

# Abstracts from 2019 Eye Bank Association of America Scientific Symposium

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## IMPACT OF SCLERAL CONTACT LENS USE ON RISK OF REQUIRING CORNEAL TRANSPLANTATION FOR KERATOCONUS

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**Purpose:** To evaluate the association of scleral contact lens (SCL) use on the need for subsequent keratoplasty for patients with keratoconus (KCN).

**Methods:** The Sight Outcomes Research Repository was used to capture data from the electronic health records of patients receiving eye care between 2012 and 2018. We identified all patients who had an International Classification of Diseases diagnostic code of KCN and no previous history of keratoplasty. A multivariable logistic regression model assessed the association between use of SCLs and the need for keratoplasty. Models were adjusted for sociodemographic factors (age, gender, ethnicity, insurance status, and community of residence), maximum corneal keratometry value, and use of rigid gas permeable (RGP) contact lenses.

**Results:** A total of 1,683 patients with KCN met inclusion criteria. After controlling for all other factors in our model, use of a SCL was associated with 75.8% decreased hazard of a patient requiring keratoplasty (HR=0.242, 95% CI 0.09-0.62,  $p<0.005$ ). Factors associated with an increased hazard of keratoplasty were black race (versus white) (HR=1.75, 95% CI 1.03-2.97,  $p=0.04$ ), steeper keratometry (HR=1.312, 95% CI 1.16-1.49,  $p<0.0001$ ), and lower socioeconomic status (HR=1.011, 95% CI 1.00-1.02,  $p=0.01$ ).

**Conclusion:** Patients who use SCLs for KCN have lower risk of undergoing a corneal transplantation while RGP use had no effect.

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## KIDNEY DISEASE: A RISK FACTOR FOR CORNEAL TISSUE CONTAMINATION

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**Co-Authors:** Lucero Pedro-Aguilar, MD; Martha P. Jaimes-Gutierrez, MSC; Enrique O. Graue Hernández; and David Rivera de la Parra

**Purpose:** To assess the contamination rate and identify donor risk factors on the contamination of donor corneas in a single Eye Bank in Mexico City.

**Methods:** We performed a retrospective review of donor clinical records between 2011 and 2017. Demographic information from the corneal donors, such as systemic diseases or kidney disease (KD), cause of death, death to preservation time and positive donor rim culture results were recorded for statistical purposes. Descriptive statistics, potentially associated factors and contamination correlation between the samples were analyzed.

**Results:** A total of 444 donor rim cultures were included. Pathogens were obtained in 17 samples (3.8%); 9 (53%) of them attributed to Gram-negative bacteria. *S. epidermidis* was the most common Gram-positive isolated microorganism and *Pseudomonas aeruginosa* the most common Gram-negative microorganism. Donor rim cultures were 5 times more likely to be positive in donors with KD (15.38%) than those with no KD (2.76%), ( $p=0.002$  95% CI, 1.02-1.13).

**Conclusion:** The usefulness of routine culturing reveals conflicting data. Our findings suggest a higher contamination rate between donors with KD and those without KD. Our proposal is that due to KD, this group of patients may have had previous pathologic exposures that increase the risk of colonization. Although positive cultures do not predict clinical infection, performing routine donor rim cultures in donors with risk factors for contamination such as systemic immunosuppression, uncontrolled chronic diseases, KD or long hospital stay could be useful to reduce contamination rates in donor corneas.

\*Resident

## COMPARISON OF THE PERFORMANCE OF TRADITIONAL CORNEAL STORAGE MEDIA WITH A NEW CORNEAL STORAGE MEDIA WITH ANTIMYCOTIC TABLET

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**Purpose:** To compare the performance of Kerasave and Optisol-GS corneal storage media across metrics of donor evaluation and processing.

