcus* spp

Non-fermenting micro-organisms: *Pseudomonas* spp, *Acinetobacter* spp, *Stenotrophomas maltophilia*, *Sphingomonas paucimobilis*, *Burkholderia cepacia*

Sporulating micro-organisms: *Bacillus* spp (*B. anthracis*, *B. cereus*), *Clostridium* spp

Enterobacteriaceae: *Escherichia coli*, *Enterobacter* spp, *Salmonella* spp, *Shigella* spp, etc.

Gram-negative anaerobic micro-organisms: *Bacteroides* spp, *Prevotella* spp, *Porphyromonas* spp, *Fusobacterium* spp

Yeasts and filamentous moulds

3.5 Microbiological examination of specific tissues

3.5.1. Skin allografts

3.5.1.1. Introduction

Healthy human skin is constantly colonised by (non-)pathogenic micro-organisms associated with skin (transient, resident and temporary resident flora). This is why topical disinfection and antibiotic treatment are frequently applied to decontaminate skin allografts. Given that the efficacy of antibiotics is now in doubt, great care will have to be taken with the procedure for cleansing and disinfecting donor skin prior to its collection.

Up to 20% of microbial cutaneous flora are beyond the reach of routine disinfection methods. Those bacteria are located in pilo-sebaceous units or at places where lipids and superficial cornified epithelium protect them (Selwyn & Ellis, 1972). Topical antiseptics eradicate only the superficial cutaneous flora, which are subsequently replaced by micro-organisms from deeper-lying layers (Schindler et al., 2006).

Many of these species isolated from cultures of skin fragments (taken during collection and/or final processing) are coagulase-negative staphylococci (Heck et al., 1981; Ireland & Spelman, 2005; May et al., 1985; Obeng et al., 2001; van Baare et al., 1998; White et al., 1991). They are low-virulence micro-organisms which colonise normal skin. Some highly virulent *S. aureus* have been isolated in between 9 and 24% of positive skin cultures (Ireland & Spelman, 2005; May et al., 1985; van Baare et al., 1998).

3.5.1.2. Sampling for microbiological examination

Samples of skin are taken from the source material in order to verify the bioburden, following an (optional) decontamination stage, and immediately from the final product.

The donor skin is collected using a dermatome and then packed into a sterile container filled with a sterile transport medium with or without antibiotic. A sample of skin may be taken during collection for use as a sample culture (1 to 2% of the total surface area of the skin collected). The microbiological cultures carried out during skin collection give an early idea of the initial cutaneous contamination and of the species present on the donor skin (bioburden) (Neely et al., 2008).

Skin allografts may be stored in various ways: glycerolisation and cryopreservation in liquid nitrogen are the techniques used most frequently. We shall confine this part to those two methods.

- Glycerolised skin allografts

85% glycerol has a definite antimicrobial effect, particularly at temperatures above 24°C and on gram-negative bacteria (Saegeman et al., 2008).

At the end of the processing of the skin, representative residual fragments of skin are used as final test samples prior to final placement in a container. Those skin fragments representative of the grafts placed in separate containers comprise 1 to 2% of the surface area of donor skin. The glycerolised skin fragments need to be rinsed.

- Cryopreserved skin allografts

During the cryopreservation procedure, a sample may be collected after washing (if a maximum amount of antibiotic is eliminated) (processing sample).

The strips of skin after final processing are frozen, and a package containing skin fragments (a total of at least 1 to 2% of the surface area of donor skin) will be used for the microbiological culture of the final product (Pirnay et al., 2012). These samples are first thawed out in a water bath at 37°C and rinsed in a sterile 0.9% sodium chloride solution (or any other buffered saline solution suitable for skin) (Pirnay et al., 2012).

3.5.1.3. Micro-organism detection/reporting

3.5.1.3.1. *Classification according to bacterial virulence*

HBM establishments aim to ensure that skin allografts are free of virulent micro-organisms and/or set an asepsis limit (bioburden) for those allografts (SHC 8716, 2013). It is sensible to supply a list of acceptable (low-virulence) and unacceptable (high-virulence) micro-organisms. A typical list is provided in Table 4; if one of the highly virulent micro-organisms is isolated on the skin, that will entail the rejection, decontamination or additional sterilisation/decontamination of the allografts (Table 5) (Pellet et al., 1996). The decontamination and sterilisation procedures will need to be validated by the HBM establishment.

3.5.1.3.2. *Bioburden*

Table 5 gives an example of the steps to be taken depending on the micro-organisms identified on the skin allografts. Criteria that take account of the bioburden are relevant for skin allografts. Skin homogenates are required for quantitative cultures. Alternatively, it is possible either:

- i) to use the incubation period required for the microbiological culture to become positive as an indirect marker of the bioburden, or
- ii) to start a semi-quantitative culture by inoculating the swabs into both solid agar and an enrichment medium.

Table 5. Decision-making guide to the processing of skin allografts according to the micro-organisms found before and after processing (as amended by Pirnay et al, 2012)

Incubation period	Result	Interpretation	Action	Virulence of the species isolated	Possible action
Days 1 to 7	Growth	“High” bioburden	Identification of the species/antibiogram (optional, epidemiological reason, model of susceptibility)	High	Rejection or sterilisation/decontamination* with a validated method
				Low	Rejection or sterilisation/decontamination* with a validated method
	No growth		Continuation of incubation for 7 days		
Days 8 to 14	Growth	“Low” bioburden	Identification of species/antibiogram	High	Rejection or sterilisation/decontamination* with a validated method
	Growth	“Low” bioburden	Identification of species/antibiogram	Low	Exceptional release of skin allograft
	No growth				Release of skin allograft

* When sporulating bacteria are detected, the HBM is by definition rejected (so there is no possibility of sterilisation or decontamination).

Skin allografts used in the treatment of burn victims are often in short supply, so their availability may be vital. In a life-threatening situation and in the absence of alternatives, the physician in charge of the HBM establishment may waive the above rule after a thorough risk analysis of the contaminated HBM and its potential adverse effects. This decision is taken in consultation with the physician implanting the graft and is based on an exceptional release procedure for the HBM.

Table 6. Minimum recommendations for skin allografts.

	Source material	Final product
Material to be examined	Representative residual tissue	Representative residual tissue prior to final placement of allografts in packaging
Quantity	1 to 2% of the total surface area of skin	1 to 2% of the total surface area of skin
Evaluation criteria/detection	Important distinction between low and high-virulence micro-organisms and the bioburden, given that subsequent action depends thereon, or taking of additional measures	No micro-organisms detected

3.5.2. Placental membranes

3.5.2.1. Introduction

Placental membranes comprise two loosely interconnected tissues, the amnion and the chorion. The latter is of turbid appearance and is used for full thickness cutaneous wounds. The amnion, on the other hand, is the translucent surface used for partial thickness wounds or for ocular reconstruction.

The method of delivery, caesarean section or vaginal delivery, logically determines the degree of contamination of the placental membranes at the time of their collection.

Several antimicrobial factors have been found in amniotic fluid. The clinical significance of those antimicrobial factors has to be considered in the context of the frequent colonisation of amniotic fluid by bacteria from the upper part of the vagina (*E. coli*, group B and D streptococci, *Candida albicans*, and diphtheroids) in women whose membrane has been ruptured for more than 6 hours (Harminder et al., 2004).

