TECHNICAL GUIDELINES FOR OCULAR TISSUE*

GENERAL.

The following Guidelines take into account the following European Directives which are mandatory in those countries that belong to the European Union:

- Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.
- Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC with regards to certain technical requirements for the donation, procurement and testing of human tissues and cells.
- Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC with regards to certain technical requirements for the coding, processing, preservation, storage and distribution of human cells and tissues.
- Commission Directive (EU) 2015/565 of 8 April 2015 amending Directive 2006/86/EC as regards certain technical requirements for the coding of human tissues and cells
- Commission Directive (EU) 2015/566 of 8 April 2015 implementing Directive 2004/23/EC as regards the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells.

Suitably trained, designated and authorised personnel, following national legislation and requirements, should perform all tasks according to validated, up-to-date, document-controlled standard operating procedures (SOPs) in relation to:

1 Ocular tissue retrieval
2 Processing and storage of corneal tissue including tissue lamella preparation for new surgery techniques (e.g. DSAEK, DMEK etc.)
3 Corneal tissue evaluation and selection for transplantation
4 Scleral tissue
5 Amnion tissue
6 Tissue distribution and follow-up

1 OCULAR TISSUE RETRIEVAL.

The following should be carried out in compliance with EEBA Minimum Medical Standards:

1.1 Retrieval of the tissue should be performed by qualified and trained personnel.

1.2 Prior to the actual retrieval procedure:

- Identify the donor according to national legislation and the eye bank's standard procedures which have been approved by the Responsible Person / his designee.

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* Standards reviewed by the EEBA Technical Guidelines Special Interest Group in September 2016 [A. Gareiss-Lok (Chair, München), Stefan Ek (Moelndal), Lisa Dahlström (Örebro), Wessel Vermeulen (Beverwijk), Günter Simons (Köln). Sabine Salla (Aachen)]. Revisions to be submitted to the EEBA Business Meeting on 21 January 2017 for approval.

- Where required, ensure that consent or no objection to donation has been properly obtained and documented.
- Check donor's medical records, charts/interviews etc. for any contraindications for donation according to guidelines and/or recommendations for further risk assessment which may rule out the donation. New and emerging diseases including those that have spread to new geographical areas (e.g. Ebola, Zika, West Nile Virus etc.) need to be taken into consideration and a careful screening of donors travel history becomes necessary (see also https://ecdc.europa.eu/en for further information).
- Perform an inspection of the donor's body to check for any signs of medical contraindications according to actual guidelines and recommendations.
- If possible, perform an inspection of the ocular globe with special focus on the corneas with a view to the medical contraindications. If not possible prior to retrieval this should be done as soon the globes arrive to the eye bank.
- Document all significant and pathological findings.

1.3 Blood sample:

- While drawing/collecting blood sample, make sure correct tube sample (e.g. tube for plasma) is taken in accordance with samples of required test-kits. Correct labelling of all samples is mandatory.
- Draw a post-mortem blood sample, recording the date and time of sampling, within 24 hours post-mortem (EU-Directive requirement) and in accordance with national legal requirements (e.g. time frame of refrigeration of donor body). Be aware that used test-kits for donor testing has to be suitable/validated for usage of cadaveric blood samples.
- A suitable ante-mortem blood sample taken up to max. 7 days before death may be used for donor testing provided identification can be ensured (see EEBA Minimum Medical Standards – Donor Medical Assessment and Laboratory tests for donors, EU Directive). The date and time of sampling should be recorded to indicate that it is an ante-mortem sample and taken within the time-frame.
- In the event that the donor has received blood thinning medications, such as during blood transfusions (blood products, blood substitutes, colloids and crystal colloids) and/or dialysis intervals, the risk of haemodilution must be assessed in accordance with national requirements/legislation (see also EEBA Minimum Medical Standards page2 *Testing of Donor*).

1.4 Retrieval.

Ensure that the retrieval is performed within the post mortem time limits approved by the Responsible Person / his designee. Details of post mortem time limits within EEBA member eye banks can be found within EEBA annual Directory.

- Retrieve either the whole eye by enucleation or the corneoscleral disc by *in situ* excision using validated aseptic procedures.
- Place the whole eye in a fixed position in a moist chamber, or immerse the corneoscleral disc in an appropriate corneal storage solution.
- Make sure that all tissues are labelled (e.g. SEC-coding) for clear identification at every time point.
- Indicate the lot number (including expiry date) of all materials / medical devices used.

