Joint EBAANZ/Corneal Society submission seeking exemption for ‘Sclera’ released for surgical use from:

- mandatory NAT testing for HIV, HBV and HCV; and
- mandatory serological testing for HTLV-1 & 2 and syphilis

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1. PURPOSE OF SUBMISSION

The purpose of this joint submission from EBAANZ/Corneal Society is to seek exemption for the Class II biological ‘Sclera’ released for surgical use (grouped in the Product Type ‘Ocular Tissue’) from:

- mandatory NAT testing for HIV, HBV and HCV; and
- mandatory serological testing for HTLV-1 & 2 and syphilis

The information provided in this submission is in addition to the EBAANZ submission dated 16th August 2012 (which the TGA was not in a position to evaluate for the purposes of exemption at the time of submission) and it specifically focuses on, and provides additional information regarding, sclera.

Background

In August 2012 the Eye Bank Association of Australia and New Zealand sought exemption from the requirement of cadaveric NAT testing for HIV, HBV and HCV and exemption from cadaveric serological testing for HTLV-1 & 2 and syphilis, for release of those product types listed in manufacturers’ ocular tissue dossiers seeking ARTG registration. The detailed submission provided a risk analysis, discussed the logistics of donor testing, summarised overseas regulations and professional standards relating to these issues and examined the efficacy of testing in relation to relative risk, practical considerations and impact on the community.

At the time of this request for exemption, the requirements for donor selection, testing and minimising infectious disease transmission via therapeutic goods that are human blood and blood components, human tissues and human cellular therapy products were detailed in ARGB Appendix 4, Annex 1 (i.e. the draft Infectious Disease TGO was yet to be finalised as a Therapeutic Goods Order). As such, the TGA was not in a position to assess a submission for exemption as the infectious disease testing requirements were yet to be formalised in a Standard. In response to both the EBAANZ submission in August 2012 and a joint EBAANZ/Corneal Society submission in March 2011, the TGA provided clarification regarding the intent of the infectious disease testing requirements outlined in the draft Infectious Disease TGO. The Therapeutic Goods Committee agreed in March 2011 that serological testing for HTLV-1 & 2 and syphilis and NAT testing for HIV, HBV and HCV was not mandatory for ‘cornea only donors’. However, the intent of this exception and the term ‘cornea only donors’ was ambiguous and thus, in September 2012, the Office of Scientific Evaluation clarified the terminology ‘cornea only donors’ to mean – “persons who donate ocular tissue that will be released for the specific purpose of corneal transplantation”.

Thus, ocular tissues that are not specifically used for the purpose of corneal transplantation, e.g. sclera for other ocular surgical purposes or cornea for other ocular surgical purposes, are (by the draft ID Order Table 3) currently not considered to be exempt from NAT testing for HIV, HBV and HCV and serological testing for HTLV-1 & 2 and syphilis.

A request for the specific exemption of sclera released for surgical use from mandatory NAT testing for HIV, HBV and HCV; and mandatory serological testing for HTLV-1 & 2 and syphilis has not previously been considered by the Advisory Committee for Biologicals.
2. SCLERA- DEFINITION OF PRODUCT TYPE

- The released product is a whole scleral ball minus the contents of the eye, or a piece of sclera. This is the outer layer of the eye with the sclera’s contiguous cornea removed. It arises from the same donation as that of the cornea (i.e. the products ‘corneo-sclera disc (hypothermic)’ and ‘corneo-scleral disc (normothermic)’ released for the purpose of keratoplasty) (indeed the contiguous sclera forms the rim of the product that is released for keratoplasty).

- The use and efficacy of donated human sclera in oculoplastic reconstructive surgery and glaucoma surgery is well established\(^1, 2\). In oculoplastic reconstructive surgery, human sclera is often used following enucleation and in conjunction with the use of an orbital implant. Human sclera is also used in scleral patch graft surgery, usually in association with glaucoma drainage surgery, scleral thinning, or after a pterygium excision\(^3\).

- Some ophthalmologists describe the sclera as an avascular tissue\(^4\). This is no doubt in reference to the essential non-viable and non-functional state of the sclera when used for surgical purposes, and it can also be used to describe the scleral stroma (the bulk of the sclera) which contains no blood vessels other than a few vessels which traverse through the sclera to supply other parts of the eye\(^5\). However, the episclera (the thin outer covering of the sclera) is atypically vascularised showing a specialised morphology characterised by arterio-venous loops with an absence of capillaries. The presence of these atypical structures do not appear to contribute to the functions normally associated with vasculature (nutrition, inflammation, infection etc.) but appear to be solely related to the maintenance of a steady intraocular pressure.

- The sclera’s low and unique specialised vascularity (or avascular nature, depending on opinion and definition) no doubt contributes to the fact that there are no reports in the medical/scientific literature of disease transmission or adverse events involving the surgical use of sclera.