3.5.2.2. Sampling for microbiological examination

Different techniques are used to process and store placental membranes: drying, lyophilisation, cryopreservation at a temperature of - 65 °C or below, glycerolisation and storage at between 2 and 8 °C (SHC 8716, 2013).

A representative microbiological sample is collected from the source HBM in order to evaluate the bioburden, possibly during processing and from the final product:

- For the final product of non-irradiated placental membranes (per donor): representative sample at the time of packaging, comprising a total of 1% to 2% of the total surface area of the placental membrane, collected from the entire surface;
- For the final product of irradiated placental membranes (per donor): the quantity of residual tissue fragments in the packed samples should be equivalent to the quantity present in the packed samples for release.
 - o 2 packed samples containing residual tissue fragments from a batch of irradiated placental membranes if ≤ 50 samples packed per batch;
 - o 5% of the packed samples containing residual tissue fragments from a batch of irradiated placental membranes if > 50 samples packed per batch.

3.5.2.3. Micro-organism detection/reporting

The microbiological examination of source material should not result in the detection of micro-organisms that are unacceptable for placental allografts (Table 4). If micro-organisms are present, the placental allografts should be subjected to a validated sterilisation process.

In life-threatening circumstances or in the event of an ocular application in conditions presenting a danger to the eye and in the absence of any alternative, the physician responsible for the HBM establishment may waive the above rule after a thorough risk analysis of the contaminated HBM and its potential adverse effects. This decision is taken in consultation with the physician implanting the graft and is based on an exceptional release procedure for the HBM.

Table 7. Minimum recommendations for **placental membranes**.

	Source material	Final product
Material to be examined	Representative microbiological sample of placental membranes	<p><u>Non-irradiated membranes:</u> Representative sample of all the grafts packaged separately, collected at the time of the final packaging of the grafts.</p> <p><u>Irradiated membranes:</u> Packed samples of the final product.</p>
Quantity	1% to 2% of the entire surface of the placental membrane	<p><u>Non-irradiated membranes:</u> 1% to 2% of the entire surface of the placental membrane</p> <p><u>Irradiated membranes:</u></p> <ul style="list-style-type: none"> • 2 packed samples from a batch of irradiated membranes if ≤ 50 samples packed per batch • 5% of the samples packed per batch of irradiated membranes if > 50 samples packed per batch
Evaluation criteria/detection	No highly virulent micro-organisms detected, or taking of additional measures	No micro-organisms detected

3.5.3. Musculoskeletal allografts

3.5.3.1. Introduction

Musculoskeletal allografts consist mainly of bones, tendons and cartilage. More specifically, they include: femoral heads, long bones of the upper and lower extremities, pelvis; diaphyseal/metaphyseal or/and epiphyseal bone segments; isolated anatomical bones (for example, talus, scapula); hemi-joints with cartilage; tendons or hemi-tendons with or without bone insertion; *fascia lata*; menisci; cranial bones and facial bones.

Musculo-skeletal allografts are collected aseptically and are normally free of micro-organisms. During removal, there may be contamination caused by micro-organisms from the gastrointestinal tract (for example, Gram-negative, anaerobic, enterococci, *Clostridium* spp) and micro-organisms on the skin or in the ambient air (for example, staphylococci or corynebacteria).

Among other things, the tests to be carried out on musculoskeletal allografts depend on the type of processing the grafts are subjected to before they are released (chemical treatment, demineralisation, gamma irradiation, cryopreservation with or without a cryoprotective agent (-40°C or lower), organotypic culture or lyophilisation).

3.5.3.2. Sampling for microbiological examination

It is mandatory to collect a few samples (residual fragment and/or swab over the entire surface of the long bones) during removal to assess the bioburden of the collected tissues. This assessment may be decisive as regards the subsequent treatment to be applied to the allografts.

In addition to samples collected during removal and those that might be collected during the processing of the musculoskeletal tissues, it is also required to collect samples after the final processing of the tissues.

For frozen musculoskeletal allografts, it is necessary to collect eventually for each individually packed graft at least a residual fragment and a swab of the entire surface area of each individually packed graft. Rinsing liquid may be used instead of a swab.

Frozen musculoskeletal allografts produced in batches are tested by taking representative samples according to a validated procedure.

For irradiated musculoskeletal fragments, at least 2 packed samples containing residual tissue fragment must be tested per processed batch (if ≤ 50 containers) or 5% of packed samples containing residual tissue fragment if the batch contains more than 50 packed samples (containers). The quantity (weight) of residual tissue fragment in the packed samples should be equivalent to the quantity (weight of the final packed graft in the package) in the packed samples to be released.

For organotypic culture the recommendations described in the chapter on cells (3.5.8.) should be applied.

Lyophilisation is not a sterilisation technique.

3.5.3.3. Micro-organism detection/reporting

The micro-organisms identified on musculoskeletal allografts at the time of removal/before processing will determine, depending on their virulence (*Enterobacteriaceae*, sporulated or non-sporulated), what steps need to be taken: destruction, treatment with gamma rays or a new decontamination step. In any case, a new microbiological examination should be carried out after each new decontamination or safety procedure.

Table 4 provides a list of unacceptable micro-organisms that require the tissue to be destroyed if they are detected prior to processing.

For the autologous implantation of a cranial flap, the physician responsible for the HBM establishment may, if there are no alternatives, waive the previous rule after a thorough risk analysis of the contaminated HBM and its adverse effects. This decision is taken in consultation with the transplanting physician and is based on an exceptional release procedure for the HBM.

Table 8. Minimum recommendations for **musculoskeletal allografts**.

	On the source material	On the final product
Material to be examined	Representative samples of musculoskeletal grafts taken during harvesting (residual fragment and/or swab) over the entire surface of long bones	<u>Non-irradiated musculoskeletal grafts:</u> Residual fragment and/or rinsing liquid and/or a swab rubbed over the entire surface area of each individually packed graft. <u>Irradiated musculoskeletal grafts:</u> packed samples of the final product.
Quantity	Sample per individual package of musculoskeletal grafts	<u>Non-irradiated musculoskeletal grafts:</u> 1 sample (residual fragment and/or rinsing liquid and/or a swab) for each individually packed graft. <u>Irradiated musculoskeletal grafts</u> <ul style="list-style-type: none"> • 2 packages per batch of ≤ 50 containers; • 5% of packages per batch of > 50 containers.
Evaluation criteria/detection	No highly virulent micro-organisms detected or taking of additional measures	No micro-organisms detected

3.5.4. Heart valves / vascular allografts

3.5.4.1. Introduction

Heart valves or vascular allografts are removed aseptically and are normally sterile. During removal, there may be contamination caused by gastrointestinal, cutaneous or environmental micro-organisms. The following micro-organisms, in decreasing order, are isolated from cardiovascular allografts: coagulase-negative *Staphylococcus* spp., *Propionibacterium* spp., *Enterobacteriaceae*, *Staphylococcus aureus*, *Streptococcus viridans*, *Candida albicans*, *Bacillus* spp., *Corynebacterium* spp., *Enterococcus faecalis* (Fan et al., 2011).

Cardiovascular allografts may be decontaminated during transport or during the preparation process using a cocktail of antibiotics. The tissues are then rinsed and cryopreserved.

