GENERAL.

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


Suitably trained personnel should perform all tasks according to validated, up-to-date, document-controlled standard operating procedures (SOPs) during:
1. Tissue retrieval.
2. Tissue processing and storage.
3. Tissue selection.

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 personnel.
1.2 Prior to the actual retrieval procedure:
   - Identify the donor according to local legislation and eye banks standard procedures which are approved by the Responsible Person / Medical Director.
   - Where required, ensure that consent or no objection to donation has been properly obtained (and that it is safe to proceed).
   - Perform a gross inspection of the donor’s body to check for any signs of medical contra-indications.
   - Perform a gross inspection of the ocular globe with special concentration on the corneas with a view to the medical contra-indications.
   - Record all significant and pathological findings.
1.3 Blood sample:
   - Draw a post-mortem blood sample recording the date and time of sampling within 24 hours post-mortem (EU-Directive requirement) and according to local legal requirements (e.g. time frame of refrigeration of donor body).

* Standards reviewed by the EEBA Technical Guidelines Special Interest Group in September 2012 [A. Gareiss-Lok (Chair, München), Stefan Ek (Moelndal), Kim Nielsen (Aarhus), Lisa Dahlström (Örebro)]. Revisions agreed by the EEBA Business Meeting on 19 January 2013.
• A suitable ante-mortem blood sample taken up to 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.

• In the event that the donor has received blood transfusions (blood products, blood substitutes, colloids and crystal colloids), the risk of haemodilution must be assessed in accordance with local requirements/legislation.

1.4 Retrieval.
Ensure that the retrieval is performed within the post mortem time limits approved by the Responsible Person / Medical Director / Medical Advisor.

<table>
<thead>
<tr>
<th>Data submitted for EEBA Directory 2011</th>
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<tbody>
<tr>
<td>Delay in hours from death to retrieval (organ-cultured &amp; hypothermic stored tissues)</td>
</tr>
<tr>
<td>Corneoscleral disc excision in situ (hrs) (data from 30 eye banks)</td>
</tr>
<tr>
<td>Enucleation (hrs) (data from 46 eye banks)</td>
</tr>
</tbody>
</table>

• 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.

• 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.).

1.5 Transport the tissue to the eye bank for further processing as soon as possible in accordance with a validated procedure.

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 / Medical Director. 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. Time point is documented if necessary. 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 (usually class A in 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.

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 obtained in one of the
  above mentioned ways, using manual or 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 individual eye
  bank.
  An inspection of the endothelium is mandatory and a cell loss during storage
  must be taken into account except when tissue is designated for emergency or
  anterior lamellar grafting. The Responsible Person / Medical Director or 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. It is recommended not to exceed the prescribed storage time.
  An inspection of the endothelium is mandatory and a cell loss during storage
  must be taken into account except for tissue designated for emergency or
  anterior lamellar grafting. Instruction for surgery-use with recommendation of
  microbiological testing of corneal storage medium and/or remaining sclera rim at
  time of surgery should be added.
• Storage of the corneoscleral button by organ/tissue culture: It is recommended to
  keep the storage time as short as possible considering the quarantine period
  with a maximum of 5 weeks for selected surgery cases. It is at the discretion of
  the Responsible Person / Medical Director to prolong the storage time provided
  that documentation (evidence or validation of the procedure) is presented to
  support this procedure. Inspection of the endothelium is mandatory 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 cornea in the storage medium is at the
  discretion of the Responsible Person / Medical Director. The efficacy of this
  quarantine period and the microbiological testing method should be evaluated
  and validated due to 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 / Medical Director and the indications of the manufacturer. Take into consideration that corneal endothelium might be stressed if tissue is to be transferred into other solution/media.

- Cryopreservation may be used for non-viable tissue for tectonic grafting.

<table>
<thead>
<tr>
<th>Data submitted for EEBA Directory 2011</th>
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<tbody>
<tr>
<td>Storage time corneoscleral button in days</td>
</tr>
<tr>
<td>Hypothermic storage (data from 17 eye banks)</td>
</tr>
<tr>
<td>Organ/Tissue culture (data from 51 eye banks)</td>
</tr>
<tr>
<td>In transport solution (data from 49 eye banks)</td>
</tr>
</tbody>
</table>

3 CORNEAL TISSUE EVALUATION AND SELECTION FOR TRANSPLANTATION1.

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 new variety of different surgery techniques those tissues can be also used for transplantation of only specific and intact layers. Therefore communication between surgeon and eye bank becomes more necessary to release tissue for its specific purpose as a recommendation from the eye bank. The final decision of usage and suitability always remains the responsibility of the 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) – take into account that the epithelium may slash out/fall off during storage.
- The corneal stroma (full-thickness graft, superficial or deep anterior lamellar graft); transparency is crucial.
- The endothelium, essential for maintaining corneal transparency (full-thickness graft, posterior lamellar graft).
- The corneal thickness may give additional information and therefore is recommended. E.g. thickness below 0,4μm may indicate any unusual thinning (e.g. keratoconus, refractive surgery, wearing of hard contact lenses); thickness beyond 0,7 μm may indicate unusual swelling within corneal layers (e.g. contamination, weak pump function).

