Investigation of Contamination of Corneal Tissue Processed within the Utah Lions Eye Bank

Trevor Annis,[1] Kaidi Wang MD,[1] Mark Mifflin MD,[1] Amy Lin MD[1]

ABSTRACT

Introduction: The number of fungal endophthalmitis cases following endothelial keratoplasty (EK) increased in the mid-2010s and has plateaued in recent years. The cause remains unclear.

Objectives: Four post-EK endophthalmitis cases occurred at the ULEB within a 10-month period. The primary aim of this study was to examine the safety and quality of the cornea preparation and handling processes carried out at the ULEB.

Methods: This was a prospective observational study conducted in two parts. In the first part, study technicians tested 27 corneas for fungal or bacterial contamination before preparation for EK. In the second part, technicians tested ten corneas for fungal or bacterial contamination via swab before and after a one-month storage period in Optisol-GS at room temperature.

Results: Part one: Zero of the 27 corneas were positive for contamination before preparation for EK. Part two: One of the corneal tissues was positive for bacterial and fungal growth before the storage period. After the storage period, the contaminated cornea and its mate tested positive only for *Candida* species.

Conclusion: Part one: All corneal tissues were free from contamination, indicating that ULEB tissue procurement procedures are safe and do not introduce contaminants. Part two: Positive fungal culture results from the same donor suggest that donor characteristics, rather than eye bank factors, were the cause of the contamination. We speculate that preexisting fungal contamination may be detected via storage in Optisol-GS for one month at room temperature and that the addition of antifungal agents to corneal storage media should be considered.

Keywords: Corneal transplant, endothelial keratoplasty, eye bank, fungal endophthalmitis

Corneal transplantation is the most frequently performed transplant procedure.[1] In 2019, US eye banks provided 85,601 total grafts for corneal transplantation worldwide. Of these, 35,919 grafts were used for penetrating keratoplasty (PK), while 35,555 grafts were used for endothelial keratoplasty (EK).[2] Despite the high numbers of corneal tissues used for transplantation, the number of complications remains low. In 2016, the Eye Banking Association of America (EBAA) published a summary of adverse reactions related to corneal transplantation. The report showed that in the United States from 2007 to 2014, adverse reactions from corneal transplantation occurred 0.139% of the time (494 occurrences of 354,930 total corneal grafts), and primarily consisted of primary graft failure (319 cases; 0.090%), endophthalmitis (99 cases; 0.028%), and infectious keratitis (66 cases; 0.019%).[3] The data from the June 2020 meeting of the Medical Advisory Board of the EBAA show that adverse reactions related to transplantation continue to rise. From 2017 to 2019, adverse reactions occurred 0.315% of the time (483 occurrences of 153,564 total corneal grafts), with 226 primary graft failures (0.147%), 43 cases of endophthalmitis (0.028%), and 41 cases of infectious keratitis (0.027%).[4]

One of the most feared postoperative complications of corneal transplantation is endophthalmitis, a severe form of ocular inflammation due to infection of the intraocular cavity that can lead to pain and irreversible visual loss, despite timely diagnosis and treatment.[5,6] In cases of post-keratoplasty endophthalmitis, the disease is commonly due to gram-positive bacteria, gram-negative bacteria, or fungus.[3,7] Figure 1 illustrates that although the overall number of endophthalmitis cases has declined in recent years, the proportion of fungal endophthalmitis cases has not. In 2013, the medical advisory board of the EBAA reported on a non-statistically significant rise in the correlation of eye-bank prepared tissues with post-corneal transplant fungal infections.[8] Three years later, the EBAA reported that *Candida* species were the most common causative pathogens for endophthalmitis after endothelial keratoplasty (EK).[3] Many studies and case reports corroborated this mid-decade rise in post-keratoplasty fungal endophthalmitis and keratitis.[9-15]

Author Affiliation: [1]University of Utah

Acknowledgments: The authors of this work would like to acknowledge the Utah Lion's Eye Bank for supporting and conducting this study.

This work is supported in part by an Unrestricted Grant from Research to Prevent Blindness, New York, NY, to the Department of Ophthalmology and Visual Sciences, University of Utah.