**Methods:** Forty paired corneas were swabbed for microbiological testing prior to recovery. Donor decontamination occurred per eye bank SOP, tissue was recovered and placed into a CVC containing Kerasave (Alchimia) or Optisol-GS (B&L) at 2-8°C. Tissue simulated the eye bank process for PK, DSAEK, and DMEK. Endothelial density (ECD), central corneal (or DSAEK graft) thickness (CCT), slit lamp exam (SL), and endothelial cell mortality (ECM) were obtained following incubation. These points in time varied for PK and DMEK/DSAEK groupings. Media was collected at the time of tissue processing for sterility testing, and at time of final evaluation.

**Results:** Initial swab tests showed 90% and 86% contamination of corneas being stored in Kerasave and Optisol-GS respectively, 24% and 19% of which were due to fungal contamination. Kerasave was free of fungi, and fungi was detected in one Optisol-GS media sample post-DSAEK processing. In the PK group, CCT was on average  $45 \pm 3 \mu\text{m}$  greater in Kerasave than in Optisol-GS at day 1 ( $p=0.006$ ); the difference in CCT was not statistically significant at day 6 ( $40 \pm 13 \mu\text{m}$ ,  $p=0.311$ ) and day 12 ( $14 \pm 5 \mu\text{m}$ ,  $p=0.765$ ). In PK, DSAEK, and DMEK simulation, no significant differences were observed in ECD or ECM between Kerasave and Optisol GS, or among different time points within the same group.

**Conclusion:** Key metrics of corneas stored in Kerasave and Optisol-GS and processed for DSAEK and DMEK were comparable. In the PK group, the degree of swelling was greater for Optisol-GS than Kerasave while initial CCT was greater in Kerasave.

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## THE EFFECT OF ARTIFICIAL ANTERIOR CHAMBER PRESSURE ON MICRON REMOVAL DURING MICROKERATOME GRAFT PREPARATION

*Marguerite DelVecchio, Eversight*

**Co-Authors:** Michael Titus, CEBT; Holly Hartman; Kayla Jones, CEBT; and Kristen McCoy, CEBT, CTBS

**Purpose:** The purpose of this study is to evaluate the results of varying artificial anterior chamber pressure (AACP) on micron removal during microkeratome graft preparation.

**Methods:** Corneas were prepared for DSAEK by two technicians with variable amounts of pressure behind the cornea for each cut ranging from 90 mmHg to 140 mmHg. The pressure was controlled using the Moria ACP console. Optical coherence tomography (OCT) was used to measure thickness before and after preparation. Micron removal data was calculated by subtracting the graft thickness from the full thickness of the cornea. Data was analyzed for successful outcome by determining surgical suitability. To analyze correlation between pressure and micron removal, data was segregated by technician and microkeratome head (350  $\mu$  or 400  $\mu$ ). Groups with the largest data sets were analyzed.

**Results:** The study included over 500 donor corneas with full thickness measurements that ranged from 386 microns to 697 microns, while graft thickness ranged from 42 microns to 176 microns. There was no difference in the success rate among groups at different pressure levels with the overall success rate at 98%.

Microkeratome head data for Technician One with the 350  $\mu$  head showed moderate correlation between the depth of the cut and the AACP ( $r=0.654$ ,  $n=190$ ,  $p<0.001$ ). Technician Two with the 400  $\mu$  head also showed moderate correlation ( $r=0.665$ ,  $n=71$ ,  $p<0.001$ ). For each mmHg increase in AACP, micron cut depth increased by 0.76  $\mu$  and 1.08  $\mu$  for Technicians One and Two respectively.

**Conclusion:** Preliminary data analysis shows a trend as AACP increases, micron removal also increases during microkeratome graft preparation. This data indicates an additional variable that can be controlled and modified to achieve target graft thickness. Other donor characteristics such as age and diabetes may have an impact on micron removal and will be analyzed and included at time of presentation.

