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RESEARCH

Donor Endothelial Specular Image Quality in Optisol GS and Life4˚C

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ABSTRACT

PURPOSE: Identify warming time needed to obtain analyzable specular endothelial images in Optisol GS- and Life4˚C-stored corneas and identify other factors that contribute to endothelial image quality.

METHODS: Twenty-five transplant quality human corneal donor pairs were obtained. One cornea was placed in Optisol GS, the other in Life4˚C, in their respective viewing chambers, and both were stored at 2°C to 8°C for 48 hours. Specular microscopic images of the central endothelium were then obtained immediately following placement at room temperature, and additional images were taken every 30 minutes for a total of 3 hours. Image quality was graded by a single masked reader using a 6-point scale; an analyzable image was defined as grade >3.

RESULTS: Internal chamber temperature reached room temperature at 1.5 hours for both groups. The Optisol GS group had a higher average image grade than the Life4°C group at all time points; however, this difference was only significant at 1.5 hours and 2 hours: 2.8 ± 1.6 (SD) for Optisol GS versus 2.1± 1.1 for Life4°C at 1.5 hours (*P*=0.007), and 3.5 ± 1.5 versus 2.8 ±1.1 at 2 hours (*P*=0.007). Average image grade became analyzable at 2 hours for Optisol GS (3.5 ± 1.5) and at 2.5 hours for Life4°C (3.2 ± 1.1). Donor age and death–to-preservation time were not found to affect image quality.

CONCLUSIONS: Optisol GS-stored corneas should be warmed at room temperature for at least 2 hours, while Life4°C at least 2.5 hours, to achieve a good-to-excellent quality specular image of the donor endothelium.

KEYWORDS: corneal preservation medium, endothelium, specular microscopy

In addition to the microbiologic safety characteristics, the qualitative and quantitative assessment of endothelial cell quality by eye banks is an important factor in corneal graft selection. The human corneal endothelium is characterized by a quasi-regular array of hexagonal cells with nearly the same area. This regularity is lost with age, trauma, and disease.[1] Characteristics such as endothelial cell density (ECD), regularity of size as measured as the coefficient of variation, and regularity of cell shape or the percentage of hexagonal cells are indirect measures of the functional status of the endothelium and may predict clinical outcomes in keratoplasty.[2-5] The precise and accurate assessment of ECD and morphometric parameters aids in proper donor selection and a successful clinical outcome. In the Specular Microscopy Ancillary Study (SMAS), Lass et al[6] examined image quality and its effect on the accuracy of ECD measurement of donor corneas. After analyzing 688 images, they found greater variability between the eye bank- vs. reading center-determined ECD with images graded as fair by the reading center versus no significant difference found when images graded as excellent by the reading center were examined. This study concluded that image quality correlates with counting accuracy.[6]

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The accurate determination of ECD has become even more important for a number of reasons including: (1) examining surgical techniques and impact on cell loss with endothelial keratoplasty, including Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) and Descemet Membrane Endothelial Keratoplasty (DMEK),[7-10] (2) increased use of precut tissue prepared by the eye bank for endothelial keratoplasty and assessment of the post-cut ECD, (3) increasing exchange of tissue between eye banks both domestically and internationally and use of ECD as a quality measure for the eye banks and surgeons, and (4) the introduction of a new 4˚C preservation medium, Life4˚C and its accompanying Transend storage chamber (Numedis, Isanti, MN) and interest in this new medium's and storage chamber's influence on obtaining excellent endothelial images in storage and postoperatively. The medium and chamber have been approved by the US Food and Drug Administration (FDA) for up to 14 days' corneal storage, similar to Optisol GS (Bausch + Lomb, Rochester, NY). Finally, accuracy in donor ECD determination is important for the ongoing National Eye Institute funded Cornea Preservation Time Study (CPTS), a multi-center study examining not only the effect of preservation time (1-7 days vs. 8-14 days) on graft success following endothelial keratoplasty after three years, but also ECD and endothelial cell loss at three years. Thus, the importance of obtaining the highest quality donor central endothelial specular images is critical for the success of the study.

Two important factors contributing to image quality are the medium and tissue temperature. The timing of endothelial image capture following transfer of the donor in its viewing chamber from cold storage to room temperature has been arbitrary, resulting in part in greater variability in image quality and the potential impact on the accuracy of ECD determination. We believe our study is the first to systematically examine the effect of warming time on donor endothelial specular image quality. We wish to identify the minimum warming time needed to obtain good quality specular images on average as well as identify other factors that may contribute to donor endothelial image quality.

