Eye Bank Association of Australia and New Zealand request for exemptions for ocular donors from:

- cadaveric NAT testing for HIV, HCV and HBV
- cadaveric serology testing for HTLV-1, HTLV-2 and syphilis

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Chair, EBAANZ
1. PURPOSE FOR SUBMISSION

The purpose of this submission is to seek 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.

Background

When developing the current draft of the Therapeutic Goods Order (TGO), ‘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’, the regulators took into account international best practice for eye banking and information provided by EBAANZ to the TGA in August 2009.

Based on this information which detailed residual risk and logistics, the TGO was drafted so that the Donor Testing Requirements for release (TGO Table 3) for “cornea only donors” were specifically limited to anti-HIV 1 & 2, anti-HCV and HBsAg.

In recent discussions between EBAANZ and the TGA it was clarified that the Donor Testing Requirements (Table 3) refer not to the type of donor or the product that is transplanted, but instead refer to the product that is released. However, viable cornea for transplant is never released as “cornea only”. Cornea is always released together with some of its contiguous sclera (depending on the product and the requirements of the surgeon it may be a rim of sclera or the whole eye (refer to Section 2 of this submission for product definitions). This is because until the actual surgery and due to its fragility, the cornea is never directly handled and is only ever held by its supporting eye or its scleral rim. In addition it is a requirement that the surgeon, as part of the operative procedure, cuts the cornea to a diameter appropriate for the recipient and for the procedure being performed (i.e. all of the sclera is removed). This is the international practice of eye banking.

Thus, the “cornea only donor” designation in Table 3 of the TGO becomes redundant, and the original analysis, purpose and decision of the regulators in limiting donor testing for ocular tissue has been lost.

Rather than amending the TGO standard at this late stage to read “ocular tissue” (which we have been informed by the TGA is likely to take some time), you have suggested instead we submit an exemption for our released products from some of the requirements of the Donor Testing Requirements (Table 3): Deceased Donors. In particular, exemption is required from NAT testing for HIV, HBV and HCV and from serological testing for HTLV-1 & 2 and syphilis for corneoscleral disc, sclera-only and whole globe product release types. This submission supplements our submission of August 2009 and provides the necessary supporting information (updated) upon which these exemptions are based.
2. DEFINITION OF PRODUCT TYPES

**Corneoscleral disc**

- The released product is cornea with a rim (usually 2-4 mm) of contiguous sclera.
- The cornea from this released product may be used for penetrating keratoplasty, deep lamellar keratoplasty or endothelial keratoplasty (i.e. all forms of corneal transplantation). Only the cornea, or a layer thereof is transplanted (all of the sclera has been removed).
- The cornea itself is avascular. This avascularity no doubt contributes to the fact that the cornea demonstrably presents the lowest risk of all forms of transplantation *(refer to Section 4 of this submission)*. The episclera (outer surface of the sclera) of the rim is vascularised but shows a specialised morphology characterised by arterio-venous loops with an absence of capillaries; here the vasculature function is related to maintenance of intraocular pressure. The scleral stroma (bulk of the sclera) contains few blood vessels, with the exception of the input and output vessels supplying other parts of the eye traversing through it.
- As part of the operative procedure, the surgeon cuts the cornea to a diameter appropriate for the recipient and for the procedure being performed, thereby removing all of the sclera prior to transplantation.

**Sclera only**

- The released product is a “scleral shell”. 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.
- The sclera from this released product may be used in glaucoma shunt surgery, oculoplastic procedures following enucleation due to disease or trauma, or tectonic patch graft procedures involving thinned or ruptured sclera.
- The sclera’s anatomy is as previously described in *corneoscleral disc*.
- The sclera’s low and unique specialised vascularity no doubt contributes to the fact that there are no reports in the medical/scientific literature or similar of disease transmission or adverse events involving sclera grafting.