 ISSN 2161-5546

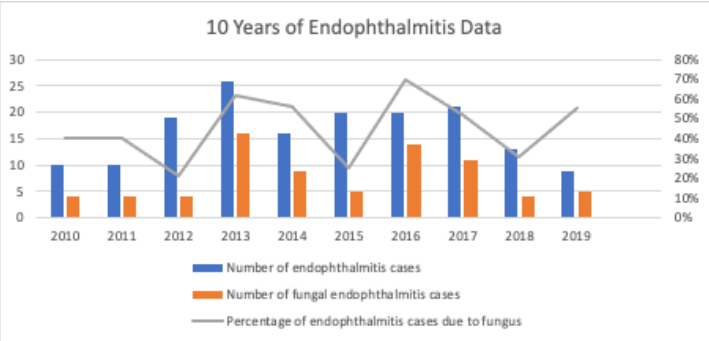

Figure 1: *A Decade of Endophthalmitis Cases In the United States*

In the United States, eye banks prepare, store, and distribute corneal tissues. Local eye banks and the EBAA thus find themselves responsible for ensuring transplant quality and safety. In 2015 and 2016, four cases of post-EK endophthalmitis occurred in patients who received corneal tissues processed at the Utah Lions Eye Bank (ULEB) in Murray, Utah, USA. Two of these cases were bacterial, and two were fungal. To determine if ULEB procedures may have led to contamination of the tissues, and to examine the safety and quality of the organ preparation and handling processes conducted at the ULEB, we conducted this prospective observational study.

The study was divided into two portions. The first portion was an examination of the quality of tissues intended for Descemet stripping and automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK) received by the ULEB for one month. This effort aimed to determine if the corneal tissues were arriving at the eye bank already contaminated with fungus. The second portion of the study evaluated if ULEB tissue-processing procedures, together with an incubation period of one month in Optisol-GS (Bausch + Lomb, Bridgewater, NJ) at room temperature, led to higher rates of fungal contamination.

MATERIALS AND METHODS

At our institution, data collected as part of quality assurance for corneal transplantation is, by definition, exempted from institutional review board appraisal. Patient consent was waived because the rim cultures were from donor tissue.

Part 1: Twenty-seven total tissue samples were evaluated over the month-long study period, six of which were tissues destined for DMEK preparation, and 21 of which were tissues destined for DSAEK preparation. Before any preparation of the tissues for EK occurred, the technicians removed the corneal tissues from their storage chambers in a sterile environment and used an Eswab® (Copan Diagnostics, Lombardy, Italy) collection kit to swab the entire anterior surface of the corneal rim/conjunctiva. The swabs were then sent to the laboratory for processing, and the corneas were prepared for their respective surgical procedures in the usual fashion.

Part 2: In the second portion of the study, five pairs of corneas, ten in total, were recovered with transplant intent. The recovery technicians gathered the corneas in situ, in the same fashion used for the collection of all ophthalmic tissues intended for transplant. The recovery technician placed 5-10 drops of 10% povidone-iodine solution into the canthus and fornices of the donor's eyes for two minutes, and then rinsed the surface of the eye thoroughly with sterile eyewash solution. After recovery, the tissues were placed into Optisol-GS for transportation to the ULEB. After procurement, the tissues were evaluated for quality and safety based upon either slit lamp or specular examination, a review of possible systemic infection in the donor, and an analysis of death-to-preservation time. In this study, all of the donor tissues were deemed not suitable for transplant (NSFT) based upon low endothelial cell counts.

Once the tissues were determined NSFT and authorized for research/training use, the study technicians were notified. Technicians conducted all procedures on a clean bench space in a dedicated eye tissue laboratory. The study technician first performed an aseptic hand antisepsis scrub and donned sterile attire. The sterile field was arranged following the ULEB standard operating procedure manual, and the corneas were removed from the viewing chamber, right eye first. The technician then obtained the ESwab® collection kit and swabbed the entire anterior rim/conjunctival surface placing the swab in the collection kit. This process was then repeated for the second cornea (left eye). The technicians conducted the entire process under the observation of a member of the ULEB's quality assurance team. After process validation, the corneas were stored in sterile viewing chambers in Optisol-GS at room temperature in a secure room at the ULEB for one month (range: 28–32 days). At the one month mark, the technicians obtained the sequestered corneas from their storage location, and in steps identical to the above process, the corneas were again swabbed and evaluated for microbial growth.