3.5.4.2. Sampling for microbiological examination

- At the beginning of tissue processing, a portion of the transport liquid is sampled from the container for the HBM (at least 1% of the total volume);
- During processing: a sample (at least 20 mL) of the last preparation medium is collected;
- After processing, the microbiological examination involves
 - o 1 representative fragment of the final residual tissue per individually packed graft
 - o AND, in the case of heart transplants
 - o A sample of the cryoprotective solution is also collected from inside the internal container just before the internal container is sealed definitively for each of the allografts packed individually.

3.5.4.3. Micro-organism detection/reporting

Microbiological examination during removal or before processing may reveal the presence of low-virulence micro-organisms.

Table 4 provides a list of unacceptable micro-organisms that normally require the tissue to be destroyed if they are detected prior to processing.

Cardiovascular allografts are often in short supply and may be lifesaving; there are often no acceptable therapeutic alternatives for the recipient. The physician responsible for this HBM may in such cases be justified in waiving this rule. When faced with a bioburden consisting of micro-organisms listed in Table 4 for the source material, he or she may legitimately take, after a thorough risk analysis, documented and validated measures or additional steps in the process and/or test to ensure the microbiological safety of the allografts supplied. In such cases he or she must always inform the physician implanting the material.

Table 9. Minimum recommendations for **cardiovascular grafts.**

	On the source material	On the final product
Material to be examined	Representative microbiological sample	Representative microbiological sample
Quantity	1% of the total volume of the transport liquid 20 mL of the last preparation solution	<ul style="list-style-type: none"> • 1 fragment per individually packed graft AND, for heart transplants, <ul style="list-style-type: none"> • A sample of the cryoprotective solution collected just before the internal container is sealed definitively
Evaluation criteria/detection	No highly virulent micro-organisms detected or taking of additional measures	No micro-organisms detected

3.5.5. Tympanums and ossicles

3.5.5.1. Introduction

After collection, tympano-ossicular tissue is transported in a buffered 2.7%-4% formaldehyde solution (pH 5-6) and subsequently stored at the HBM establishment at a temperature of between 2°C and 8°C. Processing is done either during collection or after short-term storage in a buffered formaldehyde solution. After final packing, the allografts are kept for at least 14 days in a formaldehyde solution (at room temperature for the first 2-5 days, and subsequently at between 2°C and 8°C). In order to guarantee the pH of the buffered formaldehyde solution, it is recommended that small containers be used.

At the aforementioned concentration, formaldehyde is a bactericide, fungicide, virucide and slow sporicide. The presence of organic material may have an inhibitory effect on formaldehyde. Very extensive contact times need to be applied.

3.5.5.2. Sampling for microbiological examination

Tympano-ossicular grafts are stored for at least 14 days in 2.7%-4% formaldehyde. These longer contact times can be considered to obviate the need for culture of the source material and culture of the final tympano-ossicular grafts.

Microbiological tests may be carried out on a representative sample of the last storage liquid (at least 1% of the total volume).

3.5.5.3. Micro-organism detection/reporting

Tympano-ossicular allografts must be free of micro-organisms (aerobic and anaerobic bacteria, yeasts and filamentous moulds) at the time of their release.

In the event of positive culture results, the allograft is to be either destroyed or subjected to a validated inactivation procedure prior to release.

Table 10. Minimum recommendations for **tympano-ossicular grafts**

	Source material	Final product
Material to be examined	No microbiological examination if the procedure including immersion for 14 days in formaldehyde is complied with. <u>As an option</u> : sample of the last storage liquid.	
Quantity		At least 1% of the total volume (optional).
Evaluation criteria/detection		No micro-organisms detected.

3.5.6. Corneas/scleras

3.5.6.1. Introduction

Ocular source HBM is in principle contaminated. *Streptococcus* spp., *Propionibacterium* spp., *Staphylococcus* spp. and diphtheroids are common contaminants on the edges of the cornea (Farrell et al., 1991). However, several case reports also describe ocular infection with *Candida* spp and with non-fermenting micro-organisms (SHC 8763, 2014).

Additional microbiological safety measures (such as decontamination of the cornea donor's peri-ocular skin at the time of collection) are recommended. At the time of collection, the whole globe may be disinfected using antiseptic and/or antibiotic solutions. The storage and decongestant media may contain antibiotics.

Corneas may be stored in a storage medium at between 30°C and 37°C (organ culture) for a maximum of 5 weeks (including the period in the medium containing macromolecules) or in a storage liquid at a temperature of between 2°C and 8°C for up to 7 days (hypothermic storage). If organ culture is used, the cornea is transferred shortly before the actual transplantation to a medium containing macromolecules (SHC 8716, 2013).

Scleras may be decontaminated in a solution containing antibiotics before being transferred to the final storage medium (at least 70% ethanol). 70% ethanol is active against bacteria, mycobacteria, filamentous moulds, yeasts and viruses, but is not a sporicide.

3.5.6.2. Sampling for microbiological examination

3.5.6.2.1. Source material

Skin samples are taken from the source material to determine the bioburden. This presupposes microbiological examination of the residual material or rinsing liquid (1% of the total volume, at least 100 µL).

3.5.6.2.2. Corneas – final product

At least one sample must be collected for microbiological examination from the storage medium (organ culture and hypothermic storage) and from the transport medium (organ culture), 24 hours at the earliest after the cornea was transferred to it. This sample comprises 1% of the storage or transport medium used for the cornea (at least 100 µL).

During storage and until just before implantation, the colour and clarity of the medium must also be checked.

3.5.6.2.3. Scleras – final product

It is desirable to check a representative sample of the final rinsing liquid (1% of the total volume) or of a fragment of tissue when storage is in 70% ethanol. Alternatively, a microbiological examination is carried out on 1% of the total volume of the 70% ethanol solution (at least 100 µL, 1/10 dilute ethanol). Ethanol has a very powerful disinfecting effect. Culture in dilute 70% ethanol may lead to bacteriostasis, necessitating a validated neutralisation procedure.

The results of culture of the cornea medium may be used as an alternative, given that it is not easy to collect a fragment of sclera. Micro-organisms that may be present are perfectly capable of multiplying in the cornea medium.

3.5.6.3. Micro-organism detection/reporting

Before the cornea is delivered, it is necessary to verify that the latest microbiological examinations are still negative. This (provisional) result is notified to the transplant physician prior to implantation. However, the culture media remain in incubation for a longer period [a total of at least 7 days (and 21 days for moulds) at 25°C-37°C (depending on whether the culture system is automated or visual)]. If a positive microbiological result does appear, this shall be reported immediately to the transplant physician, in accordance with the establishment's procedures.

The results of microbiological culture of corneas should preferably be taken into consideration when assessing the acceptability of sclera grafts. If pathogenic micro-organisms are found on a cornea, not only the cornea, but also the sclera from the same donor should be destroyed.