**Whole globe**

- The whole eye is released. This is a vascularised organ. However, only the cornea or a layer thereof is transplanted. Depending on the transplant to be performed, the ophthalmic surgeon dissects from the whole globe an anterior layer of the cornea, a corneoscleral disc for further trephining to the required diameter, or directly trephines the required diameter cornea directly from the eye. Sometimes a corneoscleral patch may be dissected by the surgeon and grafted for tectonic/cosmetic purposes. Sometimes a portion of the limbus may be dissected and used for limbal transplantation to treat ocular surface disorders.
- This is the product release type now generally reserved for specialised transplant surgery such as limbal transplantation or traditional (superficial) anterior lamellar keratoplasty. However, it is still a valid product release for all types of transplant surgery and indeed was the only type of “product-release” for corneal transplantation until the mid 1970’s. Outside of the “developed countries” it remains the most common form of product release.
- Until the 1970’s all corneas for transplantation were preserved and released in this form. Even when used this way corneal transplantation demonstrably presents the lowest risk of all forms of transplantation *(refer to Section 4 of this submission)*.
3. DONOR ASSESSMENT

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 TGA Australia 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. Furthermore, the physical assessment requirements in the EBAANZ Standards – Section 8, involves scrutiny 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 (Edition 2, 2009) specifies 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 TGA Australia cGMP, Human Blood and Tissues (2000) also states that eye tissue is exempt from HTLV-I and syphilis testing.

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

EBAANZ member banks report on the number of positive serology results (non-confirmed 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 show 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 colleagues⁴. In addition, figures are available for Australia in relation to incidence rate in the blood donor population for viraemic markers⁶, and these can be applied using Zou’s method to estimate the an 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 colleagues⁶.

The same methodology of residual risk estimate has also recently been performed and published for musculoskeletal tissue donors in Australia⁷. 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 Australia⁸. In this time, unconfirmed positive serology results reported were HIV - one, HBV - nine and HCV – seven. Applying Zou’s reported and validated figures of percent false positives for each of these results (HIV – 89%, HBV – 68% and HCV- 28%)⁴ the Australian eye donor prevalence rates are listed in Table 1.

Table 1. Australian Eye Donor Prevalence Rates

<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>

†fractional values are presented as a result of the estimation of numbers following Zou’s⁴ estimations.
Table 2. Prevalence Rates among different donor populations

<table>
<thead>
<tr>
<th></th>
<th>Prevalence per 100,000 persons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Australian eye donors</td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>5.93</td>
</tr>
<tr>
<td>HBsAg</td>
<td>155.17</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>271.55</td>
</tr>
</tbody>
</table>

† Published in Yao et al. for 1993-2004, * Published in Zou et al. for 2001-2002.

These comparative prevalence rates 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 United States general population is estimated at around 600/100,000 people compared with Australia 69/100,000 (and New Zealand 10/100,000)⁹. 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).

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 United States blood donors to assign estimated incidence rates among tissue donors⁴. Yao and colleagues made the same extrapolation between Australian musculoskeletal and Australian blood donors⁷. 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* (no./100,000 person-years)</th>
<th>Estimated Incidence rate in eye donors (no./100,000 person-years)</th>
</tr>
</thead>
<tbody>
<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. Seed⁶ provides an adjusted incidence figure to account for underestimation

* derived from Yao et al. 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\(^6\), which is that also used by Zou\(^4\) and Yao\(^7\).

- 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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>172 years</td>
</tr>
</tbody>
</table>

†from Dodd et.al\(^10\).

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\(^1\).
Table 6: Residual risk of transmission after serology testing for Australian corneal transplants

<table>
<thead>
<tr>
<th></th>
<th>Estimated probability of an infected donor (no./100,000 eye donors)</th>
<th>Theoretical rate of transmission in corneal transplantation (% inoculated)</th>
<th>Probability of transmission† (no./100,000 eye donors)</th>
<th>Residual risk of transmission after serology testing 1 in</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>0.0001013</td>
<td>987,361,769</td>
<td>1 every 987,361 yrs</td>
</tr>
<tr>
<td>HBV</td>
<td>0.2085</td>
<td>3-60</td>
<td>0.010 – 0.125</td>
<td>799,360 - 9,992,006</td>
<td>1 every 799 - 9,992 yrs</td>
</tr>
<tr>
<td>HCV</td>
<td>0.5792</td>
<td>1.8</td>
<td>0.0167</td>
<td>5,994,858</td>
<td>1 every 5,994 years</td>
</tr>
</tbody>
</table>

† This takes into account approximately 1.6 corneal transplants from each Australian eye donor.

The residual risk of transmission of these viruses through the use of sclera can be calculated in the same fashion as above. The residual risk probability of an infected donor is the same as that calculated above, as sclera is derived from the same eye donors. While sclera is not entirely avascular like the cornea, the likelihood of transmission is considered the same as that of percutaneous transmission with infected blood (similar to a needle stick injury with infected blood). Thus the figures above for corneas (an overestimate of risk for corneal transmission based on percutaneous transmission rates) are also good calculated values for sclera.