METHODS

The two most utilized FDA-approved preservation media at 4°C, Optisol GS with its storage chamber and Life4˚C and its storage chamber, Transend, approved for up to 14 days in storage, were studied. Table 1 shows the similarities and differences between the two media. The two are similar, except Life4˚C contains glutathione, recombinant human insulin, ATP precursors, vitamins, and trace minerals. Their storage chambers, however, are significantly different with the Optisol GS chamber holding 20 mL of medium, whereas the Life4˚C chamber, Transend, holds 30 mL (Fig. 1). The platforms for the two media supporting the donor cornea also differ. Both chambers have optically clear plastic tops enabling specular microscopy for the donor cornea to be performed endothelial side up.

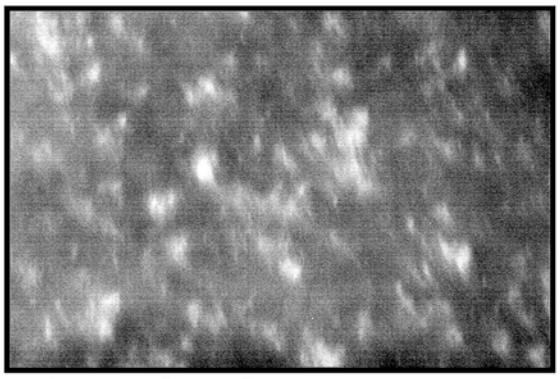
Fig. 1. Side view of Optisol GS and Life4°C viewing chambers filled with their respective media.

Table 1. Components of Life4°C and Optisol GS corneal storage media

Components		Life4°C	Optisol GS
Base medium	Minimum essential medium	Yes	Yes
Glycosaminoglycan	Chondroitin sulfate	Yes	Yes
Buffers	Sodium bicarbonate, HEPES buffer	Yes	Yes
Deturgescent agent	Dextran 40	Yes	Yes
Antibiotics	Gentamycin, streptomycin	Yes	Yes
ATP precursors		Yes	Yes
Non-essential amino acids		Yes	Yes
Human recombinant insulin		Yes	No
Glutathione		Yes	No
Additional Supplements: • Stabilized L-Glutamine • Additional antioxidants • Membrane stabilizers • Micronutrients • Trace elements • Vitamins • Coenzymes		Yes	No

Since measuring internal temperature in the viewing chamber would disrupt normal eye bank operating procedures and risk contamination, an initial experiment was designed to examine the relationship of the change in external to internal temperature once the viewing chamber with medium is placed at room temperature after having been at 2°C to 8°C for 48 hours. The internal temperature was measured with an Onset HOBO U14-002 Data Logger with Temperature Smart Sensor (Bourne, MA). The external chamber temperature and corneal thickness were measured with a Konan KSS-EB10 reader (Irvine, CA) attached to its specular microscope. The change in internal versus external temperature was determined for Optisol GS and Life4˚C in their respective storage chambers. The donor corneas were stored at 2°C to 8°C for 48

Fig. 2. Specular image quality grading scale. In this study analyzable was defined as grade >3.0 enabling ECD determination by Konan center method.[1]