Table 11. Minimum recommendations for **corneas and scleras**

	Source material	Final product
Material to be examined	Residual fragment or liquid	<u>Cornea</u> <ul style="list-style-type: none"> • storage medium (organ culture and hypothermic storage method) and transport medium in the event of organ culture <u>Sclera</u> <ul style="list-style-type: none"> • 70% ethanol, or • last rinsing medium, or • cornea storage medium (hypothermic storage method), or • cornea transport medium (organ culture), or • residual fragment of sclera.
Quantity	Residual material or 1% of the rinsing liquid (at least 100 µL)	<u>Cornea</u> At least 1% of the volume of the cornea transport and/or storage medium (not less than 100 µL) <u>Sclera</u> <ul style="list-style-type: none"> • 70% ethanol: at least 1% of the volume (with final 1/10 dilution of the ethanol) (at least 100 µL of sample); • at least 1% of the last rinsing volume of the sclera (sample of at least 100 µL).
Evaluation criteria/detection	Ocular tissues are generally contaminated. If necessary taking of additional measures.	No micro-organisms detected.

3.5.7. Reproductive cells

3.5.7.1. Introduction

Spermatozoa and oocytes are collected from seminal fluid (1) and follicular fluid following a follicular puncture (2), respectively. In certain cases, (parts of) gonads are collected (3).

3.5.7.1.1. Seminal fluid

Seminal fluid containing spermatozoa, obtained through masturbation, is by definition non-sterile and contains mainly bacteria that belong to the skin and/or the faecal flora (coagulase-negative staphylococci, enterococci). The commensal flora may, depending on their concentration, have an impact on medically assisted procreation (MAP). A strict sperm collection procedure must be applied by the patient in order to guarantee optimum hygiene conditions and to reduce the risk of contamination to a minimum.

These micro-organisms are generally no longer found after selection and washing of the spermatozoa (Cottel et al., 1997). If the sperm is of sufficient quality, it is advisable to select spermatozoa through discontinuous density gradient centrifugation in order to eliminate these micro-organisms (Nicholson et al., 2000).

Sperm may also contain pathogenic micro-organisms such as *Staphylococcus aureus*, *Escherichia coli*, mycoplasmas (mainly *Ureaplasma urealyticum*) and *Chlamydia trachomatis*. If pathogenic micro-organisms are found in the sperm during the diagnostic examination, antimicrobial treatment of the donor may follow, to enable the spermatozoa to be used in a subsequent in vitro therapeutic phase.

A survey conducted by the ABEF (*Association Belge des Embryologistes Francophones*) at a number of MAP centres in Belgium showed a mean bacterial contamination rate of less than 5 per thousand in embryos being cultured during the process of *in vitro* fertilisation (IVF). A study by Kastrop et al (2007) of the risks of microbial contamination of embryo cultures during MAP also shows a contamination rate of approximately 0.5% per IVF cycle, mainly caused by *E. coli* (58.9%) and *Candida* spp. (25.3%). Such contamination is observed only in those cultures where spermatozoa are placed in the presence of oocytes for IVF. No contamination has been detected when the intracytoplasmic sperm injection (ICSI) technique was used.

3.5.7.1.2. Follicular fluids

Oocytes are obtained using a sterile process of ultrasound-guided transvaginal follicular puncture. Although the genital tract is not sterile (Pelzer & Allan, 2012), the presence of micro-organisms from the vagina (lactobacilli, *Candida* spp, etc) in the culture media is extremely rare and has been very little reported in literature (Kastrop et al., 2007).

The usual sterilisation processes (using alcohol, etc) cannot be applied to reproductive cells, but the processing media generally contain antibiotics.

The embryos or sperm are deposited in the uterus, which presents a lower risk of contamination than implantation or the application of other kinds of HBM. Furthermore, in the context of donation between partners, the risk of contamination is lower than it is during sexual intercourse.

3.5.7.1.3. Gonadal tissue

Gonadal tissue fragments are collected surgically (aseptically). A distinction has to be made between, on the one hand, ovarian or testicular fragments which will be frozen as they are with a view to preserving fertility prior to gonadotoxic treatment, and, on the other, those testicular fragments (or epididymal samples) from which spermatazoa are to be extracted, and the final product of which is a suspension of spermatazoa which may be considered as sperm and which needs to be examined as such.

3.5.7.2. Sampling for microbiological examination

It is impossible to conduct a microbiological analysis of reproductive tissues or cells before use for fresh material, which is applied immediately after preparation. The results are known only after it has been used in the recipient, so they are retrospective. They may nonetheless be used to check the aseptic/decontaminating nature of the technique applied, in parallel with the periodical microbiological tests that are conducted in a clean room (SHC 8699, 2012).

If the material has been subjected to cryopreservation, a microbiological analysis may be conducted prior to implantation of the HBM.

Reproductive cells and tissues are valuable genetic material of which it is rarely possible to preserve part for analysis. Only for sperm is there generally (although not always) a sufficient volume available for a small proportion of it to be used for microbiological analysis. One alternative may be analysis of the HBM's transport/processing media. However, there is not sufficient information in the literature about the conducting of microbiological examinations on the last media with which the reproductive cells or tissues concerned were in contact during preparation (oocyte rinsing medium following collection, embryo culture media, etc).

Where gonadal fragments are concerned, which are generally autografts, a microbiological examination has to be conducted on each donation prior to cryopreservation (residual tissues or ovarian/testicular rinsing/final processing media: 1% of the total volume, adjusted to 100 µL). As this is crucial autologous material, it will always be kept, whatever the result of the microbiological examination. It is nevertheless important to keep track of the microbiological results as a means of surveillance, and to avoid possible cross-contamination.

Where sperm for allogeneic use is concerned, microbiological testing (including the detection of *Chlamydia trachomatis* through PCR in the urine) must be carried out for each donor, on raw semen during the selection tests (prior to donations) and/or periodically during donations or where indicated (1% of the total volume, adjusted to 100 µL) (RD, 2009).

Where donations of sperm between partners are concerned, the literature offers no arguments for microbiological testing other than that carried out at the beginning of the infertility investigation (SHC 8292, 2009).

3.5.7.3. Micro-organism detection/reporting

Raw semen for allogeneic use is, by definition, not sterile, but will be rejected only if the sperm contains highly virulent micro-organisms (Table 4).

In addition to these microbiological analyses carried out on the reproductive material itself or on the preparation media, a visual check may be made of the HBM during its processing

(culture), since reproductive material is frequently observed macroscopically (turbid) and microscopically.

Rapid microbial growth is encouraged by the culture conditions (pH, temperature, etc.) and completely and adversely affects the HBM, which, as a result, will not be used. It may be the case that only part of the HBM shows clear contamination. It is then for the HBM establishment manager to evaluate the risk and decide whether to apply the material that does not show clear contamination or to reject it.

Table 12. Minimum recommendations for **reproductive cells/tissues.**

	Material
Material to be tested	<p><u>Gonadal sample</u> at the time of collection or following preparation:</p> <ul style="list-style-type: none"> • ovaries: rinsing/processing medium or residual gonadal tissue; • testicles: rinsing/processing medium. <p><u>Sperm for allogeneic donation</u>: sample of the sperm of each donor during the selection tests (prior to donation) and/or periodically during the donations or where indicated. Does not apply for donations between partners.</p>
Quantity	<p><u>Gonadal sample</u>: at the time of each donation, at least 1% of the total volume of the rinsing/processing medium (adjusted to 100 µL) or the residual tissue.</p> <p><u>Sperm</u>: at least 1% of the total quantity of unprocessed sperm, adjusted to 100 µL.</p>
Evaluation criteria/detection	<p><u>In the event of donations between partners or autografts</u>, microbiological examination is important particularly to avoid any cross-contamination and to ensure surveillance.</p> <p><u>In the event of allogeneic sperm donation</u>, material contaminated by highly virulent micro-organisms is rejected.</p>

3.5.8. Cells other than cord blood cells, donor lymphocytes, haematopoietic stem cells and ATMPs

3.5.8.1. Introduction

The transplantation of cells of human origin is an ever-developing, yet complex, sector offering a very wide range of ways to treat diseases. Human cells are isolated from an apheresis product or from organ or tissue fragments.