## EFFICIENCY OF THE NARROW-BAND ULTRAVIOLET (UV) LIGHT IN ANTIFUNGAL AND ANTIBACTERIAL DECONTAMINATION OF DONOR CORNEAS – PRELIMINARY RESULTS

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**Purpose:** FDA approved cornea storage media do not contain an antifungal additive. The efficacy of Narrow-Band 222 nm UV light in reducing potential corneal fungal and bacterial contamination was evaluated and the corneal endothelial safety was studied

**Method:** A 222 nm UV Light generator was utilized. Plates with *Candida Albicans* (CA), MRSA were exposed to UV light for 5, 10, 20, 30, 40, 60 sec from distance 5.0 cm, 5.5cm, 6.0 cm. Plate inhibition activity was measured. Corneoscleral rims(n=6) were cultured prior to contamination, post contamination with CA and post UV light application. Corneal endothelial safety was evaluated (n=5) by specular microscopy at 10 min, 1day and 3 days post irradiation on each cornea

**Results:** There was no growth of CA, MRSA at 40 and 60 seconds and observed partial inhibition at 20 seconds. No endothelial toxicity was noted at 60 seconds of UV exposure.

**Conclusion:** Our study confirms the antifungal and antibacterial efficacy of 222 nm Far Ultraviolet Light. It is safe for the endothelium at 60 seconds at the prescribed distance

## THE EFFECTIVENESS OF CHLORHEXIDINE GLUCONATE IN THE DECONTAMINATION OF DONOR CORNEAS FOR TRANSPLANTATION

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**Purpose:** Surface decontamination of donor eyes is a standard operating procedure that eye banks advocate prior to enucleation and this antiseptis procedure has been shown to be very effective in reducing the microbiota. The objective was to evaluate the antiseptic effect of 0.05% chlorhexidine gluconate in reducing the microbiota of the eyeballs before enucleation.

**Methods:** The antiseptic was applied in thirty eyes at the action times of 5, 10 and 15 minutes and for each action time 10 eyes were used. Swabs were collected before application of the solution, after the time of action and at the time of preservation of the corneal tissue. After identification of the microbiota, an antibiogram test with gentamicin was performed.

**Results:** The results showed that in the second collection, after the use of the antiseptic, there was a reduction of the microbiota of 50% in the total of Gram-positive bacteria, 76.2% in the Gram-negative bacteria, and 100% in the isolated fungi. Chlorhexidine gluconate was shown to be more effective in Gram-negative bacteria, with a significant value ( $p = 0.01$ ). In the third collection, the residual effect had an average time of 2 hours and 11 minutes, the reduction of the microbiota was 100%. In the antibiogram test, 88% of the microorganisms were sensitive to gentamicin.

**Conclusion:** Chlorhexidine gluconate can be used in the decontamination of donated corneas, and five minutes are sufficient for its action. Its cationic characteristics allow affinity to the skin and mucous membranes, conferring greater adhesion and permanence at the application site and its residual time has increased the decontamination power of the donor eyeballs for corneal transplantation.

## LEARNING CURVE FOR IN SITU CORNEOSCLERAL EXCISION BY HEALTH CARE PROFESSIONALS WITHOUT PREVIOUS SURGICAL TRAINING: COMPARISON OF THREE DIFFERENT LEARNING APPROACHES

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**Purpose:** To determine the learning curve for in situ corneoscleral excision by health care professionals without previous surgical training, comparing three different approaches in a porcine model.

**Methods:** The study was conducted from March to July 2017 in one ophthalmological center in Mexico City. Participants were randomized to three different learning groups. In each group, subjects repeated the technique in pig eyes until satisfactory performance was achieved. Every practice in consecutive eyes was distributed over different times among groups. For group one, the whole practice was undertaken in one day having one practice every hour; for group two, one practice was performed every 24 hours and for group three, one practice was performed every 7 days. Descriptive statistics were performed, through measures of central tendency and dispersion; as well as an analysis of variance (ANOVA) for independent groups, using statistical software STATA version 12.0.