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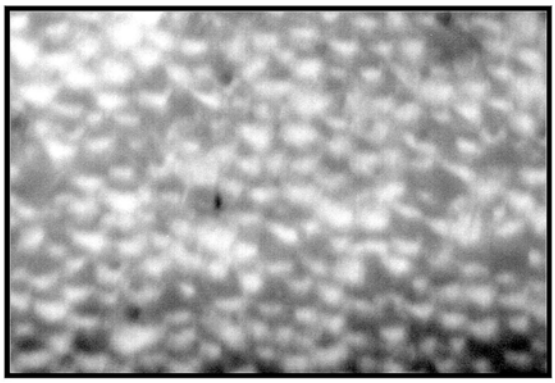
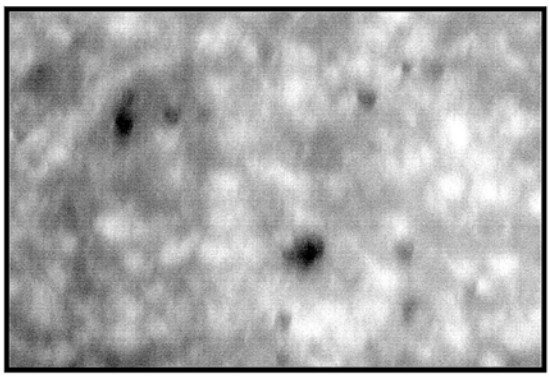
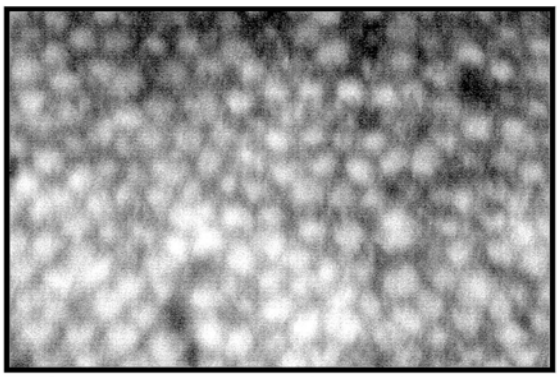


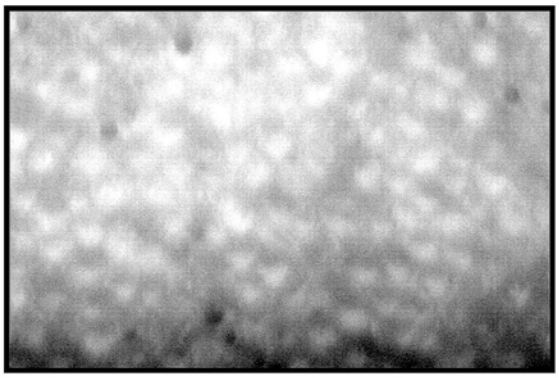
Grade 3: ≥50 contiguous clear cells with significant distortion and/or unclear borders



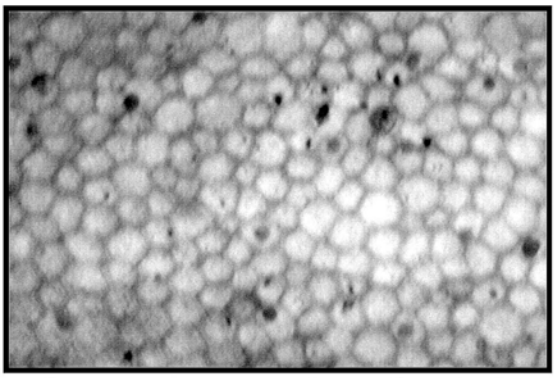
Grade 1: <50 contiguous clear cells with significant distortion and/or unclear borders



Grade 4: ≥50 contiguous clear cells with some distortion and/or unclear borders



Grade 2: <50 contiguous clear cells with some distortion and/or unclear borders



Grade 5: ≥50 contiguous clear cells with clear borders and/or NO distortion

hours, then removed from cold storage, placed on the laboratory counter in the Cleveland Eye Bank (Cleveland, Ohio), and allowed to warm to room temperature (23°C). Temperature readings were taken at time zero and at 15-minute intervals for 3 hours.

Next, paired donor corneas suitable for transplantation were collected by the Cleveland Eye Bank following their standard protocol: corneas were removed by *in situ* corneal excision in the field using a sterile technique to ensure transplant suitability. One cornea was placed in Optisol GS in its storage chamber, the other in Life4˚C in its storage chamber, and they were stored at 2°C to 8°C for 48 hours prior to testing. Along with their suitability for transplantation, to qualify for the study both corneas had to have intact epithelium, and the endothelium could not have guttae. The Eye Bank Association of America standards for human corneal donors were followed.[11] The corneas were removed from cold storage and allowed to passively warm to room temperature over 3 hours. External chamber temperature and corneal thickness measured optically with the Konan KSS-EB10 specular microscope were recorded, and specular microscopic imaging of the central donor cornea endothelium was obtained at time zero and every 30 minutes until the 3-hour time point with the Konan specular microscope with contrast settings adjusted for use with both Optisol GS and Life4˚C. The images were deidentified and graded on a 6-point scale by a single masked reader. Figure 2 lists the scoring criteria used for grading of images and examples of images for each grade. Analyzable was defined as a rating > 3.0, enabling ECD determination by Konan center method.[1]

Table 2. Donor parameters

Mean age (years ± SD)	56 ± 13
Range (years)	21 to 75
Race	
Caucasian (N, %)	20 (80)
African American (N, %)	5 (20)
Cause of death (N)	
Heart disease	13
Respiratory disease	3
Cerebral vascular accident	3
Cancer	3
Trauma	1
Other	2
Mean death-to-preservation	
Hours:minutes ±SD	10:22 ± 4:05

SD = Standard Deviation, n=25 pairs.