3.5.8.2. Sampling for microbiological examination

Source material: microbiological analyses may be carried out on:

- the donor organ transport medium;
- a small quantity of cellular material after collection.

Cell isolates

Certain types of human cells, such as beta cells and hepatocytes, can be transplanted immediately (< 24 hours) after isolation of the primary cells from human donors (Matsumoto et al., 2005; Puppi et al., 2008). In such cases, prospective microbiological screening based on the growth of micro-organisms is impossible, given that the culture results will not be available at the time when the graft is to be implanted. In those circumstances, visual macroscopic and microscopic examinations need to be made during the production process and at regular intervals.

At the end of the production process, just prior to the final cell product, a sample of the storage medium should be collected for retrospective screening for aerobic and anaerobic bacteria, yeasts and filamentous moulds.

Storage of primary cells

Human cells may be kept for several days in a storage medium prior to transplantation in a human being (Keymeulen et al., 2006) or prior to cryopreservation. A sample of the storage medium must at least be collected immediately prior to the final cell product for screening for aerobic and anaerobic bacteria, yeasts and filamentous moulds.

This microbiological screening comes too late for cells that were transplanted immediately after storage. In such circumstances, it is recommended that a microbiological sample be taken at an earlier stage. The time at which this sample is taken and the sensitivity of the analytical technique are crucial in order to obtain valid microbiological results. It follows that this is an exceptional release procedure.

The taking of a sample immediately after isolation of the primary cells may give rise to false negative results, because the potential contamination is heavily diluted by the different washing stages carried out after enzymatic digestion of the donor material (Bucher et al., 2005; De Corte et al., 2012; Lehec et al., 2009; Tew et al., 2008). In addition, the cell storage conditions generally constitute an ideal environment for microbial growth (pH, culture medium, temperature). Hence the timing for collecting the samples may be deferred for 1 to 3 days without any loss in detection efficiency. This deferral results in a sharp decrease in the risk of false negative results (Dreier et al., 2008).

Cell cultures

It is recommended that, for cultures of cells prepared in several passages (such as mesenchymal stem cells, keratinocytes), freezing be considered as a final product for the microbiological examination. Microbiological examination to detect aerobic and anaerobic bacteria, filamentous moulds and yeasts on the final product is mandatory.

According to Master Cell Banken, the presence of mycoplasmas should also be investigated (HSC 8318, 2007).

3.5.8.3. Microbiological culture methods

For those cells adhering to the culture dish, the culture medium (supernatant) may be pooled prior to the collection of a sample for microbiological examination. If the culture is prepared in several flasks, it is necessary to pool the supernatant from the various storage media and to take a sample representative of the whole.

For cells in suspension, the storage medium may be collected after a brief period of sedimentation, although this also increases the risk of false negative culture results due to the sedimentation of the bacteria. If the culture is prepared in several flasks, it is necessary to pool the supernatant of the various culture media and to take a sample representative of the whole.

Generally speaking, at least one examination must be conducted (on the supernatant or the final product), preference being given to testing a sample of the final product.

A sample of 1% (at least 100 µL) must be inoculated for the microbiological control test (European Pharmacopoeia 7.0, 2011, and subsequent editions; Kielinski et al., 2005; Plantamura et al., 2012). If the sample cannot be inoculated immediately, it must be stored at 2°C-8°C in order to avoid phagocytosis.

3.5.8.4. Micro-organism detection/reporting

No micro-organisms may be detected in the cell cultures.

In principle, release can take place in quite specific/clearly defined circumstances on the basis of partial provisional results (i.e. the latest examination). Before the cells are delivered, it must be ascertained whether the most recent microbiological tests are still negative. The (provisional) results are communicated prior to implantation to the transplant physician. The culture media nevertheless remain in incubation for a longer period [a total of at least 7 days (and 21 days for moulds) at 25°C-37°C (depending on whether the culture system is automated or visual)]. If a positive microbiological result were to be recorded, the transplant physician should be notified immediately in accordance with the procedures in force in the establishment.

Table 13. Minimum recommendations for **cells**.

	Source material	Final product
Material to be examined	Transport medium or cell material	<p><u>Adherent cells</u>: supernatant from the cell storage medium. If several flasks are to be used for culture, pooled supernatant.</p> <p><u>Cells in suspension</u>: cell storage medium following a brief sedimentation period. If several flasks are to be used for culture, pooled supernatant.</p>
Quantity	Minimum 1% of the total volume (at least 100 µL).	Minimum 1% of the total volume (at least 100 µL) of the storage medium.
Evaluation criteria/detection		No micro-organisms detected.

3.5.9. Haematopoietic stem cells, donor lymphocytes and cord blood

3.5.9.1. Introduction

Cell products containing haematopoietic stem cells, donor lymphocytes and cord blood may, after collection, be subject to slight microbiological contamination. There have been, however, very few reports of immediate reactions or severe delayed reactions linked to the bacterial contamination of administered cord blood or stem cells (Honohan et al., 2002, Klein et al, 2006).

Haematopoietic stem cells may, after mobilisation by means of a haematopoietic growth factor, be collected from peripheral blood by apheresis. To this end, the donor or patient (in the case of autologous transplantation) has a needle inserted into a vein in his or her arm, following disinfection of the skin. Donor lymphocytes are collected in a similar way (without mobilisation). In both cases, the cells are ultimately available in the form of cell concentrate. When bone marrow is collected, a needle is inserted into the donor's or patient's hip bone following disinfection of the skin.

As is the case for donor lymphocytes, stem cells from bone marrow and peripheral blood may be processed in a closed system and frozen, as already mentioned for cord blood. A volume reduction prior to freezing is sometimes indicated, but is not always carried out.

When **cord blood** is collected from the umbilical vein, the first step is disinfection (for example by means of 70% ethanol, povidone-iodine, 0.5% chlorhexidine gluconate). During processing (in a closed system), several centrifugation and separation steps (minimal handling) lead to production of the final cell product, which is stored in a cryopreservation solution. That final cell product is frozen according to a tested protocol and stored at a temperature below -130°C.

3.5.9.2. Sampling for microbiological examination

A microbiological culture must be performed for each cell product collected.

Where cord blood is concerned, we refer to the latest version of the relevant standards: NetCord FACT- D10.2.6.: Microbiological cultures using a system validated for the growth of aerobic and anaerobic bacteria and fungi.

In order to lose as little as possible of the stem cell material, the residual red blood cell fraction and/or the supernatant plasma fraction following processing and prior to freezing of the final product is/are used. The study by Honohan et al. has shown that these residual fractions give results comparable to those from the culture of the stem cell concentrate after thawing. The size of the fractions to be tested ranges from (a minimum of) 1 mL up to 5 mL (Honohan et al., 2002; Sparrow, 2004).