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). Therefore the residual risk probability for HTLV is likely to be the least of any of these viruses.
5. TRANSMISSION OF HIV 1 & 2, HEPATITIS B, HEPATITIS C, HTLV I & II AND SYPHILIS VIA TRANSPLANTATION OF OCULAR TISSUE (CORNEA AND SCLERA)

When assessing reports of disease transmission via ocular tissue transplantation it is worth considering that successful corneal transplantation and whole eye donation (and release) was first described in 1905. It is the most common form of cadaveric donation and transplantation. World-wide numbers of accumulated corneal transplants performed are not available, but recent estimates by the Global Alliance of Eye Bank Associations and reports to the World Health Organisation estimate it to be between 150,000 to 200,000 transplants per year for the past 25 years (M. Masci, P. Dubord, M. Mannis, G. Pollock, R. Vajpayee, E. Pels, L. Noel: personal communication).

HIV 1 and 2

HIV has never been reported to be transmitted via transplantation of cornea, sclera or any other ocular tissue. This includes all product release types (listed in Section 2 of this submission).

There are three reports in the literature detailing nine patients who received corneas from HIV-positive donors\(^1\),\(^5\),\(^6\),\(^7\). None of the corneal recipients seroconverted or became ill, although all other organ and tissue recipients from these donors seroconverted.

HIV 1 has been documented to be in tears\(^8\) and corneal discs\(^9\),\(^10\),\(^11\),\(^12\),\(^13\),\(^14\) but only a small percentage of donors with antibody HIV have detectable genome in the cornea\(^15\). The potential for transmission of HIV via corneal transplantation is considered to be lower than that of percutaneous transmission and is likely to be more analogous to transmission through mucous membrane contact\(^16\). The incidence of seroconversion after exposure to HIV-positive blood is 0.3% after a percutaneous exposure (e.g. needle-stick injury) and 0.09% after mucous membrane exposure. The risk of seroconversion after exposure to other tissues or fluids, while not quantified, is felt to be considerably lower\(^17\). In comparison, transmission approaches 100% through blood transfusion.

The potential for transmission of HIV via the surgical use of sclera is thought to be similar to percutaneous transmission\(^1\).

Hepatitis C

Hepatitis C virus (HCV) has never been reported to be transmitted via transplantation of cornea, sclera or any other ocular tissue. This includes all product release types (listed in Section 2 of this submission).

There are reports in the literature detailing of six patients who received corneas from three HCV seropositive donors (at least two of whom had viral RNA in their serum). None of the corneal recipients seroconverted after transplantation\(^1\).

The risk of contracting hepatitis C after exposure to HCV-positive blood is 1.8% after a percutaneous exposure (e.g. needle-stick injury) and is considered rare after a mucous membrane exposure\(^1\).

Polymerase chain reaction assays indicate that only 20-26% of seropositive cornea donors have viral RNA in their serum, and initial attempts to detect viral RNA in the cornea were unsuccessful\(^1\). However more recently there was a report of HCV RNA detection in 24% of corneas obtained from seropositive donors\(^2\). However, the potential for transmission of HCV via corneal transplantation is
considered to be lower than that of percutaneous transmission and, like HIV, is likely to be more analogous to transmission through mucous membrane contact\textsuperscript{25}.

The potential for transmission of HIV via the surgical use of sclera is thought to be similar to percutaneous transmission\textsuperscript{25}.

**Hepatitis B**

Transmission of hepatitis B virus (HBV) has been documented in two corneal recipients from two separate donors\textsuperscript{30}. Since serologic screening for HBsAg was introduced (in the late 1980’s) there have been no reported cases of transmission.

HBV has never been reported via transplantation of sclera or any other non-corneal ocular tissue.

Recipients of one cornea from each donor developed clinical and serological evidence of HBV infection 2 months and 14 weeks after penetrating keratoplasty. The recipient of the fellow cornea from one donor died from a CVA 4 months after surgery without undergoing serologic testing. The recipient of the fellow cornea from the other donor never developed clinical characteristics of hepatitis but tested positive for prior exposure to HBV 2 years after penetrating keratoplasty\textsuperscript{30}.

These cases occurred in 1984 and 1985, before screening for HBV was required. Since then there have been no cases of hepatitis B transmission via transplantation of corneal tissue.