- Disposable materials are preferred. If re-usable instruments are used, it is recommended that the identification code of instruments or instrument sets is recorded to be able to track which instruments were used with regard to the possibility of slow diseases transmission (e.g. slow growing bacteria/virus/fungus, prions etc.).

Transport the tissue to the eye bank for further processing as soon as possible in accordance with a validated procedure (e.g. validated transport box etc.).

2 PROCESSING AND STORAGE OF CORNEAL TISSUE.

2.1 General.

- Use only reagents and materials from suppliers that meet the documented requirements and specifications approved by the Responsible Person / his designee. CE/pharmacopeia-labelled materials/chemicals are recommended.
- All procedures must be documented in written and periodically revised SOPs, including method and dates for decontamination, endothelial evaluation and microbiological testing of the tissue. Where necessary the time point should also be documented. Use aseptic techniques while processing the tissue in the eye bank.
- The required air quality standard of the environment (air particle/CFU-count) in which the corneal tissue will be processed should be defined and monitored routinely (usually class A within the laminar flow in a class D background).
- Considering that:
  - post-mortem eye tissue is generally contaminated,
  - the amount of remaining contaminating microbes is dependent on pre-storage decontamination procedures, antibiotics during storage, and storage procedure.

  Each bank should collect data to demonstrate and document that the defined standard of the environment achieves the required quality and safety of the corneal tissue (e.g. monitoring plan).

2.2 The following methods for preparation of the cornea are accepted:

- Excision of the corneoscleral button from enucleated whole eyes *in vitro*.
- Excision of the corneoscleral button from the donor eyes *in situ*.
- Lamellar tissue preparation of the corneoscleral button using manual, automated methods or lasers.

2.3 The following storage methods are generally accepted for the viable cornea:

- Hypothermic storage of the whole eye. Maximum recommended storage time is 72 hrs for selected surgeries. New surgical techniques may lead successfully to longer storage times which should be left to the discretion of the Responsible Person / his designee of the individual eye bank.
  An inspection of the endothelium is mandatory and the cell loss during storage must be taken into account, except when tissue is designated for emergency or anterior lamellar grafting. The Responsible Person / his designee needs to assure that all the necessary serological tests on the donor have been

performed within this time period. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining eye tissue at time of surgery should be added.

- Hypothermic storage of the corneoscleral disc in a corneal storage solution: Maximum storage time depends on the storage medium used; see instructions of the manufacturer. Any exceedance of the prescribed storage time should be left to the discretion of the Responsible Person / his designee of the individual eye bank in consultation with the transplanting surgeon (e.g. usage for tectonic graft /stromal patch to avoid enucleation of patient's eye if no alternative transplant tissue is available).
  An inspection of the endothelium is mandatory and the cell loss during storage must be taken into account, except for tissue designated for emergency or anterior lamellar grafting. Due to the short time of storage, it is not possible to wait for the final result of sensitive microbiological testing of the culture medium using traditional microbiological testing (e.g. blood-culture bottles) but there are alternative testing-methods available. However, sampling of the culture medium one day after the start of the storage period, or just before delivery for clinical use is recommended. The efficacy of the used microbiological testing method should be evaluated and validated due to the presence of antibiotics within the storage media. The treating physician/receiving transplanting centre should be informed as quickly as possible in the event of a 'late' positive result. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining scleral rim at time of surgery should be added (see 3.7).
- Storage of the corneoscleral button by organ/tissue culture: It is recommended to keep the storage time as short as possible with a maximum of 34 days for selected surgery cases. Any exceedance of the recommended storage time should be left to the discretion of the Responsible Person / his designee of the individual eye bank in consultation with the transplanting surgeon (e.g. usage for tectonic graft /stromal patch to avoid enucleation of patient's eye if no alternative transplant tissue is available). Inspection of the endothelium is mandatory and should be preferred in any case at the end of the storage period except for tissue designated for emergency or anterior lamellar grafting.
  A minimum storage period is mandatory to allow for proper microbiological testing thus minimizing the risk of contamination. The time period required to perform microbiological tests of the storage medium is at the discretion of the Responsible Person / his designee. The efficacy of this quarantine period and the microbiological testing method should be evaluated and validated considering the effectiveness of antibiotics within the storage media.
  Microbiological testing of media samples is mandatory, sole visual inspection of the medium for a change in colour or transparency is not acceptable. Medium change during storage using aseptic procedures is at the discretion of the Responsible Person / his designee and/or the indications of the manufacturer - taking into consideration that corneal endothelium might be stressed if tissue is to be transferred into other solutions/media. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining scleral rim at time of surgery should be added (see 3.7).
- The procedure and technique used to prepare lamellar tissue in the tissue bank should be evaluated, validated and regularly revised and if possible, including microbiological testing (e.g. testing of transport media and/or lamella/remaining

piece of tissue after preparation) intervals decided by the Responsible Person / his designee. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining scleral rim/tissue at time of surgery should be added (see 3.7). NOTE: some EU countries require separate/specific 'allowances' for lamella preparation in the eye bank.