Alternatively, microbiological examination may be conducted on at least 1% of the total volume of the cell suspension (without cryoprotectant or fractionation) or at least 1% of the total volume of the final product to which a cryoprotectant has been added (not less than 100 µL). The cryoprotectant has no bacteriostatic effect on the microbiological culture (Larrea et al., 2004).

3.5.9.3. Microbiological culture methods

For haematopoietic stem cells, donor lymphocytes and cord blood, it is possible to inoculate a sample into aerobic and anaerobic blood culture bottles which are incubated for seven days in a continuous monitoring system at 35°C-37°C.

3.5.9.4. Micro-organism detection/reporting

No micro-organisms may be detected in haematopoietic stem cells and cord blood.

3.5.9.4.1. Haematopoietic stem cells

The FACT-JACIE standards (International standards for cellular therapy product collection, processing, and administration – version 5.2, chapter D4.9) mention that the quality management plan must include procedures and protocols on the use of stem cell products with positive microbial culture results for infusion during transplantation into allogeneic or autologous patients. If there is microbiological contamination of a frozen cell product, the HBM manager must consult the responsible physician and possibly the microbiologist in order to decide whether the release and administration of the cell product is indicated/justified. In that case, the degree of susceptibility must be ascertained.

If a microbiological contamination is observed following injection of the HBM, the responsible physician shall be informed thereof so as to make appropriate arrangements, depending on the micro-organism identified.

3.5.9.4.2. Cord blood

The NetCord-FACT International Standards for Cord Blood recommend that units presenting a positive microbiological culture be destroyed if they are intended for unrelated allogeneic use. If these units are being stored for use within the family, a susceptibility test must be performed on the micro-organism(s) isolated on the one hand, and, on the other, the risks and benefits at the time of grafting must be evaluated. The transplant physician is to be informed of the positive result of those tests.

Table 14. Minimum recommendations for **haematopoietic stem cells/cord blood.**

	Source material	Final product
Material to be examined	Cell suspension (without fractionation or cryopreservation).	Residual quantity (Erythrocytes or plasma fraction). OR Product to be frozen after the addition of a cryoprotectant to the final product prior to cryopreservation.
Quantity	<u>Erythrocytes or plasma fraction</u> : 1-5 mL. <u>Cell suspension</u> : at least 1% of the total volume (not less than 100 µl)	<u>Final product with cryoprotectant</u> : at least 1% of the total volume (not less than 100 µL).
Evaluation criteria/detection		No micro-organisms detected.

4. REFERENCES

- American Association of Tissue Banks, Standards for Tissue Banking, 10th ed - McLean, VA: American Association of Tissue Banks, April 2002.
- Barbour SA, King W. The safe and effective use of allograft tissue--an update. *Am J Sports Med* 2003;31(5):791-7.
- Beres et al. Introduction of iFA Plus and iFN Plus Neutralizing media for BacT Alert 3D Systems. Poster BioMérieux; 2013.
- Bucher P, Oberholzer J, Bosco D, Mathe Z, Toso C, Buhler LH, et al. Microbial surveillance during human pancreatic islet isolation. *Transpl Int* 2005;18(5):584-9.
- Carroll PB, Ricordi C, Fontes P, Rilo HR, Phipps J, Tzakis AG, et al. Microbiologic surveillance as part of human islet transplantation: results of the first 26 patients. *Transplant Proc* 1992;24(6):2798-9.
- Casadevall A, Pirofski LA. Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. *Infect Immun* 2000;68(12):6511-8.
- Casadevall A, Pirofski LA. The damage-response framework of microbial pathogenesis. *Nat Rev Microbiol* 2003;1(1):17-24.
- Cottell E, Lennon B, McMorro J, Barry-Kinsella C, Harrison RF. Processing of semen in an antibiotic-rich culture medium to minimize microbial presence during in vitro fertilization. *Fertil Steril* 1997;67(1):98-103.
- De Corte P, Verween G, Verbeken G, Rose T, Jennes S, De Coninck A, et al. Feeder layer- and animal product-free culture of neonatal foreskin keratinocytes: improved performance, usability, quality and safety. *Cell Tissue Bank* 2012;13(1):175-89.
- Deijkers RL, Bloem RM, Petit PL, Brand R, Vehmeyer SB, Veen MR. Contamination of bone allografts: analysis of incidence and predisposing factors. *J Bone Joint Surg Br* 1997;79(1):161-6.
- Dreier J, Stormer M, Pichl L, Schottstedt V, Grolle A, Bux J, et al. Sterility screening of platelet concentrates: questioning the optimal test strategy. *Vox Sang* 2008;95(3):181-8.
- European Pharmacopoeia, 7.0, 2.6.12. Microbiological examination of non-sterile products: microbial enumeration tests, 2010.
- European Pharmacopoeia 7.0. 2.6.27 Microbiological control of cellular products 2011. p 191-2. Internet:
http://www.pharmabooks.com.br/livros/images/livros/Index_7th_Edition_70.pdf
- European Pharmacopoeia 7.1. 2.6.1 Sterility. 2011. P. 2071-82. Internet:
https://www.edqm.eu/store/images/majbdd/201111211218560.Index_7.5_E.pdf
- European Pharmacopoeia, 7.0, Microbiological control of cellular products, 2011.
- EU – European Union. Commission Directive 2006/17/EG. Implementing Directive 2004/23/EC of the European Parliament and the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. *Official Journal of the European Union* 2006.
- Fan Y-D, Van Hoeckk B, Holovska V, Jashari R. Evaluation of decontamination process of Heart valve and artery tissues in European Homograft Bank (EHB): a retrospective study of 1.055 cases. *Cell Tissue Bank* 2012;13:297-304.