**Results:** Twenty-four subjects were included in this study. The mean age was  $24.8 \pm 1.83$  years. In our study, the average number of attempts needed to learn the correct corneal recovery technique was  $3.0 \pm 1.2$  times. The average time to learn to perform the technique for each group was: group one  $25.27 \pm 7.41$  minutes; group two  $20.53 \pm 5.40$  and group three  $24.12 \pm 5.46$ . The number of attempts required to learn to perform a satisfactory technique was: group one  $4.25 \pm 0.70$  times; group two  $3.87 \pm 0.83$  times; group three  $1.87 \pm 0.64$  times. In the analysis of variance, we did not find a significant difference in the average time to perform the technique, however, a smaller number of attempts were found in group three, which realized weekly ( $p < 0.01$ ), with an average of  $1.87 \pm 0.64$  times.

**Conclusion:** After a short technical learning period, any health personnel without previous surgical training is able to perform an adequate corneal recovery. Our results suggest that weekly practice of the in situ corneoscleral excision technique, until the student meets the criteria of suitability, reduces the number of attempts required to achieve it, up to an average of two eyes.

The standardization of Procurement Protocols by Eye Banks and cornea excision performed by health personnel may increase the corneal donation rates as a result of a greater number of professionals trained to carry out the procurement, optimizing human resources and reducing the time between death and recovery. The proper technique of in situ excision allows the procurement of corneal tissue of the highest quality, which could optimize the resources to increase the amount of viable tissue for transplantation purposes.

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## ETHICS IN EYE BANKING: UNDERSTANDING ATTITUDES TOWARD INDUSTRY CHANGES

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**Co-Authors:** Yousuf Khalifa, MD; Gerard Vong, PhD; and Rebecca Pentz, PhD

**Purpose:** The eye bank industry faces unprecedented changes. The introduction of for-profit entities poses a novel ethical challenge for eye banking, and understanding the perspectives of the relevant professionals is a necessary prerequisite for discussion and policy making.

**Methods:** A survey composed of semi-structured interview questions and Likert scale statements was used to assess opinions on eye banking among corneal surgeons (CS) and eye bank leadership (EBL) in the USA. Surveys were cognitively tested with relevant professionals and revised as needed. Participants were recruited using email listservs, personal contact via phone/email, or via rolling ball method. Completion of the survey indicated consent. Surveys were conducted in person or by phone. All data was de-identified and kept confidential.

**Results:** Nearly all interviews with CS ( $n=41/50$ ) and EBL ( $n=20/25$ ) are complete. The majority of CS (63.4%) and EBL (60%) agreed that they have ethical concerns with for-profit entities in eye banking. The concerns most strongly expressed via Likert statements were loss of donor trust (68.3% CS, 55% EBL) and exploitation of donor generosity (60.9% CS, 65% EBL). This was consistent with the qualitative data. Other themes of note include conflicts of interest, eye bank consolidation, and collaboration in innovation.

**Conclusion:** Examination of attitudes toward the changing eye bank industry reveals many ethical concerns. Discussion of these concerns should be encouraged amongst invested parties, and regulatory bodies should consider the positions of these stakeholders in the goal of ethical policy creation. More research is needed to assess the impact of for-profits on donors/donation rates.

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## IMPACT OF QUALITY INDICATORS AND QUALITY ASSURANCE ON UTILIZATION OF CORNEAL TISSUES IN A COMMUNITY EYE BANK

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**Purpose:** To study the impact of quality indicators and quality assurance on utilization of corneal tissues in a community eye bank.

**Methods:** For the period 2008 to 2010, corneas recovered for transplant but discarded by eye bank were grouped into four categories based on reason for discard. These categories were "Tissue Quality," "Seropositive," "Medical History," and "Blood Sample Issue." Gap analysis was done and areas of improvement identified. Factors under the control of the eye bank were targeted for improvement. As part of the Quality Assurance Program (QAP), three specific interventions were undertaken. The donor selection criteria were expanded with documentation and strict adherence. Second, was the identification and implementation of Quality Indicators (QI) based on "result quality" and "process quality". Lastly, the effective staff competency management processes were instituted. Performance from two periods Pre-Intervention (2008-2010) and Post-intervention (2012-2017) was compared.