Statistical analysis was performed with the paired Wilcoxon rank sum test for non-parametric data to evaluate differences in image quality between the Optisol GS and Life4˚C groups. Multiple comparisons at each of the seven time points were taken into consideration utilizing the Bonferroni correction with a *P*-value ≤0.007 considered significant. Chi square analysis using image quality grade data from the 2.5-hour time point was used to evaluate the effect of donor age and death-to-preservation time on image quality in each of the two media, since both media provided acceptable quality images at this time point.

RESULTS

Twenty-five paired corneas were studied. Demographic data are presented in Table 2. Mean donor age was 56.4 years (range, 21 to 75 years). Eighty percent of the donors were Caucasian and 20% were African American. The mean time from death to preservation was about 10 hours (range, 3.35 to 19.7 hours). Internal chamber temperature reached room temperature (defined as 23°C) for both Optisol GS and Life4˚C by 1.5 hours. External chamber temperature readings were obtained at each time point to ensure similar warming rates between the corneas. There was no statistically significant difference in the average internal chamber temperature between Optisol GS and Life4˚C at 1.5 hours ($P = 0.7$).

Table 3. Image quality grade Optisol GS vs. Life4°C

Time*	Average grade in OptisolGS	SD	Average grade in Life4°C	SD	P-value	Difference	% Difference
0:00	1.3	1.3	1.0	1.0	0.083	0.3	0.27
0:30	1.2	1.1	1.0	1.0	0.327	0.2	0.13
1:00	1.8	1.3	1.5	1.5	0.103	0.3	0.18
1:30	2.8	1.6	2.1	1.0	0.007	0.7	0.24
2:00	3.5	1.5	2.8	1.1	0.007	0.7	0.21
2:30	3.7	1.4	3.2	1.1	0.045	0.5	0.14
3:00	3.9	1.3	3.2	1.1	0.016	0.7	0.17

Table 3. *Hours:minutes, SD = Standard Deviation.

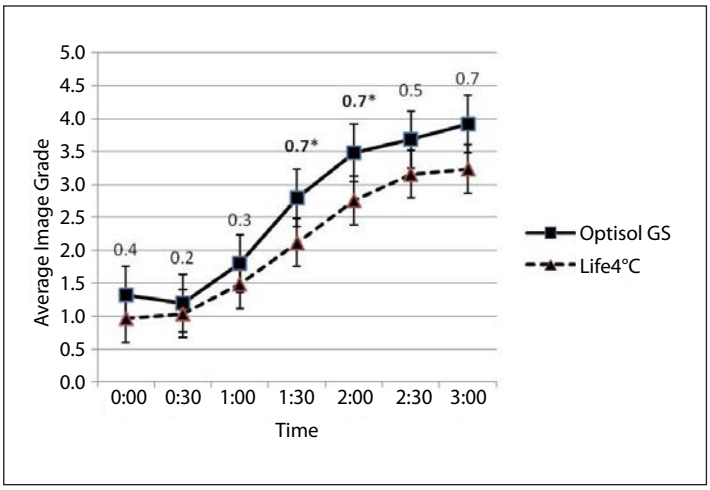


Fig. 3. Difference in mean quality grade of Optisol GS and Life4°C stored corneas over time with standard error. Time is expressed in hours:minutes. Statistically significant differences in average image grade are marked ($p<0.007$ was considered significant).