- Cryopreservation may be used for non-viable tissue for tectonic grafting. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining scleral rim/tissue postoperatively should be added (see 3.7).

Details about tissue storage time within EEBA member eye banks can be found within EEBA annual Directory.

3 CORNEAL TISSUE EVALUATION AND SELECTION FOR TRANSPLANTATION[1].

3.1 General.

The findings of the specific layers may relate to contraindications if tissue is planned to be used for full-thickness graft (PKP). Due to the variety of different and new surgical techniques such tissues can be also used for transplantation of only specific and intact layers. Therefore communication between surgeon and eye bank is very important in order to release tissue for specific purposes with the necessary recommendation from the eye bank. Nevertheless, the final decision of usage and suitability always remains at the responsibility of the transplanting surgeon.

To be able to select the tissue for the specific purpose for which it is intended, it is necessary to check and document the conditions of:

- The epithelium (full-thickness graft, superficial or deep anterior lamellar graft, limbal graft) – taking into account that the epithelium may slash out/fall off, the duration of storage is crucial.
- The corneal stroma (full-thickness graft, superficial or deep anterior lamellar graft); transparency is crucial.
- The endothelium (full-thickness graft, posterior lamellar graft) - cell density is crucial (see 3.6).
- The corneal thickness may give additional information and therefore an evaluation is recommended before and after storage/deswelling – taking into account that thickness below 400µm may indicate unusual thinning (e.g. keratoconus, refractive surgery, wearing of hard contact lenses); thickness beyond 700µm may indicate unusual swelling within corneal layers.

3.2 Macroscopic inspection.

Without optical aid inspect the donor eye for corneal transparency and document corneal pathology such as:

- Abnormalities of the external globe (e.g. hypotonic globe/phthisis bulbi, suspicious signs of conjunctiva/scleral tumour)
- Signs of previous surgery of the anterior segment.

[1] GOD Rosenwasser, WJ Nicholson. Introduction to Eye Banking: A Handbook and Atlas Proforma 2003, pp 83-127

- Epithelial abrasions, retention of excessive orbital tissue, laceration of the globe.
- Epithelial defects.
- Stromal opacities (size, location) and arcus lipoides/senilis (Gerontoxon) should be documented including size of diameter of clear zone. The minimal diameter of the clear zone is at the discretion of the Responsible Person / his designee and/or the surgeon's requirements.
- Abnormal corneal shape (keratoconus, micro- or megalocornea).
- Condition of the anterior chamber (shape, evidence of gross blood).
- Abnormalities such as pterygium extending to the optical zone.

3.3 Slit lamp examination, performed when whole eyes are enucleated or when corneoscleral buttons are excised, is recommended because it provides additional and/or crucial information.
Inspect the cornea and limbal area for features which may preclude use of tissue e.g. signs of corneal pathology or post mortem artefacts, taking into account:

- The state of the epithelium and epithelial irregularities.
- The presence of stromal opacities (e.g. macula, nebula or signs of dystrophies).
- The presence of folds in the Descemet's membrane (increasing with post mortem time, e.g. categorize into mild/medium or moderate/severe).
- Endothelial precipitates.
- Corneal guttae.
- Abnormal corneal shape (keratoconus, micro- or megalocornea).
- Attention should be paid to the following items (in case of corneoscleral button excision, this is difficult to detect):
  - Condition of the anterior chamber (shape, evidence of gross blood).
  - Signs of prior surgery in the anterior segment (e.g. glaucoma, cataract extraction).
  - Signs of any refractive surgery (PRK, Lasik etc.) [see also EEBA Minimum Medical Standards].
  - The presence of synechiae (anteriores, posteriores).
  - Signs of tumours or metastasis in the anterior ocular segment (especially at donors with diagnosis of cancer), [see also EEBA Minimum Medical Standards].

    These may only be detected by slit lamp examination.

3.4 Other microscopic examinations are mandatory by one of the following methods:

- Specular microscopy.
  The appearance of endothelial cells with specular microscopy varies with temperature, type and time of preservation and media. Evaluation of corneas at room temperature would be recommended.
- Transmitted light microscopy (bright field, phase-contrast).
  For light microscopy, it is necessary to make the endothelial cells visible by induction of swelling of the intercellular space with a hypotonic solution. The induction of the swelling and the swelling pattern is dependent on the type of medium and time of preservation. The use of a vital stain (e.g. trypan blue) may help to recognize severely damaged cells (necrotic / apoptotic) and denuded Descemet's membrane.