**Results:** In the Pre-Intervention period, the eye bank recovered 1,425 donor corneas and transplanted 762. In Post-intervention period 6,661 corneas were recovered and 4,393 transplanted. Average annual utilization rate increased from 53.78% to 66.37%

( $p=0.011$ ). Non-utilization due to "Blood Sample Issue" and "Medical History" reasons decreased from 3.49% to 0.45% ( $p=0.001$ ) and 7.20% to 0.48% ( $p=0.039$ ) respectively.

**Conclusion:** Stringent implementation of QI and QAP effectively improves utilization rate of recovered corneas. Thus, these can be effectively adopted by eye banks to increase volume of transplantable corneas specially in countries facing paucity of good quality tissues.

## THE RELATIONSHIP BETWEEN DONOR CORNEAL THICKNESS AND LONG-TERM ENDOTHELIAL CELL LOSS IN DSAEK PATIENTS

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**Purpose:** To determine if the thickness of the donor cornea affects the recipient's long-term (3 and 5 years) endothelial cell loss in patients receiving DSAEK for Fuchs' Dystrophy.

**Methods:** A consecutive series of eyes that received DSAEK for Fuchs' Dystrophy between 11/2006-5/2012 were retrospectively analyzed. Endothelial cell loss (ECL) at 3 and 5 years postoperative was calculated and compared to the donor total corneal thickness. A subgroup analysis of donor corneal thickness ( $<550\ \mu\text{m}$  and  $\geq 550\ \mu\text{m}$ ) and ECL was also performed. Pearson correlation and students t-test analysis were used to determine statistical significance.

**Results:** Overall ECL at 3 and 5 years postoperative was  $31.7\% \pm 18.9\%$  ( $n=241$ ) and  $43.9\% \pm 20.8\%$  ( $n=182$ ), respectively. Pearson correlation coefficient comparing donor thickness and ECL at 3 years was 0.072 ( $P=0.263$ ) and at 5 years it was 0.047 ( $P=0.531$ ). 3-year ECL of donor corneas  $<550\ \mu\text{m}$  and  $\geq 550\ \mu\text{m}$  thick was  $31.2\% \pm 18.2\%$  and  $35.7\% \pm 23.6\%$ , respectively ( $P=0.257$ ). 5-year ECL of donor corneas  $<550\ \mu\text{m}$  and  $\geq 550\ \mu\text{m}$  thick was  $42.8\% \pm 20.3\%$  and  $49.4\% \pm 22.8\%$ , respectively ( $P=0.121$ ).

**Conclusion:** The correlation between donor corneal thickness and ECL at 3 and 5 years postoperative were not statistically significant. There is a trend for corneas  $\geq 550\ \mu\text{m}$  to have more ECL at 3 and 5 years postoperative but this difference did not reach statistical significance and may not be clinically relevant.

## COMPARING AREA OF ENDOTHELIAL CELL LOSS: SPECULAR MICROSCOPY VERSUS TRYPAN BLUE STAINING

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**Purpose:** To compare the measured area of endothelial cell loss (ACL) on specular microscopy to the measured ACL on manual and automated analysis of trypan blue staining.

**Methods:** Donor corneas with transplant-grade endothelium were obtained for testing. For each cornea, a controlled “bullseye” pattern of cell death was created by mechanically traumatizing the central endothelium using an 18ga cannula and a Fogla dissector tip. The area of focal cell death induced by the dissector tip in the center of the bullseye was imaged using specular microscopy. ACL on specular microscopy was measured using imageJ by tracing the cell borders at the margin of the bullseye injury. Each cornea was then stained with trypan blue 0.06% for 90 seconds and imaged. ACL on trypan staining was evaluated manually, using ImageJ, and automatically, using a custom macro on an Aphelion software platform. ACL was then compared statistically between all three modalities.