 Figure 1: Anatomy of the human eye\(^6\)
3. DONOR ASSESSMENT

It is important to recognise that Donor Assessment of ocular donors includes an assessment for all products grouped in the Product Type ‘Ocular Tissue’ retrieved from the donor that may be released for surgical use. Thus the donor assessments described herein directly apply to the product ‘sclera’ released for surgical use.

It is also important to note that this submission does not seek exemption from any current donor assessment standards or testing requirements. This submission seeks to maintain current risk mitigation and risk management practices, including those that continue to manage risks from transmissions of disease that are in addition to those of HIV, HBV, HCV, HTLV and Syphilis. This submission seeks only exemptions from the new and additional requirements for sclera donor testing that have currently been identified in the draft ID TGO that the ophthalmic and eye banking professions believe, in their expert opinion, do not meaningfully contribute to risk reduction.

Eye tissue donors are currently assessed and screened in accordance with the donor selection guidelines documented in the EBAANZ Standards – Section 8 (Edition 2, 2009) and the Australian Code of Good Manufacturing Practice - Human Blood and Tissues (2000). These documents include mandatory serological testing for anti-HIV 1 & 2, anti-HCV and HbsAg. This submission is not seeking exemption from these requirements for the release of ‘sclera’. Furthermore, the physical assessment requirements in the EBAANZ Standards – Section 8, involves assessment of the donor for any physical signs of HIV disease, infectious hepatitis, and injecting drug use. A thorough review of the donor’s medical and lifestyle history with specific attention to high-risk behavioural criteria are also required as part of donor assessment criteria. Such measures halve the incidence of HIV, HBV and HCV infected individuals entering the potential eye donor population (USA figures), and these intercessions need to be taken into account when residual risk calculations are performed.

The EBAANZ Standards specify that serology testing for HTLV-1, HTLV-II and syphilis is not required for eye tissue donors which is in concordance with the 2011 Medical Standards of the Eye Bank Association of America. The Australian cGMP - Human Blood and Tissues (2000) also states that eye tissue is exempt from HTLV-I and syphilis testing.

4. LACK OF TRANSMISSION OF HIV 1 & 2, HEPATITIS B, HEPATITIS C, HTLV I & II AND SYPHILIS VIA SURGICAL USE OF SCLERA

Donor sclera has never transmitted any disease. Demonstrated risk is zero. Thus there are no reports or literature on the subject.

Accurate numbers of sclera used surgically worldwide are not available; however sclera has certainly been in widespread use from the early 1980’s. In Australia and New Zealand there are on average 500-550 pieces of sclera used each year; in Europe 1200-1400 pieces each year and in the USA 4500-8000 pieces each year. Thus in these regions alone, a conservative estimate places number of sclera used for surgical purposes since 1992 at between 124,000 to 199,000 pieces.
There are also no reports of any adverse reactions attributable to donor sclera. The Eye Bank Association of America monitors for adverse events via the Online Adverse Reaction Reporting System (OARRS) which is a system that requires all eye banks to actively seek and question surgeons about possible adverse reactions. Based on the OARRS data, the EBAA Medical Review Subcommittee 2012 report stated there have been no adverse reactions reasonably attributed to, or proved to be attributed to, the surgical use of sclera since 2006\(^{(13)}\) which is accepted within the eye banking profession to be an accurate representation of events since at least 2000. In the past 10 years that is approximately 65,000 uses of sclera without a single adverse reaction.

What we do know about transmission of communicable diseases with ocular tissue comes from corneal transplantation, of which there are over 150,000 to 200,000 transplants performed per year for the past 25 years (M. Masci, P. Dubord, M. Mannis, G. Pollock, R. Vajpayee, E. Pels, L. Noel; personal communication). HIV, HCV, HTLV and syphilis have never been transmitted via corneal transplantation. This is despite three reports in the literature detailing nine patients who received corneas from HIV-positive donors\(^{(14-16)}\). None of the corneal recipients seroconverted or became ill, although all other organ and tissue recipients from these donors seroconverted. In addition there are six patients who received corneas from three HCV seropositive donors (at least two of whom had viral RNA in their serum) but none of the corneal recipients seroconverted after transplantation\(^{(17)}\). Transmission of hepatitis B virus (HBV) has been documented in two corneal recipients from two separate donors\(^{(18)}\). Since serologic screening for HBsAg was introduced (in the late 1980’s) there have been no reported cases of transmission.

Thus, the possibility of transmission of HIV, HBV, HCV, HTLV or Syphilis from any donated ocular tissue must be considered extremely unlikely. Additional evidence suggests that transmission of disease through the surgical use of sclera is most likely not possible.