3.5 Areas of interest during microscopic examination:

- Endothelium:
  - Appearance of swelling pattern of the intercellular space if applicable.
  - Quantity and extent of Descemet's folds.
  - Presence and distribution of dead cells resulting for example from trauma or post-mortem cell decay etc.
  - Density, size and shape of endothelial cells.
    - The endothelial cell density should be assessed according to a validated and regularly checked method, either counting directly with help of a graticule or afterwards on a photograph or with a calibrated software program.
      Caution is warranted for automatically obtained cell counts as in most cases interactive manipulation of the image is required for a reliable cell count and reliable results of the morphometric analysis for cell size, variation in cell size, % hexagonals and other shape parameters.
    - Cell counting should be done in different areas, centrally and peri-centrally up to 2-3 mm from the centre being aware that cell density varies from center to periphery.
    - Polymegathism refers to variation in cell sizes; it could be graded from trace, mild, moderate to severe – a common nomenclature and valuation procedure within each facility is recommended.
    - Pleomorphism refers to variation in cell shape and the deviation from the normal hexagonal shape.
  - Signs of significant cell loss.
  - Presence of corneal guttae.
  - Intracellular morphological characteristics of endothelial cells (e.g. granulation, vacuolation).
- Stroma:
  presence of stromal opacities, stromal deposits, abnormal morphology of keratocytes. The assessment of stromal condition has to be considered on the basis of final usage and patients diagnosis (e.g. patient with stromal defects/scaring etc.).
- Epithelium:
  Check integrity of the epithelium, the presence of partial (vertical or horizontal) erosions, or the absence of epithelium. The valuation of epithelium condition has to be considered on the basis of final usage and patient diagnosis (e.g. patient with limbal defects etc.).

3.6 Exclusion criteria for the corneal endothelium, in case the endothelial layer is **included** as functional layer in the graft:

- Tissue where the viability is severely affected by trauma, post-mortem effects, indicated by dead cells and/or inflammation (presence of inflammatory cells).
- While the specific influence of morphometric parameters for the endothelium on graft outcome remains uncertain, cut off points are at the discretion of the Responsible Person / his designee. Based on literature a cell density of less than 2000 cells/mm², moderate to severe signs of polymegathism and pleomorphism, signs of significant cell loss during organ/tissue culture or the presence of dead cells are generally considered as contraindications for long term graft survival.

3.7 Clinical Use

A preservation and expiry date for the cornea has to be indicated. If medium changes are performed, these dates should be indicated, as well as the date and time of transfer to transport medium.

Instruction for surgery-use with advice of microbiological testing of corneal storage medium and/or remaining scleral rim/tissue postoperatively is highly recommended, especially due to the fact that tissue is not considered to be sterile (and storage medium is not bactericidal).

4 SCLERAL TISSUE.

4.1 Tissue selection, contraindications for sclera donation:

- Age is at the discretion of the Responsible Person / his designee
- Malignancies (especially if used as keratolimbal graft)
- Pathology of the eye: pterygium, abnormal shape, staphyloma.
- Previous surgery: Cryo-surgery (pterygium), Ablation surgery (cerclage surgery).

4.2 Processing:

Prepare the sclera after removal of the corneoscleral button; remove the remaining contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). If requested, and compliant with national legislation and the eye bank's standard procedures, cut the tissue into the required number of pieces. Use aseptic techniques. The documentation of used instruments, medical devices etc. is mandatory.

4.3 Storage (complete or in separately packed pieces).

Generally accepted storage methods are:

- At room temperature:
  - Dehydrated in 70% ethanol or higher, glycerine
  - Aqueous solution after denaturation with ethanol
  - Fixed in formalin
  - Freeze dried or frozen
- In the refrigerator (+2°C - +8°C) in hypothermic storage solution (e.g. Optisol-GS, Eusol, Corneal Chamber), 70% ethanol or dehydrated in saline supplemented with antibiotics.

4.4 Decontamination and Microbiological control:

Decontamination in an antibiotic bath for 20 minutes before storage in glycerine, or a quarantine period in ethanol 70% for 14 days before renewal of the ethanol 70%, is recommended in addition to the performance of microbiological tests of storage solution and/or piece of tissue before final storage and release for surgery. The efficacy of the microbiological testing method should be evaluated and validated.