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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)
- Signs of previous surgery of the anterior segment.
- 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 / Medical Director 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 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 Descemet folds (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).
- Also pay attention 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 medical standards.
  - The presence of synechiae (anteriores, posteriores).

These may only be detected by slit lamp examination.

Data submitted for EEBA Directory 2011: Out of 65 banks providing these data for EEBA Directory 2011, 40 reported to use a slit lamp.

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 type of
medium and time of preservation. The use of a vital stain (e.g. trypan blue) may help to recognize dead 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 e.g. 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 at 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 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.
  - Epithelium:
    - Check integrity of the epithelium, the presence of partial (vertical or horizontal) erosions, or the absence of epithelium. Valuation of epithelium condition has to be considered on the 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 / Medical Director. 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.

<table>
<thead>
<tr>
<th>Minimum accepted cell count for grafts including the endothelium</th>
<th>Number of banks (data from 61 banks)</th>
<th>Cell loss accepted after preservation / at time of release (data from 44 banks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1800</td>
<td>1</td>
<td>- 35 banks accept 5-20% cell loss ;</td>
</tr>
<tr>
<td>2000</td>
<td>45</td>
<td>- 6 banks accept only 2-5% cell loss</td>
</tr>
<tr>
<td>2100</td>
<td>1</td>
<td>- 3 banks accept 25-50% cell loss</td>
</tr>
<tr>
<td>2200</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2300</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

3.7 Clinical Use

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

Microbiological testing of medium and/or remaining scleral rim 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, contra indications for sclera donation:

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

4.2 Processing:

Prepare the sclera after removal of the comeoscleral button; remove the remaining contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). If requested cut the tissue in pieces. Use aseptic techniques.
4.3 Storage (complete or in separately packed pieces). Generally accepted storage methods are:
- At room temperature:
  - Dehydrated in 70% ethanol or higher, glycerin
  - 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 saline with antibiotics

4.4 Decontamination and Microbiological control:
Decontamination in a gentamycin 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.

4.5 Clinical use:
A preservation and expiry date for scleral tissue shall be indicated. A suitable reconstitution protocol for further surgical use must be documented and made available to the surgeon or surgical centre.

5 AMNIOTIC TISSUE.

5.1 Tissue selection:
Amniotic membrane is procured from living donors and thus implies a different procurement system including a written informed consent and an extensive medical questionnaire system. A second serology testing of the living donor is necessary after six months of quarantine unless NAT testing has been 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 (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 medium or glycerol:
- In a freezer at - 80°C
- In liquid nitrogen, vapour phase or
- Freeze dried
- On a carrier in humid environment (< -20°C)
5.4 Microbiological testing:
   During the entire procedure samples of the different rinsing solutions and finally
   also pieces of tissue are taken for microbiological testing.

5.5 Clinical use:
   A preservation and/or expiry date for amniotic tissue shall be indicated (according to
   local legal requirements). A suitable reconstitution protocol for further surgical use
   must be documented and made available to the surgeon or surgical centre.

6 TISSUE DISTRIBUTION AND FOLLOW-UP

6.1 Data concerning the donor and in case of the cornea the microscopic evaluation
   should accompany the donor tissue.

6.2 Shipping containers for transport of tissues need to be labelled according to local
   legal requirements with at least the following information:
   • Name, address of shipper
   • name and address of receiving hospital/surgeon
   • precise content (type of tissue)
   • storage information (e.g. ‘do not freeze’)
   • transport information (e.g. ‘keep upright’)

6.3 Surgeons should fill in the form accompanying the graft with the name of the
   recipient and all other requested data in order to facilitate tracking of donor tissue.

6.4 Surgeons should report to the eye bank all serious adverse reactions and/or
   events, such as 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 / Medical Director 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 concerning transplantation
   centre must be informed.
   Eye banks are required to seek this information on a regular basis. This in
   addition to the notification of serious adverse reactions and serious adverse
   events to the competent authority as required by EU regulations.