5. PREVALENCE, INCIDENCE AND RISK IN THE AUSTRALIAN EYE DONOR POPULATION

Calculations of prevalence, incidence and residual risk for the product ‘sclera’ released for surgical use are the same as those for corneal donation because they arise from the same donor(s). The residual risk estimates calculated below refer to eye donors and thus they hold true for all products grouped in the Product Type ‘Ocular Tissue’.

The only exception is residual risk estimates of transmissibility of infectious disease. Most importantly, and as discussed at the end of this section, the residual risk estimates for transmissibility of disease by sclera are actually lower than those for corneas.

EBAANZ member banks report on the number of positive serology results (non-confirmed potential donor screening tests) returned for markers of HIV, HBV and HCV in Australia and New Zealand.

In 2009 EBAANZ performed residual risk calculations based on the reported serology results covering September 2007 through to June 2009. Recent data from 2009 to 2012 shows a trend to decreased numbers of reactive serology donors despite the increase in the number of donors. This indicates the residual risk has decreased since 2009 but the figures have not yet been calculated. Therefore, the following is a summary of the residual risk calculations and results from the EBAANZ submission in July 2009.
While the screening test results as reported have not all been confirmed to be truly reactive
the rates of confirmed positive results can be estimated by subtracting the number of false
positive results (determined on the basis of specificity analyses of data from the United
States). Thus prevalence in the Australian eye donor population can be calculated. This is
the method used in the seminal paper by Zou and colleagues\(^9\). In addition, figures are
available for Australia in relation to incidence rate in the blood donor population for viraemic
markers\(^19\), and these can be applied using Zou’s method to estimate the incidence rate
among Australian eye donors. In turn, the estimated probability of undetected viraemia or
residual risk (the probability that any eye donor was in the vireamic window period with an
infection that was undetected by screening tests at the time of eye donation) can be
calculated using the Model B mathematical modelling equation described by Seed and
colleagues\(^19\).

The same methodology of residual risk estimate has also recently been performed and
published for musculoskeletal tissue donors in Australia\(^20\). This enables a direct comparison
of residual risk across Australia’s blood, eye and musculoskeletal tissue donor populations.

**Calculating Prevalence**

From September 2007 through to June 2009 there were 1,856 eye donors in Australia. In
this time, unconfirmed positive serology results reported were: HIV - one, HBV - nine and
HCV – seven (refer Table 1). Applying Zou’s reported and validated figures of percent false
positives\(^9\) for each of these results equates to: HIV – 89%, HBV – 68% and HCV- 28% and
the Australian eye donor prevalence rates are listed in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Estimated Positive</th>
<th>Number of Donors</th>
<th>Prevalence per 100,000 persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>1</td>
<td>0.11</td>
<td>1856</td>
<td>5.93</td>
</tr>
<tr>
<td>HBsAg</td>
<td>9</td>
<td>2.88</td>
<td>1856</td>
<td>155.17</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>7</td>
<td>5.04</td>
<td>1856</td>
<td>271.55</td>
</tr>
</tbody>
</table>

\(\dagger\) Fractional values are presented as a result of the estimation of numbers following Zou’s\(^\circ\) estimations.

<table>
<thead>
<tr>
<th></th>
<th>Prevalence per 100,000 persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian eye donors</td>
<td>5.93</td>
</tr>
<tr>
<td>Australian first-time blood donors(\dagger)</td>
<td>5.12</td>
</tr>
<tr>
<td>Australian musculoskeletal donors(\dagger)</td>
<td>64.44</td>
</tr>
<tr>
<td>United States tissue donors(\dagger)</td>
<td>92.58</td>
</tr>
<tr>
<td>HBsAg</td>
<td>155.17</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>271.55</td>
</tr>
</tbody>
</table>

\(\dagger\) Published in Yao et.al.\(^\circ\) for 1993-2004, * Published in Zou et.al.\(^\circ\) for 2001-2002.
These comparative prevalence rates in Table 2 follow the lower prevalence rates for each marker in the general population of Australia compared to the United States. For example, the prevalence of HIV in the Australian general population is estimated at 115/100,000, compared with an estimated 150/100,000 in the United Kingdom and 456/100,000 in the United States\(^{(21)}\). The exception to the relativity of these rates is the high prevalence rates in the Australian musculoskeletal population when compared to the Australian blood and eye donor populations (and compared to the HBsAg of United States tissue donor population) (Table 2).

**Calculating Estimated Incidence**

Incidence rates are not available for eye donors or tissue donors because this type of donation is a single non-repeatable event (therefore no time period can be assigned). To overcome this Zou and colleagues extrapolated incidence rates from Untied States blood donors to assign estimated incidence rates among tissue donors\(^{(9)}\). Yao and colleagues made the same extrapolation between Australian musculoskeletal and Australian blood donors\(^{(20)}\). This calculation involves adjusting the rates to reflect the different prevalence rates among the tissue donors and the populations used for comparison (a prevalence ratio). The same prevalence ratio can be applied to the Australian eye donor population to estimate the incidence rates. The prevalence ratio (calculated from Table 2) and calculated incidence ratios for Australian eye donors are presented in Table 3.