RESULTS

Part 1: In this portion of the study, all of the tissues examined revealed no prior contamination, with zero of the 27 swabs sent to the lab resulting in bacterial or fungal growth before DMEK/DSAEK preparation.

Part 2: Table 1 summarizes the results of this portion of the study. At the initial swab, one cornea was positive for

 ISSN 2161-5546

contamination with *Propionibacterium* species and "yeast," which was not further classified by the laboratory. The remaining corneal swabs were all negative for growth at this time. At the one month follow up swab, two corneas, both from the same donor, were positive for growth of *Candida* species. This included the cornea which was initially contaminated by yeast and *Propionibacterium* species, as well as the mate cornea from the donor's other eye. At one month, *Propionibacterium* species did not grow from the cornea that was initially positive. The range of what was considered one month was 28–32 days, with a mean of 29.6 days.

Table 1: *Culture Results of Pre- and Post-Storage Period Corneal Swabs*

Tissue number	Date of pre-swab and storage	Pre-swab results	Date of post-swab	Post-swab results	Reason not suitable for transplant
Donor 1, right eye	1/20/2017	No growth	2/21/2017	No growth	Low cell count
Donor 1, left eye	1/20/2017	No growth	2/21/2017	No growth	Low cell count
Donor 2, right eye	1/25/2017	No growth	2/24/2017	*C. glabrata*	Low cell count
Donor 2, left eye	1/25/2017	*Propionibacterium* species, Yeast	2/24/2017	*C. glabrata* & *C. albicans*	Low cell count
Donor 3, right eye	2/22/2017	No growth	3/22/2017	No growth	Low cell count
Donor 3, left eye	2/22/2017	No growth	3/22/2017	No growth	Low cell count
Donor 4, right eye	2/22/2017	No growth	3/24/2017	No growth	Low cell count
Donor 4, left eye	2/22/2017	No growth	3/25/2017	No growth	Low cell count
Donor 5, right eye	2/9/2017	No growth	3/9/2017	No growth	Low cell count
Donor 5, left eye	2/9/2017	No growth	3/9/2017	No growth	Low cell count

DISCUSSION

There has been much speculation about the cause of the rise of post-keratoplasty fungal endophthalmitis cases in recent years. One oft-cited potential cause of this rise is the predominance of the use of Optisol-GS. In the US, Optisol-GS is the most popular media for the storage of corneal grafts intended for transplant. It is a cold-storage medium that contains the antibiotics gentamicin and streptomycin but does not contain antifungal agents. It is postulated that the lack of antifungal agents in Optisol-GS has contributed to the rise in the incidence of post-surgical fungal complications by killing bacteria and creating a non-competitive environment for fungi to flourish. There have recently been several studies[16-19] evaluating the safety and efficacy of the addition of antifungal agents to Optisol-GS. However, as of yet, there is no Food and Drug Administration-approved corneal storage media formulated with an added antifungal agent.

Endothelial keratoplasty-prepared tissue requires an additional warming period when compared to penetrating keratoplasty-prepared tissue. This extra warming period is necessary to allow for specular microscopy to be performed, a study which is needed to evaluate the integrity of corneal endothelial cells before and after tissue processing. However, it is thought that these additional warming cycles might also allow fungal organisms to reproduce and multiply in the storage media, especially since the antibiotics in Optisol-GS are more active at warmer temperatures and thus have greater potential to reduce bacterial contamination.[20] In 2015, the medical advisory board of the EBAA issued an alert regarding the effect of temperature on fungal contamination of Optisol-GS. In this alert, the board speculated that the repeated warming cycles which are necessary for the preparation of EK tissues allow for increased fungal growth and thus increase the inoculum of fungal agents at the time of surgery. Despite this warning, no changes to the EBAA's medical standards were recommended, but EBAA members were encouraged to minimize the duration of time corneas are kept unrefrigerated.[21]

