

EBAANZ MEDICAL QUALITY STANDARD 2ND EDITION APRIL 2009

Addition to Section 12: Serology and Microbiology Testing

12.4 BIOBURDEN OF OCULAR TISSUE

12.4.1 GENERAL

- The product 'Human ocular tissue' is not sterile and cannot be subject to terminal sterilisation.
- Surgical retrieval of ocular tissue starts at the procurement/retrieval site (e.g. mortuary).
- The ocular surface is highly likely to be contaminated by environmental micro-organisms, due to the absence of blinking and tear film production following death (1, 2).
 - Microbial/bioburden testing at this stage is unnecessary and will yield no beneficial or interpretive result. This is supported by published evidence showing that preoperative donor corneoscleral rim cultures, are unreliable predictors of endophthalmitis complicating corneal transplantation (3, 4).
- Decontamination of ocular tissue starts at the point of controlled air quality (eg biological safety cabinet).
 - Immersing the ocular tissue in a disinfectant such as povidone-iodine or chlorhexidine and a sterile saline rinse, has been shown to reduce bioburden (1, 2, 5, 6, 7, 8, 9).
- Bioburden testing of ocular tissue has been shown to be an unnecessary processing step. Current TGO 88 guidelines recognise human ocular tissue as being non-sterile, due to 'inherent microflora that will not be eradicated by processing or manufacturing steps.'
 - The unique nature of ocular tissue renders the results and significance of bioburden testing invalid, and any subsequent decisions based on the results of such testing are unsound (4, 10, 11).
- Table 1 explores in further detail microbial factors for ocular tissue.

12.4.2 OCULAR RETRIEVAL

- Enucleations are not required to be undertaken in any specialised air-controlled environments, as it will not eliminate or reduce the environmental microbial flora already present on the cornea and sclera (see 12.4.1)
- Strategies to reduce microbial flora may still be employed at this stage including irrigation with sterile saline (12) or betadine swabs/drops. This is a risk reduction strategy and does not replace decontamination procedures in the laboratory (figure 1).

12.4.3 PHYSICAL EVALUATION

- Ocular tissue must undergo slit lamp evaluation of whole eyes, as part of quality control and the physical evaluation process.
- This process must occur prior to decontamination.
- An area with specified air quality is not required (see 12.4.2 and figure 1).

12.4.4 PROCESSING

- Following post-procurement evaluation, whole eyes must be subjected to a decontamination protocol prior to processing.
- This decontamination protocol must be conducted within an air controlled environment to reduce the bioburden on the ocular surface before processing of the Corneoscleral disc (figure 2).

12.4.6 MICROBIAL TESTING

- Aseptic operations during processing (decontamination stage) should have limits set for microbial/environmental testing.
- Monitoring schedules should be selected for sample size, timing and frequency.
- The data should be used to establish trends to demonstrate a continuous level of environmental control.
- Any change in supposed typical microflora found should be monitored and be subjected to further analysis.

Factor	Risk				
	Low				High
Duration of exposure of procured tissues/cells during procurement	no exposure (closed system)	≤1h	1-2h	2-3 h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment	1 person	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	Only substances intended to reduce microbiological contamination (e.g. glycerol, antibiotics, betadine swabs/drops)	only washing intended to reduce microbiological contamination	no reduction
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

Figure 1: adapted from EDQM 4th edition (13)

Probable risk assessment: It is considered a low risk procedure and is therefore reasonable to conclude, that it is not considered necessary to procure and physically assess eyes in a location with controlled, defined air quality. However, steps must be taken to reduce the bioburden on the ocular surface before/during procurement and before excision of the corneoscleral disc.

Factor	Risk				
	Low				High
Duration of exposure of procured tissues/cells during procurement	no exposure (closed system)	≤1h	1-2h	2-3 h	≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment	1 person	2-3 persons	4 persons	5 persons	≥ 6 persons
Reduction of bioburden during or after procurement	closed system	validated antibiotic/substances treatment	Only substances intended to reduce microbiological contamination (e.g. glycerol, antibiotics, betadine swabs/drops)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue or cell due to the limitations of the sampling method	-Visible contamination indicator - microbiological testing of preservation medium	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well-vascularised site	large durable implant in a well-vascularised site	direct application into the bloodstream (infusion)

Figure 2; adapted from EDQM 4th edition (13)

Probable risk assessment: It is considered a low risk procedure and is therefore reasonable to conclude, that when aseptic technique and bioburden reduction strategies are used, the overall process is low risk.

Criterion	Ocular tissue-specific
<p>Risk of contamination of tissues or cells during processing</p>	<ul style="list-style-type: none"> • Processing whole eyes in a tissue establishment, allows control of air quality (e.g. biological safety cabinet with HEPA-filtered air). • Decontamination of the eyes before processing is a necessary step. It is reasonable to assume, that bacteria and fungi will be present on the ocular surface, due to the absence of blinking and tear film production following death. • Corneas may be removed from their storage medium just prior to surgery. They are therefore re-exposed to the environment and an appropriate controlled air quality system must be applied (e.g. laminar flow cabinet in a room with HEPA-filtered air).
<p>Use of antimicrobials during processing</p>	<ul style="list-style-type: none"> • Corneoscleral discs may be stored in media containing antibiotics and antimycotics. The medium may also contain a marker (e.g. phenol red) that changes colour with a fall in pH caused by growth of micro-organisms. • Turbidity of the storage medium is also an indication of contamination. • Storage of corneas in organ culture, allows for the testing of medium samples for microbial growth during storage, as well an effective antimicrobial activity in the medium, owing to the higher storage temperature than that used for hypothermic storage.
<p>Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method</p>	<ul style="list-style-type: none"> • There is typically no microbiological testing of hypothermic corneal storage media. Even if a sample of hypothermic medium is taken, the time available before transplant is limited to just a few days, which reduces the chance of detecting contaminants. • For organ-cultured corneas, there is a greater chance of detecting contamination because of the extended, albeit still limited, storage period. A second sample of storage medium may be taken after transfer of an organ-cultured cornea to medium, to reverse stromal oedema and for transport to the recipient hospital. However, the time before transplantation is only a few days and a negative-to-date release will apply. • There is a known risk that contamination may not be detected until after transplantation.
<p>Risk of transfer of contaminants at transplantation</p>	<ul style="list-style-type: none"> • Corneal tissue for the great majority of transplant procedures cannot be sterilised because living cells are required for a successful graft outcome. • The most frequently isolated micro-organisms in controlled areas used for aseptic processing are bacteria from the human skin (e.g. Coagulase-negative staphylococci). Such micro-organisms are native human skin flora, or native environmental inhabitants, where most do not cause serious disease (14). Table 1 explores contamination risk under several circumstances. • Post-operative endophthalmitis caused by micro-organisms transferred with the graft is therefore a risk and is a defined serious adverse reaction. • Prophylactic medications are prescribed as standard clinical care. • Post-operative endophthalmitis caused by micro-organisms transferred with the graft is therefore a risk and is defined as a serious adverse reaction (see table 1). It is however extremely rare; out of 39500 registered grafts spanning 35 years, <0.01% had a report of post-operative endophthalmitis (15). Additionally, it has been reported that adverse events following corneal transplantation are more commonly associated with recipient and surgical factors rather than eye banking practices (16). Attributing a cause is not always straightforward owing to the, albeit slight, risk of post-operative infection associated with any intraocular surgical procedure.

Table 1: microbial factors for ocular tissue; adapted from EDQM 4th edition (13)

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