**Table 3: Incidence in Australian Eye Donors**

<table>
<thead>
<tr>
<th>Prevalence ratio</th>
<th>Incidence rate in blood donors*</th>
<th>Estimated Incidence rate in eye donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no./100,000 person-years)</td>
<td>(no./100,000 person-years)</td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>1.16</td>
<td>0.30</td>
</tr>
<tr>
<td>HBsAg†</td>
<td>1.14</td>
<td>1.13</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>1.26</td>
<td>2.40</td>
</tr>
</tbody>
</table>

\(^{†}\)transient nature of HBsAg makes estimations difficult. Seed\(^{(19)}\) provides an adjusted incidence figure to account for underestimation.

*derived from Yao et.al.\(^{(20)}\) for 2003-2004
**Calculation of residual risk**

The estimated probability of viraemia at the time of donation can be calculated using the Incidence-window period Model B mathematical modelling equation described by Seed and colleagues\(^{19}\), which is that also used by Zou\(^9\) and Yao\(^{20}\).

- Assumes that Window Period transmissions represent the major component of the residual risk
- Probably holds true for HIV and HCV, but less so for HBV where chronic infection can be marked by transient HBsAg detection
-  
  \[ P = \lambda \times WP \]
  
  where
  
  \[ P \] = probability donor gave infectious donation during window period
  
  \[ \lambda \] = the incidence
  
  \[ WP \] = window period (in days)

Results for Australian eye donors using serologic testing methods are presented in Table 4.

**Table 4 – Residual risk after serologic testing in Australian Eye Donors**

<table>
<thead>
<tr>
<th>Window period† (days)</th>
<th>Estimated Incidence (no./100,000 person-years)</th>
<th>Estimated probability (no./100,000 eye donors)</th>
<th>Odds of infected donor being missed</th>
<th>Expected no. in Australian eye donors (@1000/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>22</td>
<td>0.35</td>
<td>0.0211</td>
<td>1 in 4,739,336</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 every 4,739 years</td>
</tr>
<tr>
<td>HBsAg</td>
<td>59</td>
<td>1.29</td>
<td>0.2085</td>
<td>1 in 479,613</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 every 479 years</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>70</td>
<td>3.02</td>
<td>0.5792</td>
<td>1 in 172,651</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 every 172 years</td>
</tr>
</tbody>
</table>

† from Dodd et.al\(^{22}\).

These results compare to the published United States estimates for the Tissue donor population/100,000 donors of HIV 1.815 (1 in 55,096), HBV 2.962 (1 in 33,760) and HCV 2.374 (1 in 42,122), and the Australian musculoskeletal donor population of HIV 0.78 (1 in 128,000), HBV 0.53 (1 in 188,000) and HCV 1.82 (1 in 55,000).
Calculation of residual risk with Nucleic Acid Testing (NAT)

NAT testing for these viral markers reduces the estimated “window-period” and thus reduces the calculated theoretical residual risk (Table 5).

Table 5 – Residual risk after NAT testing in Australian Eye Donors

<table>
<thead>
<tr>
<th></th>
<th>Window period† (days)</th>
<th>Estimated Incidence (no./100,000 person-years)</th>
<th>Estimated probability (no./100,000 eye donors)</th>
<th>Odds of infected donor being missed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>7</td>
<td>0.35</td>
<td>0.0067</td>
<td>1 in 14,897,959</td>
</tr>
<tr>
<td>HBsAg</td>
<td>20</td>
<td>1.29</td>
<td>0.0707</td>
<td>1 in 1,414,728</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>7</td>
<td>3.02</td>
<td>0.0579</td>
<td>1 in 1,726,584</td>
</tr>
</tbody>
</table>

†from Jackson et al [23].

Calculation of Residual Risk of Transmission (HIV, HBV, HCV) through sclera for ocular surgical purposes

No disease transmission has ever occurred through the surgical use of sclera, and thus the risk of seroconversion after surgical exposure sclera from a donor infected with HIV, HBV or HCV has to be based on published rates of seroconversion from similar inoculation scenarios.