In Europe and New Zealand, organ culture storage (OCS) is the most common form of corneal tissue storage. Organ culture storage contains streptomycin, penicillin G, and amphotericin B. Streptomycin and penicillin G are antibacterial agents, while amphotericin B is an antifungal. Organ culture storage is a normothermic media for the storage of corneal tissues, meaning that no refrigeration of tissues is required when this medium is used. Organ culture storage has an advantage of increasing cornea collection rate, eye donor pool, and storage times. However, corneas stored with OCS tend to be associated with higher rates of contamination ranging from 1.8% to 19.4%[22]. Our study suggests that the use of Optisol-GS for tissue storage at room temperature for a month allows for baseline fungal contamination that was undetectable at the time of organ procurement to proliferate enough to be detected via swab one month later.

The cornea which was culture-positive for *Propionibacterium* growth at the time of the first swab was negative for it at the time of the second swab. This may not be surprising, as *Propionibacterium* species, including *P. acnes*, are known to be slow growers. Its absence at follow up might also be due to Optisol-GS suppression of the bacteria, which further supports the hypothesis that the media selects for fungal organisms by inhibiting bacteria. However, a connection between bacterial suppression and fungal growth cannot be supported by the data presented here.

 ISSN 2161-5546

It is unclear by what mechanism Donor 2's right cornea became contaminated with *Candida* species. It is likely that this donor had fungal contamination of both eyes before death, as the left eye tested positive for fungus at the time of the first swab. However, if this is true, it suggests a failure of the swab to detect low levels of contamination of the right eye. It is also possible that a fungal contaminant from the left eye was transferred to the right eye during the procurement or swabbing process, although this seems improbable as in each case the right eye was procured and swabbed before the left eye. The idea that *Candida* may be present in undetectable numbers at the time of EK preparation supports consideration for the storage and swabbing of unused corneal mate tissues in a manner similar to that described above. This testing and storage could lead to the identification of fungus in the mate tissue and increased vigilance for fungal infection in the postoperative period. It may also encourage the consideration of prophylactic topical antifungal use.

This study is limited by sample size. There are stages in the process where bias could have been introduced. The technicians involved were aware that the corneas being prepared were engaged in a quality assurance study, and they were under the watchful eye of an eye bank quality assurance employee. This observation might have resulted in the technicians taking more care or precautions than they otherwise might have. However, technicians followed standard policies without deviations. Additionally, the ULEB routinely conducts periodic testing for microbial contamination, and *Candida* species have never been cultured from any laboratory surface.

While the primary intent of the study was quality control and not external validity, the results are compelling and suggest that fungal growth in donor corneas at the Utah Lions Eye Bank is a result of the presence of fungus on the ocular surface of the donor prior to death and not from contamination during recovery or at the eye bank. Additional study in the feasibility, safety, and effectiveness of mate tissue storage, as well as the addition of antifungal additives to storage media, is warranted.