Mean image quality rating became analyzable (>3.0) by 2 hours after the donor cornea in its chamber was placed at room temperature in the Optisol GS group (3.5 ± 1.5) (± SD) and by 2.5 hours in the Life4˚C group (3.2 ± 1.1) (Table 3). At 3 hours, Optisol GS-preserved corneas reached a mean image quality rating of 3.9, whereas Life4˚C-preserved corneas reached a mean image quality rating of 3.2. Average image quality grade was higher in the Optisol GS group at all time points; however, this difference achieved statistical significance at 1.5 hours and 2 hours only. There was a statistically significant difference in average image quality grade between Optisol GS and Life4˚C at 1.5 hours and 2 hours, with higher average grades in the Optisol GS group (2.8 ± 1.6 versus 2.1 ± 1.1 at 1.5 hours, P=0.007; 3.5 ± 1.5 versus 2.8 ±1.1 at 2 hours, P=0.007). There was no significant difference in average image quality grades between Optisol GS and Life4°C at any of the other time points (Fig. 3).

There was no significant difference in average image quality grade at 2.5 hours between the donor age ≤60 years group and the >60 years group in either Optisol GS- or Life4°C-stored corneas. Similarly, there was no significant difference in average image quality grade at 2.5 hours for death-to-preservation time ≤12 hours and >12 hours for either Optisol GS- or Life4°C-stored corneas.

DISCUSSION

Advances in corneal preservation media have allowed for extended preservation time which has increased the availability of viable graft material. The formulation of corneal preservation media has evolved over time to include many factors aimed at not only extending preservation time but also maintaining endothelial functional status. The standard for 4°C corneal preservation media and most commonly utilized in the United States for the past two decades has been Optisol GS. Components include chondroitin sulfate (which contributes to endothelial cell integrity, may serve an anti-oxidant function, and increases endothelial cell survival),[12-14] 1% dextran, amino acids, glutamine, energy sources, and the antimicrobials, gentamicin and streptomycin sulfate. Life4˚C's introduction is the first new major 4°C preservation medium approved by the FDA in the last 20 years for storage of human corneas suitable for keratoplasty for up to 14 days under refrigeration. It offers potential advantages supporting endothelial and epithelial function over Optisol GS with the addition of glutathione (free-radical scavenger), recombinant human insulin (cell metabolism enhancer), ATP precursors, vitamins, and trace minerals. Coupled with a larger volume storage chamber, Life4˚C potentially provides better dilution of toxic cell derived metabolites in the medium (Fig. 1 and Table 1). Despite these potential advantages affecting cellular metabolism, we were surprised to find that once placed at room temperature, Optisol GS-stored corneas were able to yield analyzable specular donor endothelial images sooner than the Life4˚C-stored corneas by at least 30 minutes. The Optisol GS corneas required being at room temperature for at least 2 hours to achieve this image quality while the Life4°C corneas required at least 2.5 hours. In addition, there was a statistically significant difference in mean image quality rating between the two media starting at 1.5 hours, and this statistically significant difference persisted through the 2-hour observation period. The Bonferroni correction is a conservative estimation of significance when multiple comparisons are performed. There may have been differences between the two media at later time points as well if a larger sample size or a less conservative correction estimate was utilized.

The corneal endothelium provides a barrier function against imbibition of water by the corneal stroma, which relies on maintenance of a homogenous lamellar organization of collagen fibers for optical clarity. The Na^+-and K^+-dependent ATPase and Na^+/H^+ exchanger (both present in the basolateral membrane),

and conversion of CO_2 to HCO_3 by carbonic anhydrase contribute to movement of water out of the stroma and across the endothelial cells back to the aqueous humor by maintenance of an osmotic and electrochemical gradient.[15] Inhibition of the Na^+K^+ ATPase by ouabain results in corneal swelling that increases in a dose-dependent manner.[16] Since these cellular processes are energy-dependent, cold storage of the cornea leads to a loss in energy-dependent ion activity, turgescence, disruption of the lamellar organization of collagen in the stroma, and scattering of incident light, leading to obscured endothelial cell detail and poor image quality on specular microscopy.[1,15-16]

Endothelial cells are metabolically active at 4°C; however, changes in membrane lipids, hindrance of transport mechanisms, and changes in enzyme-substrate interactions and energy-coupling mechanisms lead to corneal swelling.[17] Passive swelling during cold storage due to changes in the organization of apical junction complexes as well as hypothermic morphologic changes in endothelial cell structure also contribute to turgescence, thus poor image quality. These changes to cellular adhesion proteins and endothelial cell morphology were found to be reversible upon warming.[18] The return to room and then body temperature in the presence of adequate metabolic factors (eg, adenosine, glutathione, sodium, bicarbonate) leads to reactivation of the endothelial pump machinery, subsequent deturgescence, and improved image quality.[19,20]