In one study less than 10% of HBsAg-seropositive donors had detectable HBsAg in their corneas\textsuperscript{31}, and in a similar study no viral genome was detected\textsuperscript{32}. The chance of a cornea being infectious prior to the appearance of sAg in the blood is considered to be very small\textsuperscript{32}.

**HTLV I & II**

HTLV-I or HTLV-II has never been reported to be transmitted via transplantation of cornea, sclera or any other ocular tissue. This includes *all* product release types (*listed in Section 2 of this submission*).

The prevalence of HTLV-I in the U.S. blood donor population has been reported to be 25/100,000\textsuperscript{14}, far less than that of HIV, HBV or HCV. HTLV-III (yet to be associated with any disease state) has been isolated from the cornea\textsuperscript{19,20}, although no transmission of this virus has been reported.

**Syphilis**

Syphilis has never been reported to be transmitted via transplantation of cornea, sclera or any other ocular tissue. This includes *all* product release types (*listed in Section 2 of this submission*).

In animal experiments, transmission of syphilis by corneal transplantation has not been demonstrated\textsuperscript{33}. Other experiments with donor corneas from rabbits infected with *Treponema Pallidum* showed that rabbit corneal tissue contains few, if any, *T.Pallidum* organisms under corneal preservation conditions, and expert opinion concluded that it is highly unlikely that any treponemes present in human corneas would survive to cause infection in recipients\textsuperscript{34}. 
6. LOGISTICS OF NAT TESTING IN AUSTRALIA

Logistics of NAT testing relate to:

a. Availability
b. Turn-around times
c. Testing requirements
d. Sample volume

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, and routine turn-around times (not counting transport time) were 7-11 days.

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 turn-around times and sample validity (refer to following points in this section).

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 (refer to following points in this section).

b. Turn-around times

Unlike all other TGA Class 2 biologicals, donated corneas (released as corneoscleral discs or whole eyes) are extremely “time-sensitive”; much like whole organs. The Australian Corneal Graft Registry 2007 report shows that approximately 70% of all corneal transplants were performed between 0-4 days after the death of the donor. Almost 10% were performed within 24 hours. Less than 3.5% were performed at 7 days or more. This is because with increased storage times surgical handling is more difficult, the post-operative course is extended, and transplant outcomes are less successful. The 2010 report shows that the introduction of normothermic preservation in Australia has been able to extend these times, but hypothermic storage (corneoscleral product release) still accounts for approximately 30% of all corneas transplanted in Australia.

Corneal transplantation involving hypothermic preservation ideally requires the flexibility to transplant corneas within 24 hours after death of the donor. In addition, the product release of whole eyes requires transplantation of the cornea within 48 hours (as required by EBAANZ and TGA TGO No. 85, Standards for human ocular tissue) and ideally within 24 hours of donor death. Such routine turn-around times are available for serologic testing of HIV, HBV and HCV across Australia but not for NAT testing. The ARCBS testing service can only provide routine turn-around times (upon receipt of the sample) of 48 hours. This does not include any transport component (especially restrictive for eye donors in South Australia, Tasmania, ACT and NT), or the time from the death of the donor until receipt of the sample.
In summary, the requirement for NAT testing will increase rather than reduce risk. For a significant number of product releases the delays involved in NAT testing could potentially compromise corneal quality and viability, jeopardising the efficacy of the transplant and safety of the recipient.

c. Testing Requirements

Sample collection restrictions, storage and transport conditions and the volume required for NAT testing create significant problems for cadaveric eye 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 (refer to Section 6d of this submission). 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 demographics of eye 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.
d. **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 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.

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 required to complete serology testing. At 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.

Therefore in summary of the logistics of NAT testing:

- **In Australia, access to NAT testing within the time-frames required for transplantation would reduce the number of safe and viable corneas available for transplant in Australia and increase rather than reduce overall risk to the recipient.**

7. **FALSE POSITIVE RATE ASSOCIATED WITH NAT TESTING**

NAT HIV testing in Australia for eye donors has a significant false positive rate. The Queensland Eye Bank performed NAT for HIV and HCV on each eye donor from 1 June 2009 to 30 June 2011 (589 donors). The false positive rate for NAT HIV was 5.5% (N. Nuttall, personal communication).

If NAT testing was mandatory, this false positive rate would have resulted in approximately 100 fewer corneal transplants being performed across Australia in 2011.