- Farrell PL, Fan JT, Smith RE, Trousdale MD. Donor cornea bacterial contamination. *Cornea* 1991;10(5):38.
- Forsell JH, Liesman J. Analysis of potential causes of positive microbiological cultures in tissue donors. *Cell Tissue Bank* 2000;1(2):111-5.
- Greenwood D, Slack R, Peutherer J, Barer M. *Medical Microbiology*. 17th ed. New-York: Churchill Livingstone; 2007.
- Harminder SD, Gomes JAP, King AJ, Maharajan VS, Maharajan. The amniotic membrane in ophthalmology, *Survey of Ophthalmology* 2004;49:51.
- Heck E, Blood S, Baxter C. The importance of the bacterial flora in cadaver homograft donor skin: bacterial flora in cadaver homograft. *J Burn Care Rehabil* 1981;2(4):212-4.
- Honohan A, Olthuis H, Bernardts AT, van Beckhoven JM, Brand A. Microbial contamination of cord blood stem cells. *Vox Sang* 2002;82(1):32-8.
- Horvath LL, George BJ, Murray CK, Harrison LS, Hospenthal DR. Direct comparison of the BACTEC 9240 and BacT/ALERT 3D automated blood culture systems for candida growth detection. *J Clin Microbiol* 2004;42(1):115-8
- Ibrahim T, Stafford H, Esler CN, Power RA. Cadaveric allograft microbiology. *Int Orthop* 2004;28(5):315-8.
- Ireland L, Spelman D. Bacterial contamination of tissue allografts - experiences of the donor tissue bank of Victoria. *Cell Tissue Bank* 2005;6(3):181-9.
- JACIE - 5th edition of international standards for cellular therapy product collection, processing, and administration <https://docs.google.com/viewer?a=v&pid=sites&srcid=amFjaWUub3JnfGphY2llfGd4OjJmYzQzY2NkYjJiMjIhMmI>
- Kassis C, Rangaraj G, Jiang Y, Hachem RY, Raad I. Differentiating culture samples representing coagulase-negative staphylococcal bacteremia from those representing contamination by use of time-to-positivity and quantitative blood culture methods. *J Clin Microbiol*. 2009 47(10):3255-60.
- Kastrop PM, de Graaf-Miltenburg LA, Gutknecht DR, Weima SM. Microbial contamination of embryo cultures in an ART laboratory: sources and management. *Hum Reprod* 2007;22(8):2243-8.
- Keymeulen B, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G, et al. Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc Natl Acad Sci U S A* 2006;103(46):17444-9.
- Khuu HM, Patel N, Carter CS, Murray PR, Read EJ. Sterility testing of cell therapy products: parallel comparison of automated methods with a CFR-compliant method. *Transfusion* 2006;46(12):2071-82.
- Kielpinski G, Prinzi S, Duguid J, du Moulin G. Roadmap to approval: use of an automated sterility test method as a lot release test for Carticel, autologous cultured chondrocytes. *Cytotherapy* 2005;7(6):531-41.
- Klein MA, Kadidlo D, McCullough J, McKenna DH, Burns LJ. Microbial contamination of hematopoietic stem cell products: incidence and clinical sequelae. *Biol Blood Marrow Transplant*. 2006 Nov;12(11):1142-9.
- Lakey JR, Rajotte RV, Warnock GL. Microbial surveillance of human islet isolation, in vitro culture, and cryopreservation. *Clin Invest Med* 1995;18(3):168-76.
- Larrea L, de la Rubia J, Soler MA, Ribas P, Fernández JM, Picón I et al. Quality control of bacterial contamination in autologous peripheral blood stem cells for transplantation. *Haematologica*. 2004;89(10):1232-7.

- Lehec SC, Hughes RD, Mityr RR, Graver MA, Verma A, Wade JJ, et al. Experience of microbiological screening of human hepatocytes for clinical transplantation. *Cell Transplant* 2009;18(8):941-7.
- Martinez OV. Chapter 5. Microbiological screening of cadaver donors and tissues for transplantation. In: Phillips G, editor. *Advances in Tissue Banking*. World Scientific Publishing Co. Ptd. Ltd 2003;7:143-55.
- Matsumoto S, Yamada Y, Okitsu T, Iwanaga Y, Noguchi H, Nagata H, et al. Simple evaluation of engraftment by secretory unit of islet transplant objects for living donor and cadaveric donor fresh or cultured islet transplantation. *Transplant Proc* 2005;37(8):3435-7.
- May SR, Wainwright JF, DeClement FA. The role of sampling in the detection of microbial contamination on cadaveric allograft skin used as a biological wound dressing. *Burns Incl Therm Inj* 1985;12(1):36-48.
- McGowan KL Specimen collection, Transport, and Processing: Mycology. In: *Manual of Clinical Microbiology*. Eds: Versalovic J, Carroll KC, Funke G et al. 10th edition, vol.2, 2011, ASM Press, p. 1756-1766.
- Mermel LA, Farr BM, Sherertz RJ, Raad, II, O'Grady N, Harris JS, et al. Guidelines for the management of intravascular catheter-related infections. *Infect Control Hosp Epidemiol* 2001;22(4):222-42.
- Murray PR, Witebsky FG. The clinician and the microbiology laboratory. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of Infectious Diseases*, 7th ed; 2010;17:233-67.
- NetCord-FACT International Cord Blood Standards 4th edition: D10.2.6.2 International standards for cord blood collection, processing, testing, banking, selection and release. 2007.
- Neely AN, Plessinger RT, Stamper B, Kagan RJ. Can contamination of a patient's allograft be traced back to the allograft donor? *J Burn Care Res*. 2008;29(1):73-6.
- Nicholson CM, Abramsson L, Holm SE, Bjurulf E. Bacterial contamination and sperm recovery after semen preparation by density gradient centrifugation using silane-coated silica particles at different g forces. *Hum Reprod*. 2000;15(3):662-6.
- Obeng MK, McCauley RL, Barnett JR, Heggors JP, Sheridan K, Schutzler SS. Cadaveric allograft discards as a result of positive skin cultures. *Burns* 2001;27(3):267-71.
- Pellet S, Kearny J, Dziedzic-Goslawska A, Masselis M, Panakova E, van Baare J. European Association of Tissue Banks. *Standards for skin banking*; 1996.
- Pelzer ES, Allan JA. The isolation and identification of microorganisms in the reproductive environment: the potential impact on the IVF culture system and on IVF outcomes. *Journal of Clinical Embryology*, 2012;15(3), pp. 44-53.
- Plantamura E, Huyghe G, Panterne B, Delesalle N, Thepot A, Reverdy ME, et al. Validation of the BacT/ALERT((R))3D automated culture system for the detection of microbial contamination of epithelial cell culture medium. *Cell Tissue Bank* 2012;13(3):453-9.
- Pirnay JP, Verween G, Pascual B, Verbeken G, De Corte P, Rose T, et al. Evaluation of a microbiological screening and acceptance procedure for cryopreserved skin allografts based on 14 day cultures. *Cell Tissue Bank* 2012;13(2):287-95.