4.5 Clinical use:

A preservation and expiry date for scleral tissue has to be indicated. A suitable reconstitution protocol for further surgical use must be documented and made available to the surgeon or surgical centre including recommendation of microbiological testing of storage medium and/or remaining scleral tissue postoperatively.

5 AMNIOTIC TISSUE.

5.1 Tissue selection:

Amniotic membrane is customarily procured under strict aseptic conditions from living donors undergoing elective caesarean section and thus implies a different procurement system, including a written informed consent to retrieve amniotic membrane for therapeutic purposes and the carrying out of an exhaustive pre-natal screening of the donor's medical history for malignancies, genetic or transmissible diseases. A second serology testing of the living donor is necessary after six months of quarantine unless NAT testing has been initially performed.

5.2 Processing:

The amniotic membrane has to be processed in an aseptic manner in a laminar flow hood (similar to processing of corneal/scleral tissues after enucleation). The required air quality standard of the environment (air particle/CFU-count) in which the amniotic tissue will be processed should be defined and monitored routinely (usually class A in class D background). The whole placenta is rinsed several times and the amnion and chorion are mechanically separated according to a documented standard operating protocol. The amnion is then placed on a carrier (e.g. Merocel®) and divided in smaller pieces.

5.3 Storage:

Generally accepted storage methods are in a (cryo-)medium, in glycerol, or native without any liquids

- In a freezer at - 80º C
- In liquid nitrogen, vapour phase
  or
- Freeze dried
- On a carrier in humid environment (< -20°C)

Microbiological testing:

During the entire procedure samples of the different rinsing solutions and finally also pieces of tissue are taken for microbiological testing. The efficacy of the microbiological testing method should be evaluated and validated.

5.4 Clinical use:

A preservation and/or expiry date for amniotic tissue has to be indicated (according to national legal requirements). A suitable reconstitution protocol for further surgical use must be documented and made available to the surgeon or surgical centre including recommendation of microbiological testing of storage medium and/or remaining tissue postoperatively.

NOTE: All tissues will remain under QUARANTINE until all mentioned processing steps are fulfilled for final release of tissue. This time frame will also give appropriate timing for approval that tissue will survive during this period including adequate time for proper microbial testing for the safety of the receiving recipients. Any shortening/exceedance of recommended time frames should be left to the discretion of the Responsible Person / his designee of the individual eye bank in consultation with the transplanting surgeon (e.g. emergency usage for tectonic graft / stromal patch to avoid enucleation of patient's eye if no alternative transplant tissue is available).

6 TISSUE DISTRIBUTION AND FOLLOW-UP

Written agreements with all parties (e.g. transport company, receiving surgeon/surgery centre) should be available for clear differentiation of every party's responsibilities.

6.1 Data concerning the donor and in case of the cornea the microscopic evaluation should be annexed to the documentation accompanying the donor tissue. Minimum information should include:
donor age, date/time of death, post-mortem-time interval, date/time of any media change (e.g. into deswelling/transport media), date/time of any later 'manipulation' on donor tissue (e.g. lamella preparation), results of lab-testing (including micro-biological testing) and tissue itself (e.g. cell-count).
Transport of the tissue to the transplanting facility should be regularly revised in accordance with a validated procedure (e.g. validated transport box etc.) to make sure that tissue specification will be preserved.

6.2 Shipping containers for transport of tissues need to be labelled according to legal requirements (EU-Coding) and the EU Directives with at least the following information:

- name, address and telephone of sender
- name, address and telephone of receiving hospital/surgeon
- precise contents (type of tissue)
- unique EU-tissue code (SEC-code)
- date and time of transport
- storage information (e.g. 'do not freeze')
- transport information (e.g. 'keep upright')

6.3 Surgeons should fill in the appropriate form (e.g. recipient form) accompanying the graft with the name of the recipient and all other requested data and send it back to the providing tissue facility in order to ensure full donor tissue traceability.

6.4 Surgeons should report to the eye bank all serious adverse reactions and/or events (SAR/SAE), such as a primary graft failure, post-operative endophthalmitis or disease transmission, as these may be related to the quality of the transplanted tissue.

All reported serious adverse reactions and/or events must be investigated by the Responsible Person / his designee and, where necessary, appropriate corrective and preventive actions must be taken. If other tissue(s)/organ(s) from the same donor has/have been transplanted, the concerned transplantation centre(s) must be informed. Eye banks are required to seek out this information from their surgeons on a regular basis. Notification of

confirmed serious adverse reactions and/or events must be communicated without delay to the competent authority as required by EU regulations.