Oxidative damage and morphologic change occurs to the corneal endothelium during cold storage in a time-dependent manner (longer exposure to hypothermia leads to more damage) and some of these changes continue during warming.[19] The results of the present study suggest that for optimal specular image quality donor corneas should be warmed to room temperature and allowed to remain at that temperature for at least several hours. This amount of time, however, raises concern about eye bank personnel and surgeons regarding the effect of this warming period on endothelial cell viability and function. H. Edelhauser has addressed Optisol GS-stored endothelial cell viability after extended periods of time at room temperature. In studies of donor corneas, he found no significant difference in corneal viability, as measured by live cell/dead cell assay, electron microscopy, and precursor apoptotic activity, between those stored in Optisol GS at 4°C vs. 23°C for up to 72 hours (personal communication). He concluded that lack of refrigeration up to 72 hours is well tolerated by the human corneal endothelium. The end-point in the present study was 3 hours, well under the 72 hours found to be tolerated by corneal endothelium.

What may account for the difference in time at room temperature for these two media and their differing viewing chambers? The present study has shown that at least 2 hours of warming after cold storage is required to obtain an image quality grade >3 in the Optisol GS group, while the Life4°C group required an additional 30 minutes of warming. Differences in the two media and their storage containers could conceivably account for the disparity in time to minimum image quality rating. It has been shown that rates and amount of corneal swelling during storage at 4°C as well as endothelial pump function differ between various preservation media.[21] Life4°C's larger volume of liquid as well as slimmer, taller viewing chamber could lead to a difference in rate of warming, thus the increased time to quality image capture. The temperature data, however, in the present study does not support this hypothesis as both groups reached an internal temperature of 23°C after 1.5 hours. Life4°C medium is darker in color than Optisol GS, which may feasibly affect image quality; however, specular microscope contrast settings used in this study were corrected for the difference. Deposition of dextran in the cornea occurs during organ culture, and this accumulation can interfere with mitochondrial function and lead to stromal swelling.[22] Differences in the molecular weight of dextran used in various formulations of preservation media may result in increased uptake and consequently increased swelling. The endothelial pump relies on establishment and maintenance of an osmotic gradient to sustain stromal deturgescence. *In vivo*, the effective sodium concentration is 143 mEq/L, while osmotically active stromal sodium concentration is 134 mEq/L, leading to an osmotic pressure of 163.8 mmHg that draws water out of the cornea.[23] Differences in osmolarity of various storage media may lead to smaller osmotic gradients and decreases in the osmotic pressure that favor movement of water out of the cornea, leading to decreased rate of thinning and increased time to quality imaging.

The present study did not find a correlation between donor age or death-to-preservation time and image quality in either of the two media. A correlation may have been masked by the small sample size (n=25), and a larger sample may reveal associations that are not found here. Death-to-preservation times of greater than 6 hours have been associated with greater epithelial sloughing, and there is a positive correlation between increasing postoperative epitheliopathy and increased death-to-preservation times.[24] In the present study, however, we found no difference

in image quality grade between death-to-preservation times less than or equal to 12 hours and greater than 12 hours. Differences may be revealed by use of a larger sample size and a smaller death-to-preservation time as a cutoff.

In summary, warming time as well as choice of preservation medium was found to affect image quality, while donor age and death to preservation had no effect. The warming time required for optimal image quality (if optimal image quality were to be defined as image grade equaling 4.0 or better) is still undetermined, as well as the effect of active warming, rather than simply placing the tissue in its chamber at room temperature, on image quality. Yap et al[25] found that temperature reversal of previously cold-stored corneas at 37°C with Balanced Salt Solution Plus (BSS Plus; Alcon, Fort Worth, TX) demonstrated corneal thinning over the first 4 hours of the experiment; thus, up to a 4-hour warming time may be required for best image quality. The present study used only passive warming, and data was not collected past the 3 hour time point at which time average image quality reached only 3.9 in the Optisol group and 3.2 in the Life4°C group. Further study with extended time points would be needed to fully characterize changes in image quality from warming. Although further study is needed, we found that choice of preservation medium may affect the amount of warming time needed to achieve quality specular images. Optisol GS-stored corneas should be warmed at room temperature for at least 2 hours, while Life4°C stored corneas should be warmed for at least 2.5 hours. These times are longer passive warming times than generally practiced by eye banks.

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