**Results:** In total, 10 donor corneas were included in the study. Both automated (0.45 mm<sup>2</sup>) and manual trypan analysis (0.42 mm<sup>2</sup>) significantly underestimated the mean ACL found on specular microscopy (0.54 mm<sup>2</sup>) (p=0.00005). However, there was no statistical difference between ACL on automated trypan analysis and ACL on specular microscopy if specular microscopy ACL was measured by tracing the dead cell nuclei at the margin of injury (rather than cell borders) (0.45 mm<sup>2</sup>) (p=0.95). A regression model comparing ACL on specular microscopy to ACL on automated trypan analysis demonstrated good predictive correlation between both measurements (R<sup>2</sup>=0.93, residual Std Error=0.004).

**Conclusion:** ACL on trypan staining consistently underestimates the ACL on specular microscopy. This may be due to trypan staining the nuclei rather than the cell borders of dead endothelial cells.

## TENSION IN THE AIR: BAROMETRIC PRESSURE AND DMEK

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**Purpose:** To assess DMEK air bubbles at varying barometric pressures (BP).

**Methods:** Retrospective interventional series. All DMEK performed at a single center from January 2018 to January 2019 were reviewed. Combined cases such as triple procedures were excluded, as were eyes with glaucoma tubes/filters, imperforate/absent peripheral iridotomies, or Seidel positivity. Primary outcome measures were same-day postoperative intraocular pressure (IOP) and intracameral air fill size. Outcomes were analyzed by local BP on the day of surgery. Days with diurnal variability or “normal” BP, defined as 101.5-102 kPa, were excluded.

**Results:** A total of 499 DMEK surgeries were performed on 96 unique operating days by 5 surgeons. 34 days were excluded due to normal BP (n=31) and diurnal variation (n=3). The remaining 310 DMEK from 62 days were divided into Low (<101.5 kPa, n=26) and High (>102 kPa, n=36) groups. After excluding 163 DMEK based on the criteria above, there were 69 DMEK in the Low group and 78 in the High group. Mean (±SD) air fill size was 74.1±11.6% in the Low group and 69.6±10.3% in the High group (p=0.014). There were 6 full air fills in the Low group (8.7%) and none in the High group (p=0.0095). IOP did not vary significantly (p=0.41).

**Conclusion:** BP inversely affects DMEK air bubble size. Air may assume expansile properties at low BP. Conversely, bubbles may be smaller than desired at high BP. Prospective studies are needed to assess whether BP awareness can improve DMEK outcomes.

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## PRODUCTION OF IMPLANTABLE & SCAFFOLD-FREE DMEK TISSUE

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**Purpose:** Malfunction of the corneal endothelium can lead to corneal blinding and often require endothelial keratoplasty. In the view of limited donor availability, novel approaches for ex vivo cornea production are required. We present a ‘smart’ cell culture platform, which is first able to secure gentle tissue release from the culture substrate without any remaining supporting scaffold attached to the tissue. Such artificial sheets may be used for the restoration of a degenerated corneal endothelium to overcome the shortage of donor corneas.

**Methods:** Enzymatically cleavable hydrogel carriers were used for human corneal endothelial cell (HCEC) culture. After confluent cell sheet formation, the hydrogel culture carrier was degraded, thus releasing free floating cell sheets which were successfully transferred as a living and functional (proved by live-dead & immune staining) tissue onto decellularized corneas.

**Results:** Confluent HCEC sheets with a physiologically relevant diameter of ~1 cm were isolated after 1 week culture. The cells exhibited a regular morphology, appropriate metabolic activity as well as typical function-associated marker proteins (ZO-1, Na<sup>+</sup>/K<sup>+</sup>-ATPase) and extracellular matrix proteins (fibronectin, laminin and collagen type IV). After transplantation onto decellularized porcine corneas in vitro, the cell sheets showed attachment as well as high cell viability which highlights its transplantation potential.

**Conclusion:** This novel hydrogel platform allows for convenient tissue engineering of viable, functional and carrier-free human corneal endothelial tissue with physiological dimensions. Future applications of this technology can advance the field of ex vivo cornea production.