Blood transfusion has a rate of transmission from HCV Ab/RNA blood components of approximately 80%. For HIV Ab+ it exceeds 90% but is less than 100% (C.R. Seed, G. Pollock; personal communication). For all types of surgical uses of sclera the likelihood of transmission is significantly less than that of percutaneous transmission with infected blood, which for HCV is approximately 1.8% [22], for HIV 0.3% [22] and HBV 6-30% [24]. (These rates are likely to be overestimates as the rates from mucous membrane transmission are likely to be more applicable [22]. However accurate figures on these rates are not available). To calculate residual risk of transmission usage rates per donor are also applied to the calculation. Every eye donor in Australia donates two eyes and thus two pieces of sclera (with the odd exception) but the average usage is one piece of sclera for every four donors. Thus, taking into account a usage rate for sclera in Australia of approximately 0.25 pieces per eye donor, the residual risk calculations after serology testing, and the likely rates of seroconversion (transmissibility) one can calculate the residual risk of transmission from the use of sclera. These are presented in Table 6.
Table 6: Residual risk of transmission after serology testing for sclera for surgical purposes in Australia

<table>
<thead>
<tr>
<th></th>
<th>Estimated probability of an infected donor (no./100,000 eye donors)</th>
<th>Theoretical rate of transmission with surgical use of sclera (%) inoculated</th>
<th>Probability of transmission† (no./100,000 eye donors)</th>
<th>Residual risk of transmission after serology testing</th>
<th>Expected transmission in Australian eye donors (@1000/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>0.0211</td>
<td>0.3</td>
<td>0.0000158</td>
<td>1 in 6,329,113,924</td>
<td>1 every 6,329,113 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>0.2085</td>
<td>3-60</td>
<td>0.001 – 0.031</td>
<td>3,225,806 - 100,000,000</td>
<td>3,226 – 100,000 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>0.5792</td>
<td>1.8</td>
<td>0.00261</td>
<td>1 in 38,314,176</td>
<td>1 every 38,314 years</td>
</tr>
</tbody>
</table>

† This takes into account approximately 0.25 pieces of sclera used for surgical purposes from each Australian eye donor

Residual Risk of Transmission of HTLV and Syphilis through sclera for ocular surgical purposes

It is not possible to calculate the prevalence, incidence and residual risk for HTLV and syphilis in the Australian eye donor population as the tests for these markers are not performed for eye donors.

However, it is known that the prevalence of HTLV-I in the general population in Australia is less than that for HIV, HBV or HCV, and the prevalence in United States blood donors has been reported at 25/100,000 (four times less than that of HIV)\(^{(25)}\). Therefore the residual risk probability for HTLV is likely to be the least of any of these viruses.

For syphilis, the prevalence and incidence in the general Australian population is similar as that for HIV\(^{(21)}\) and in the USA it is lower than of HIV\(^{(26, 27)}\). While these prevalence and incidence figures do not automatically translate into residual risk estimates in the eye donor population, it has been demonstrated that *Treponema Pallidum* is neither found in or on corneas or sclera that are used for surgical purposes (including transplantation), and it does not survive *in vitro* inoculation of cornea or sclera in their preservation media\(^{(28)}\). Therefore, residual risk of transmission (even in the absence of serology screening) is likely to be at least as insignificant as any of the other diseases considered here.
Over estimation of Residual Risk of Transmission from sclera used for surgical purposes and comparison with corneal transplantation

While the residual risk of transmission of HIV, HBV and HCV through the use of sclera has been determined by utilising percutaneous transmission rates with infected blood, these figures are likely to be an overestimate. All assume an inoculation equivalent to that of a hypodermic needle containing viable infected blood. However no viable virus or bacteria originating from the donor survives the processing of sclera for surgery (refer to section 6 of this submission). This is unlike the cornea where there is a theoretical (but highly unlikely) risk that a viable virus particle could survive the corneal preservation process. Thus the calculated residual risk, the demonstrated risk and the theoretical risk for the use of sclera are all lower than that of corneal transplantation – which already has the lowest risks of any form of transplantation.

6. PRESERVATION OF SCLERA

In Australia, sclera is preserved in 95% sterile ethanol (ethyl alcohol). This solution is a virucide (for both lipid and nonlipid viruses at the exposure times involved for scleral preservation), bactericide, tuberculocide and fungicide; it is not sporicidal

Several studies have shown human glycerine, 70% ethanol or 95% ethanol to be 100% bactericidal at 14 days for preservation of human sclera. Studies have also demonstrated the effectiveness of ethanol as a virucide for preserved sclera and in other ophthalmic uses. Other studies have shown the rates of 95% ethanol permeation through preserved scleral shells with 99.95% achieved by 50 minutes exposure, supporting the previous findings of its efficacy in scleral preservation and decontamination.

Thus, the risk of any virus of HIV, HBV, HCV and HTLV or bacterium for Syphilis surviving scleral preservation are insignificant and remote. The fact that donor sclera has never transmitted a disease, and the absence of any adverse reaction reports, would practically support this conclusion beyond any reasonable doubt.