REFERENCES

1. Gain P, Julliene R, He Z, et al. Global survey of corneal transplantation and eye banking. *JAMA Ophthalmol*. 2015; 134(2):167-173. doi:10.1001/jamaophthalmol.2015.4776
2. Eye Bank Association of America, 2019 Eye Banking Statistical Report. Eye Bank Association of America; 2020. No doi/url available.
3. Edelstein SL, DeMatteo J, Stoeger CG, et al. Report of the Eye Bank Association of America medical review subcommittee on adverse reactions reported from 2007 to 2014. *Cornea*. 2016; 35(7):917-926. doi: 10.1097/ICO.0000000000000869
4. Eye Bank Association of America, Medical Advisory Board Agenda. Eye Bank Association of America; 2020. No doi/url available.
5. Sheu SJ. Endophthalmitis. *Korean J Ophthalmol*. 2017; 31(4):283-289. doi: 10.3341/kjo.2017.0036
6. Durand ML. Endophthalmitis. Clin Microbiol Infec. 2013; 19(3):227-234. doi: 10.1111/1469-0691.12118
7. Borkar DS, Wibbelsman TD, Buch PM, et al. Endophthalmitis rates and clinical outcomes following penetrating and endothelial keratoplasty. *Am J Ophthalmol*. 2019; 205:82-90. doi: 10.1016/j.ajo.2019.05.004
8. Aldave AJ, DeMatteo J, Glasser DB et al. Report of the Eye Bank Association of America medical review subcommittee on fungal infection after corneal transplantation. *Cornea*. 2013; 32(2):149-154. doi: 10.1097/ICO.0b013e31825e83bf
9. Mathes KJ, Tran KD, Mayko ZM et al. Reports of post-keratoplasty infections for eye bank-prepared and non-eye bank-prepared corneas: 12 years of data from a single eye bank. *Cornea.* 2019; 38(3):263-267. doi: 10.1097/ICO.0000000000001839
10. Brothers KM, Shanks RMQ, Hullbert S, et al. Association between fungal contamination and eye bank prepared endothelial keratoplasty tissue. *JAMA Ophthalmol*. 2017; 135(11):1184-1190. doi: 10.1001/jamaophthalmol.2017.3797
11. Mian SI, Aldave AJ, Tu EY, et al. Incidence and outcomes of positive donor rim cultures and infections in the cornea preservation time study. *Cornea*. 2018; 37(9):1102-1109. doi: 10.1097/ICO.0000000000001654
12. Koenig SB, Wirostko WJ, Fish RI, et al. Candida keratitis after Descemet stripping and automated endothelial keratoplasty. *Cornea*. 2009; 28(4):471-473. doi: 10.1097/ICO.0b013e31818ad9bc
13. Kitzmann AS, Wagoner MD, Syed NA, et al. Donor-related Candida keratitis after Descemet stripping automated endothelial keratoplasty. *Cornea*. 2009; 28(7):825-828. doi: 10.1097/ICO.0b013e31819140c4
14. Chew ACY, Mehta JS, LI L, et al. Fungal endophthalmitis after Descemet stripping automated endothelial keratoplasty – a case report. *Cornea*. 2010; 29(3):346-349. doi: 10.1097/ICO.0b013e3181a9d0c0
15. Lau N, Sesé AH, Augustin VA, et al. Fungal infection after endothelial keratoplasty: association with hypothermic corneal storage. *Br J Ophthalmol*. 2018; 0:1-4. doi:10.1136/bjophthalmol-2018-312709
16. Kaufman SC, Rhee M. Amphotericin B supplementation of cold storage media to treat fungal contamination of donor cornea transplant tissue. *Int J Eye Bank*. 2019; 7(1):1-5 https://eyebankingjournal.org/article/amphotericin-b-supplementation-of-cold-storage-media-to-treat-fungal-contamination-of-donor-cornea-transplant-tissue/
17. Layer N, Cevallos V, Maxwell AJ, et al. Efficacy and safety of antifungal additives in Optisol-GS corneal storage medium. *JAMA Ophtlamol*. 2014; 132(7):832-837. doi: 10.1001/jamaophthalmol.2014.397
18. Duncan K, Parker J, Hoover C, et al. The effect of light exposure on the efficacy and safety of amphotericin B in corneal storage media. *JAMA Ophthalmol*. 2016; 134(4):432-436. doi: 10.1001/jamaophthalmol.2016.0008
19. Ritterband DC, Shah MK, Meskin SW, et al. Efficacy and safety of voriconazole as an additive in Optisol GS: a preservation medium for corneal donor tissue. *Cornea*. 2007; 26(3):343-347. doi: 10.1097/ICO.0b013e31802d82e8
20. Kapur R, Tu EY, Pendland SL, et al. The effect of temperature on the antimicrobial activity of Optisol-GS. *Cornea*. 2006; 25(3):319–324. doi: 10.1097/01.ico.0000183492.23754.9f
21. Eye Bank Association of America, Medical Advisory Board Alert: The Effect of Temperature on Fungal Contamination of Optisol-GS. Eye Bank Association of America; 2015.http://restoresight.org/wp-content/uploads/2015/12/MAB-Alert-December-2015.pdf
22. Ling MLH, Wells M, Petsoglou C, et al. Factors affecting corneal organ culture contamination: a 6-year study at the New South Wales Tissue Bank. *Cornea*. 2019; 38(7); 829-835. doi: 10.1097/ICO.0000000000001936

 ISSN 2161-5546