- Puppi J, Tan N, Mitry RR, Hughes RD, Lehec S, Mieli-Vergani G, et al. Hepatocyte transplantation followed by auxiliary liver transplantation--a novel treatment for ornithine transcarbamylase deficiency. *Am J Transplant* 2008;8(2):452-7.
- Royaume de Belgique. Loi du 19 décembre 2008 relative à l'obtention de matériel corporel humain destiné à des applications médicales humaines ou à des fins de recherche scientifique. MB du 30 décembre 2008, p. 68774.
- Royaume de Belgique. Arrêté Royal du 28 septembre 2009 fixant les normes de qualité et de sécurité pour le don, le prélèvement, l'obtention, le contrôle, le traitement, le stockage et la distribution de matériel corporel humain, auxquelles les banques de matériel corporel humain, les structures intermédiaires de matériel corporel humain et les établissements de production doivent répondre. MB du 23 octobre 2009, n° 2009-3602/18414, p. 69409.
- Saegeman VS, Ectors NL, Lismont D, Verduyck B, Verhaegen J. Short- and long-term bacterial inhibiting effect of high concentrations of glycerol used in the preservation of skin allografts. *Burns* 2008;34(2):205-11.
- Saegeman VS, Flamaing J, Muller J, Peetermans WE, Stuyck J, Verhaegen J. Clinical evaluation of the Copan ESwab for methicillin resistant *Staphylococcus aureus* detection and culture of wounds *Eur J Clin Microbiol Infect dis* 2011;30:943-9.
- Scharp DW, Lacy PE, McLear M, Longwith J, Olack B. The bioburden of 590 consecutive human pancreata for islet transplant research. *Transplant Proc* 1992;24(3):974-5.
- Schindler OS, Spencer RF, Smith MD. Should we use a separate knife for the skin? *J Bone Joint Surg Br* 2006;88(3):382-5.
- Selwyn S, Ellis H. Skin bacteria and skin disinfection reconsidered. *Br Med J* 1972;1(5793):136-40.
- SHC – Superior Health Council. Standards de qualité particuliers pour les cellules humaines pour lesquelles aucun autre standard de qualité particulier n'existe et qui sont destinées à une application chez l'homme. Bruxelles: CSS, 2007. Avis n° 8318.
- SHC – Superior Health Council. Inactivation et sécurisation des tissus et cellules vis-à-vis des bactéries, virus et prions – Partie I: Prions. Bruxelles: CSS; 2008. Avis n° 8143.
- SHC – Superior Health Council. Standards de qualité pour les tissus et cellules reproducteurs. Bruxelles: CSS; 2009. Avis n° 8292.
- SHC – Superior Health Council. Inactivation et sécurisation des tissus et cellules vis-à-vis des bactéries, virus et prions – Partie III: Virologie. Bruxelles: CSS; 2012. Avis n° 8785.
- SHC – Superior Health Council. Recommandations en matière de validation et de contrôle de l'environnement au sein des banques et structures intermédiaires de matériel corporel humain. Bruxelles: CSS; 2012. Avis n° 8699.
- SHC – Superior Health Council. Inactivation et sécurisation des tissus et cellules vis-à-vis des bactéries, virus et prions – Partie II : Bactéries. Bruxelles : CSS; 2014. Avis n° 8763.
- SHC – Superior Health Council. Standards de qualité pour les différents types de matériels corporels humains destinés à une application humaine ; les tissus. Bruxelles : CSS ; 2013. Avis n°8716.
- Sparrow RL. Microbial screening of UC blood units by an automated culture system: effect of delayed testing on bacterial detection. *Cytotherapy* 2004;6(1):23-9.

- Sutton DA. Specimen collection, transport, and processing: Mycology. In: Murray PR and Baron, editors. *Manual of Clinical Microbiology*. 9th ed; 2007. 2:1734.
- Tew SR, Murdoch AD, Rauchenberg RP, Hardingham TE. Cellular methods in cartilage research: primary human chondrocytes in culture and chondrogenesis in human bone marrow stem cells. *Methods* 2008;45(1):2-9.
- van Baare J, Ligtoet EE, Middelkoop E. Microbiological evaluation of glycerolized cadaveric donor skin. *Transplantation* 1998;65(7):966-70.
- Verhaegen J, Lagrou K, Pyckavet M. *Medische microbiologie voor laboratoriumtechnologen, deel 2*. ACCO; 2007.
- Vigano EF, Vasconi E, Agrappi C, Clerici P. Use of simulated blood cultures for time to detection comparison between BacT/ALERT and BACTEC 9240 blood culture systems. *Diagn Microbiol Infect Dis* 2002;44(3):235-40.
- Warwick RM, Magee JG, Leeming JP, Graham JC, Hannan MM, Chadwick M et al. Mycobacteria and allograft heart valve banking: an international survey, *J Hosp Infection* 2008; 68:255-261.
- White MJ, Whalen JD, Gould JA, Brown GL, Polk HC, Jr. Procurement and transplantation of colonized cadaver skin. *Am Surg* 1991;57(6):402-7.
- Winters M. Methods of culturing, problems associated and radiation sterilisation options. In: Phillips G, editor. *Advances in tissue banking* 1 ed. Singapore: World Scientific Publishing Co Pte Ltd; 2003. p. 193-210.
- Zibari GB, Lipka J, Zizzi H, Abreo KD, Jacobbi L, McDonald JC. The use of contaminated donor organs in transplantation. *Clin Transplant* 2000;14(4 Pt 2):397-400.

5. COMPOSITION OF THE WORKING GROUP

All experts joined the working group *in a private capacity*. The names of the experts appointed by Royal Decree as well as members of the Committee and the Board are available on our website (web page: [composition et fonctionnement](#)). The general declarations of interests of the experts who approved or validated the advisory report are available on our website (web page: [Conflits d'intérêts](#)).

The following experts were involved in drawing up the advisory report :

BAUDOUX Etienne	Medicine, cell therapy	ULg
BEELE Hilde	Medicine, dermatology	UZ Gent
CAREMANS Jeroen	Biomedical sciences	UZA
DE SUTTER Petra	Reproductive medicine	UZ Gent
DE VOS Daniel	Cell technology	MHKA
DELFORGE Alain	Medicine, cell therapy	ULB
DELLOYE Christian	Medicine, orthopaedic surgery	UCL
DUFRANE Denis	Endocrine cell therapy, HBM banking, locomotor system	UCL
ECTORS Nadine	Medicine, anatomical pathology	KUL
GIET Olivier	Clinical pathologist, quality co-ordinator	ULG
GORDTS Bart	Microbiology and hospital hygiene	AZ Brugge
GUNS Johan	Medico-social sciences	UZ Brussel
HEINEN Ernst	Human histology	ULg
IEVEN Greet	Microbiology	UZA
JASHARI Ramadan	Medicine, cardio-vascular surgery	EHB/clinique St-Jean, Brussel
KLYKENS Johan	Biochemical engineering, QA/QC	UZLeuven
MARICAU Daniel	Clinical pathology	FAMHP
MUYLLE Ludo	Medicine, clinical pathology	FAMHP, UZA, UA
PIRNAY Jean-Paul	Medical sciences	MHKA
SAEGEMAN Veroniek	Medicine, clinical pathology, hospital hygiene	UZ Leuven
THONON Fabienne	Reproductive medicine, embryology	CHU de Liège
VAN DEN ABBEEL Etienne	Reproductive medicine, embryology	UZ Gent
VAN GEYT Caroline	Medico-social sciences	UZ Gent
VAN RIET Ivan	Medicine, cell therapy	UZ Brussel
VANDERKELEN Alain	Medicine, general surgery	HMRA
VANSTEENBRUGGE Anne	Reproductive medicine, embryology	CHR Namur
VERBEKEN Gilbert	Clinical pathology, QA/QC/RA	MHKA

The administration was represented by:

DE VOS Claire	Managing accredited of establishments HBM	FAMHP
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The working group was chaired by Veroniek SAEGEMAN and Hilde BEELE, the scientific secretary was Muriel BALTES.

About the Superior Health Council (SHC)

The Superior Health Council is a federal advisory body. Its secretariat is provided by the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC aims at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge.

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, staff members of scientific institutions, stakeholders in the field, etc.), 300 of whom are appointed experts of the Council by Royal Decree. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a Committee on Professional Conduct) as well as the final endorsement of the advisory reports by the Board (ultimate decision-making body of the SHC, which consists of 40 members from the pool of appointed experts). This coherent set of procedures aims at allowing the SHC to issue advisory reports that are based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

Once they have been endorsed by the Board, the advisory reports are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.shc-belgium.be). Some of them are also communicated to the press and to specific target groups (healthcare professionals, universities, politicians, consumer organisations, etc.).

In order to receive notification about the activities and publications of the SHC, please contact: info.hgr-css@health.belgium.be.