7. LOGISTICS OF NAT TESTING IN AUSTRALIA IN RELATION TO SCLERA

Logistics of NAT testing for the release of sclera for surgical purposes relate to:

a. Availability
b. Testing requirements
c. Sample volume

Unlike the time constraints that apply to the testing of donor blood for corneal transplantation, sclera can be preserved for an extended period of time and therefore time constraints are not an issue. However, because the donors of sclera are the same donors as for corneas (the tissues are contiguous), the issues of testing requirements, available sample volume and possible effects of these on supply remain relevant.
a. **Availability**

Our submission of July 2009 indicated that validated cadaveric NAT testing was not widely available in Australia. For example, at the time (2009) the closest laboratory to test blood from a donor in Perth for validated cadaveric NAT testing was in Melbourne.

Recently (August 2012) the ARCBS confirmed that they are now able to provide validated cadaveric NAT testing for HIV-1 RNA, HBV RNA and HCV RNA. However, this service is not nationally available. Adelaide ARCBS no longer has testing facilities. Therefore blood from donors in South Australia, Tasmania, ACT and the Northern Territory would have to be transported interstate for testing. This not only has significant cost implications but more importantly is significant in regard to validity and potential failure in both sample transportation and sample validity itself after a period of time.

In addition, ARCBS is unable to offer validated cadaveric serology (which is still required to be performed in conjunction with NAT testing). Therefore at least two separate samples would be required to be sent to two separate laboratories. Again this is significant in regard to cost and, more importantly, sample volume and validity issues. With samples having to be serology tested for corneal transplantation purposes (with their time constraints) and separate samples having to be sent and tested for sclera purposes this especially compounds the problem of the lack viable sample that is available.

b. **Testing Requirements**

Sample collection restrictions, storage and transport conditions and the volume required for NAT testing create significant problems for cadaveric eye donation (and thus sclera donation).

The ARCBS has advised of the following requirements for the Novartis PROCLEIX assay (the platform currently used by ARCBS):

- **Cadaveric samples must be collected within 15 hours of death if the donor has not been refrigerated within 12 hours and 24 hours after death otherwise.**

  Particularly for cases under coronial jurisdiction, where consent to proceed with donation (and blood sampling) can be delayed for extended periods, these time frames will increasingly preclude donation because the blood sample will not be valid for NAT testing.

- **Whole blood must be tested within 24 hours of collection.**

  This precludes testing of whole blood in those instances where interstate transport is required for testing (e.g. South Australia).

- **Cadaveric plasma must be tested within 72 hours. If not able to be tested within 72 hours it must be frozen <-70°C and shipped on dry ice to the laboratory.**

  This has significant logistical and cost implications for donations “remote” from a testing laboratory and especially if interstate transport is required.

- **Pre-mortem samples (often required if cadaveric sampling cannot be performed or obtained, or if plasma dilution has occurred) must be tested within 72 hours of**
collection. If this is not possible plasma frozen at collection and stored <-20°C must be provided.

This is a significant impost and precludes donation for the majority of patients where post-mortem blood cannot be obtained or is invalid due to plasma dilution. Frozen pre-mortem samples of plasma are seldom held by pathology laboratories, especially in the volumes required. The alternative of fresh refrigerated plasma restricts the samples to within 2 days prior to death (at a minimum) thus restricting the availability of plasma in the volumes required, and for plasma diluted donors restricts the availability to those donor who have only been plasma diluted in the day preceding their death.

In addition to these restrictions, heparin and other common inhibitors interfere with NAT testing. Given the unique demographics of eye donors (compared to organ and tissue donors), there are a significant number of patients that have inhibitors in their blood stream at the time of death. Also, samples for NAT testing are required in an EDTA or Plasma preparation tube. Restricting blood samples to an EDTA/PPT tube further restricts access to pre-mortem samples if they are required.

c. Sample Volume

Most importantly, the volume of sample (plasma) required for NAT testing, serology testing and testing for HTLV-I and syphilis is quite substantial and exceeds the volume usually obtained at (non-coronial) cadaveric collection, or that able to be obtained from laboratories holding pre-mortem samples. The amount of blood sample (of quality suitable for testing) taken from a cadaveric donor can be very small but usually (but not always) within the limits of serology testing. Given that priority for any available viable sample volume will be given over to serology testing for corneal transplantation purposes, the availability of sample for additional testing for scleral purposes becomes a significant issue.

Some laboratories indicate the need for up to 20mls of blood to perform the three NAT tests. One laboratory indicates at least 2ml of serum per test is required. ARCBS indicates at least 10ml of blood and at least 3.5ml of plasma is required for the Novartis assay. These requirements are in addition to the volumes requires to complete serology testing (that would be performed for corneal transplantation purposes). A minimum of 30mls of valid whole blood or 7mls of plasma (split between two laboratories) is required to complete all serology, NAT, HTLV-1 and syphilis testing. For the majority of eye donors this is not obtainable – the additional testing for scleral purposes will be unable to be performed.