8. **FINANCIAL COSTS ASSOCIATED WITH NAT TESTING 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, or the additional costs involved in providing “at-call” testing of donor serum (which some laboratories have quoted at greater than $1000 per sample). They do also 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 donor (and one transmission) 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 30% transmission rate

Additional testing and costs per donor also have a greater relative effect on the eye donation and transplantation than on any other forms of tissue donation. Most donors in Australia are eye-only donors (due to age and medical contraindications precluding other forms of donation), with two possible transplants (corneas) and two possible grafts (sclera) arising from one donor. Therefore reimbursement of the costs involved in the donation can only be divided between four possible service fees. In addition, time-constraints for donation and corneal transplantation mean that costs are often greater because it likely that “at-call” testing will be required. In contrast, other forms of tissue donation (e.g. skin, musculoskeletal, cardiovascular) quite often take place together, and especially in the case of musculoskeletal donation, one donation can result in many products. Long storage and quarantine times for other tissues also mean it is possible to pool samples for testing, avoiding high “at-call” costs of testing (or re-testing of false positive or equivocal samples). Thus the additional testing costs involved with one donor can be amortised over several types of donation, and within a single donation type (e.g. musculoskeletal) over more potential recipients and service fees. The additional cost impost to the patient, health system and manufacturer is substantially less than for eye donation.
9. COMPARISON WITH OVERSEAS REGULATORY REQUIREMENTS

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 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, in particular of the 27 member States:

- NAT HIV-1 testing [14]: Six Member States (Denmark, Estonia, Italy, Hungary, Portugal, Slovakia)
- NAT HBV testing [15]: Five Member States (Denmark, Spain, Italy, Hungary, Portugal)
- NAT HCV testing [16]: 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%. 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.

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.

United States of America

The US FDA requires NAT testing of all donors of HCT/Ps for HIV-1 and HCV (not HBV).

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 tissue 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\(^42\).

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\(^43\).

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)\(^47\).

Testing for Syphilis

There is no evidence that syphilis can be transmitted by corneal transplantation\(^33,34\). 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 cornea donors\(^45\). 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 the transplantation team\(^46\).

European Commission
Syphilis screening is required under EU Directive 2006/17/EC\(^38\). However, a reactive result to syphilis screening is not an exclusion criteria for release of product\(^41\).

Canada
Canadian regulations mandate syphilis testing for all tissues other than ocular tissues. It does not recommend syphilis testing for ocular tissue donation\(^42\).

United States
The FDA mandates syphilis screening for all donors (CFR 1271.85(a))\(^43\). However, like the EU directives, a reactive result to syphilis screening is not necessarily an exclusion criteria for release of product. The 2011 Eye Bank Association of America Medical Standards do not recommend syphilis testing of ocular donors\(^5\).

10. CONCLUSION

Overall, current measures used to evaluate eye donors 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 donors are low and are comparable to blood donor rates. In addition, the risk of transmission of these viral diseases from an eye donor is lower than that from a blood donor. This is in contrast to the low but significantly higher risk (compared to eye donors) among Australian musculoskeletal tissue donors. Additional benchmarking against United States equivalents also confirms that Australian corneal donation and transplantation is among the safest of any type of donation in the world.

Implementing NAT to screen individual eye donors is estimated to reduce the residual risk of a donor being vireamic within the “window-period”. However, this calculated reduction from an already low
level of risk 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\(^{29}\). 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 corneal transplants. Logistical issues surrounding NAT testing in Australia will reduce the number of safe and viable corneas available for transplant in Australia and will increase the overall risk to the recipient.

HTLV-I, HTLV-2 and syphilis have never been reported to be transmitted via transplantation of cornea, sclera or any other ocular tissue. By not mandating HTLV testing for ocular tissue 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. The Eye Bank Association of America does not recommend syphilis testing of ocular donors.

In summary

- In the Australian and New Zealand context, mandatory NAT testing will provide no 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 corneal transplantation.

- The low prevalence of the diseases in the Australian and New Zealand eye donor population makes the risk/benefit ratio of loss of tissue as a result of NAT testing unacceptable.

- The introduction of mandatory NAT testing in Australia will reduce the number of safe and viable corneas available for transplant in Australia and will increase the overall risk to the recipient.
11. REFERENCES


37 Therapeutic Goods Administration. TGO 85 (Standards for human ocular tissue) Commonwealth of Australia 2011.