These additional testing requirements for release of sclera (and the inability to conduct these tests for a significant numbers of eye donations) will therefore have a direct impact on supply and availability in Australia. Unlike organs or other tissues, sclera is readily available from overseas sources. An alternative source will be the importation of sclera from overseas sources under special access schemes.
8. COMPARISON WITH OVERSEAS REGULATORY REQUIREMENTS FOR SCLERA

Overseas regulatory agencies do not discern testing requirements for biologicals of the same product type (e.g. corneo-scleral disc (released for transplantation), sclera (released for surgical use)) but rather base their testing requirements on the source and type of tissue donated – in this case ocular tissue/eye donors/eye donation. Therefore, while it is the case that overseas regulatory agencies generally require NAT testing for tissues other than ocular tissue, the majority do not require NAT testing or HTLV or action on syphilis testing for ocular tissue.

Therefore the following summary relates to testing of eye donors for the purpose of release of ocular tissues for surgical use (this includes corneo-scleral discs (released for transplantation) and sclera (released for surgical use).

NAT testing for HIV, HCV and HBV

All these regulations refer to all ocular tissue (i.e. all ocular product types) released for transplantation.

European Commission
NAT testing is not mandated under EU Directives but serological testing for HIV-1 & 2, HCV and HBV\(^{(35)}\) is mandated under EU Directive 2006/17/EC.

Some Member States apply other tests in addition to those established as minimum requirements in the Directive\(^{(36)}\), in particular of the 27 member States:

- NAT HIV-1 testing: Six Member States (Denmark, Estonia, Italy, Hungary, Portugal, Slovakia)
- NAT HBV testing: Five Member States (Denmark, Spain, Italy, Hungary, Portugal)
- NAT HCV testing: Six Member States (Denmark, Germany, Spain, Italy, Hungary, Portugal)

The introduction of NAT testing in Denmark has since been rescinded following a 34% drop in eye donor numbers following NAT implementation. Since ceasing the NAT testing mandate, numbers of eye donors have risen by 101%\(^{(11)}\). In Germany (HCV only) the requirement has been under review after donation in Baden-Württemberg dropped by 25%. The Charité hospital in Berlin noted similar effects. In addition, the Swedish Competent Authority has reported results of a national study which noted that the availability of cadaver corneas in Sweden may be reduced by over 60% if a 24 hour limit for post-mortem blood sampling (NAT test requirements) was required\(^{(37)}\).

Canada
Canada separates their standards for biologicals into three categories 1) Vascularised organs, 2) Tissues, 3) Ocular tissues. It does not mandate but recommends NAT testing of HIV, HBV and HCV for all tissues other than ocular. It does not mandate but recommends NAT testing for ocular tissue donation\(^{(38)}\).

United States of America
The US FDA requires NAT testing of all donors of HCT/Ps for HIV-1 and HCV (not HBV)\(^{(39)}\).
Testing for HTLV-1

European Commission
The EU Directive 2006/17/EC requires HTLV-I antibody testing for donors living in, or originating from, high-incidence areas or with sexual partners originating from those areas or where the donor’s parents originate from those areas. However the “areas” were not defined. In response to defining the geographical areas, and also in response to considering alignment with FDA regulations (HTLV-1 testing is only required for leucocyte rich tissues in the USA) the Regulatory Committee of the EU organised a study looking at the issues. Preliminary results indicate that 1) general population prevalence in US and Europe is fairly low and probably less than 0.1% and 2) the transmission of HTLV by lymphocyte cells is valid for blood donations, but uncertain for tissues and cells. For this evidence it appears the only likely high-incidence areas are the Caribbean and Japan and HTLV-I testing will be limited to these groups.

Canada
Canadian regulation makes HTLV-I testing mandatory only for donors of leukocyte-rich tissues, and recommended for donors of tissues that are not considered to be leukocyte-rich. It does not mandate nor recommend HTLV-I testing for ocular tissue donation.

United States of America
Under FDA requirements (CFR 1271.85), screening and donor deferral for HTLV is only required for viable, leukocyte rich HCT/P donors.

The ‘Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues and Cellular and Tissue-Based Products’ published by the FDA states that neither cornea nor sclera are considered viable, leukocyte-rich HCT/Ps (and hence do not require testing for HTLV).

Testing for Syphilis

There is no evidence that syphilis can be transmitted through the use of ocular tissue (indeed this was considered and examined as long ago as 1949). Screening for RPR reactivity was first introduced as an indicator of “high-risk” activity for viral infection (HIV and HBV) prior to tests for these viruses being readily available. However it has since been shown that there is a poor correlation between reactive syphilis serology and human immunodeficiency virus testing among potential eye donors. European experts to the W.H.O. Notify project consider that in some geographic areas, the most important caveat in accepting such donors is the possibility that syphilis may be representing a high-risk donor, thus increasing the risk of transmitting other more severe infections (i.e. HIV, HTLV-1, HCV, and other infections). The decision to accept such donors depends on past medical history and the evaluation from surgical team.

European Commission
Syphilis screening is required under EU Directive 2006/17/EC. However, a reactive result to syphilis screening is not an exclusion criteria for release of product.

Canada
Canadian regulations mandate syphilis testing for all tissues other than ocular tissues. It does not recommend syphilis testing for ocular tissue donation.
**9. FINANCIAL COSTS ASSOCIATED WITH ADDITIONAL TESTING REQUIREMENTS FOR SCLERA IN AUSTRALIA**

An analysis of the costs and reduced theoretical risks in undertaking NAT testing of Australian eye donors is presented in Table 7. These figures have been updated from the 2009 submission to now take into account the lower cost of testing quoted by the ARCBS ($45 per sample). This is the approximate cost for non-urgent batched testing of donor plasma. It does not take into account the additional expenses of transport from donor site to testing laboratory. They also do not take into account the cost of serology (ELISA) testing that must be performed in conjunction with NAT testing. The figures also assume that the residual risk after NAT testing is zero (the actual calculated residual risks are listed in Table 5) – thus the calculated costs are the *minimum additional* costs of detecting one eye donor (and one transmission through the use of sclera) by NAT testing that would *not* have been detected by serology testing.

**Table 7: Minimum additional costs of NAT testing in Australia**

<table>
<thead>
<tr>
<th>Test</th>
<th>Cost of detecting one infected donor</th>
<th>Cost of preventing one transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>$45</td>
<td>$213 million</td>
</tr>
<tr>
<td>HBsAg</td>
<td>$45</td>
<td>$22 million</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>$45</td>
<td>$7.7 million</td>
</tr>
</tbody>
</table>

†assumes 60% transmission rate

**10. CONCLUSION**

Overall, current measures used to evaluate sclera for surgical purposes in Australia and New Zealand are effective. Benchmarking with anti-HIV, HBsAg, anti-HCV prevalence data from Australian blood donors confirms that prevalence rates in eye (sclera) donors are low and are comparable to blood donor rates. In addition, the risk of transmission of these viral diseases from sclera is lower than that from a blood donor.

Implementing NAT to screen individual eye donors is estimated to reduce the residual risk of a donor being vireamic within the “window-period”. However, the calculated reduction is
insignificant from what is an already low level of risk. It also comes at a cost. NAT is more complex, time consuming and expensive than serological testing. The cost-effectiveness of nucleic acid testing may not compare favourably with that of other health preventative measures such as the evidence-based application of stringent donor exclusion criteria or the employment of increased numbers of senior experienced professional staff assessing donors. This is certainly the experience with HIV and HCV NAT donor screening in the United States where its cost effectiveness has been assessed as poor. In this study the costs involved in identifying one donor, or preventing one transmission of disease cannot be justified by any public health criteria. Responsible risk management suggests that rather than continuing to focus with marginal benefit on already comparatively low risks of viral transmission there needs to be a re-focus on increasing donor rates to enable more patients to benefit from what are already demonstrably safe scleral use. Logistical issues surrounding NAT testing in Australia will reduce the amount of sclera available in Australia and in effect will increase the overall risk to the recipient. Importation of unlicensed sclera from overseas (under special access schemes) to make up for the local shortfall, will also increase risk to the community.

HTLV-I, HTLV-2 and syphilis have never been reported to be transmitted via use of sclera or any other ocular tissue. By not mandating HTLV testing for eye (sclera) donors, Australian regulatory requirements will be consistent with regulatory requirements overseas (including US FDA, European Commission and Health Canada). Although some regulatory authorities overseas mandate testing for syphilis, a reactive result to syphilis screening is not an exclusion criteria for release of product.

In summary

- In the Australian and New Zealand context, mandatory NAT testing provides no significant reduction in risk of HIV, HCV or HBV, either in relation to detection of eye donors with viraemia or in regard to the transmission of viruses through the use of sclera. In addition, HTLV and Syphilis testing of eye donors provides no reduction in risk from transmission of this virus and bacterium from sclera to recipient.

- The low prevalence of the diseases in the Australian and New Zealand eye donor population, and the good evidence that these disease are not transmitted by donor sclera, makes the risk/benefit ratio of loss of tissue as a result of NAT testing, HTLV and Syphilis testing, unacceptable.

- On a risk to benefit to cost assessment, the costs involved to conduct the additional testing cannot be justified.

- The issues of availability of NAT testing; sample requirements for testing; and the sample volume required for this additional testing, will reduce the amount of sclera available in Australia. The resulting importation of sclera from overseas to meet demand will, paradoxically, increase risk to the Australian community.
11. REFERENCES


10. Eye Bank Association of America. EBAA Medical Standards. EBAA; July 2011.


