Comparison of Endothelial Cell Measurements by Two Eye Bank Specular Microscopes

Khoa D. Tran, PhD,[1+z]* Jameson Clover, CEBT,[1+] Kelly Odell, BS,[1] Winston Chamberlain, MD, PhD[1,2] and Christopher G. Stoeger, MBA, CEBT[1]

ABSTRACT

Purpose: To compare endothelial cell measurements between a new specular microscope, the Konan CD-15/CellChekD+, and its predecessor, the Konan EKA-10/EB10.

Methods: Specular images and cell measurements were obtained on both systems by the same technicians. In one assay, the same cells were imaged and measured on both platforms. Additional corneas, including eye bank processed DMEK and DSAEK tissues, were analyzed using the standard method of acquiring three random specular images and counting 50-80 cells per image (>120 cells were measured per cornea). For all experiments, endothelial cell densities (ECD) were obtained manually using the center method and cell measurements were calculated using software included with each system.

Results: A total of 99 corneas from 58 donors were examined. Random sampling of 50 donor corneas revealed an overall average difference of 34 cells/mm2 ($p=0.14$) between the two systems. In the direct comparison assay where the same cells were selected, the overall average difference in ECD measurements between the two systems was 18 cells/mm2 ($p=0.17$, $R2=0.98$, $p<0.001$). Roughly 1-1.5% of the differences in ECD measurements between individual pairs of measurements may be attributed to the input error of the center method on both systems. ECD measurements for eye bank processed DMEK and DSAEK tissues differed by 14 and 36 cells/mm2, respectively ($p<0.05$).

Conclusions: Comparison of endothelial cell measurements between the two systems revealed insignificant differences. ECD, HEX, and CV values obtained from the two systems can be used interchangeably, and should have no negative impact on tissue evaluation as well as on-going basic and clinical research.

Key words: Cornea, Endothelial cell density, Specular microscope, Eye bank, Equipment qualification

Abbreviations: ECD: Endothelial cell densities, HEX: Hexagonal ratio, CV: Coefficient of variation, DMEK: Descemet membrane endothelial keratoplasty, DSAEK: Descemet stripping automated endothelial keratoplasty.

Specular microscopy and the quantification of corneal endothelial cell densities (ECD) are essential processes during eye bank evaluation of donor tissue. ECD measurements and several other important parameters are used to help determine tissue suitability for transplant procedures where viable cells are required.[1-4] The ability to acquire endothelial cell measurements in a consistent manner over time is also critical for on-going basic and clinical research studies.[5-7] However, as technology continues to evolve and a new generation of specular microscopes are produced, many eye banks will acquire the new microscopes to replace or complement their existing equipment. It is important to consider the impact of implementing these new systems in the midst of on-going studies within a single center, as well as multi-center collaborations. Therefore, it is essential to know whether new specular microscope systems and accompanying software packages will yield values consistent with their predecessors, allowing for these measured values to be used interchangeably.

The new Konan CD-15 specular microscope and CellChekD+ software package (referred to as CellChekD+ from

Author Affiliations: [1]Lions VisionGift, Portland, Oregon, USA.

[2]Casey Eye Institute, Department of Ophthalmology, Oregon Health & Science University, Portland, Oregon, USA.

[+]These authors contributed equally

*Corresponding Author: Khoa D. Tran, 2201 SE 11th Ave., Portland, OR. 97214, Khoa@VisionGift.org

Acknowledgments: The authors would like to thank Tawnya Lynch, Amy Ansin, Jessica Honaker, Phil Dye, and Tad Shaw for technical assistance on this body of work. We thank Susan Oak (Konan Medical USA) for thoughtful comments on the manuscript.

Author Contribution Statement: KDT and JC contributed equally. Experimental design – KDT, JC, CGS, WC; performed the experiments – JC, KDT, KO; data analysis – KDT, JC, CGS, WC; prepared the manuscript - KDT, JC, CGS. All authors reviewed the manuscript.

Disclosures: – None.

here on) offer many improvements over its predecessor, the EKA-10 with the EB10 software package (EB10 from here on). These additions include improved specular images, a larger area where specular reflections can be obtained (more peripheral regions of the cornea), and two additional ways to view donor tissues (Enhanced View and Finder View). Technological improvements aside, this new specular microscope will be used primarily to help determine transplant tissue suitability, and as such, it should provide accurate and consistent endothelial cell measurements.

The Eye Bank Association of America's (EBAA) Medical Standards require that new equipment be qualified prior to use.[8] Outside of defining equipment qualification as "Protocols designed to adequately evaluate, prior to use, whether or not pieces of equipment will perform to expectation, and normally function within the required tolerance limits" in section C3.200, the EBAA does not specify how this should be executed. For equipment such as a specular microscope, an eye bank would generally qualify it by documenting that it was calibrated according to the manufacturer's specifications. An additional step may be to compare ECD from the new microscope to a pre-existing microscope, and to define an acceptable error limit for the resulting ECD and morphometric parameters measured.

In addition to qualifying the CellChekD+ for use at our eye bank, we have developed a protocol to examine whether the CellChekD+ yields similar endothelial cell measurements as our current specular microscope, the EB10, which is maintained at regular intervals and includes calibration checks. Here, we present the results of our comparative study examining endothelial cell measurements calculated by the two systems.

METHODS

Tissue Selection and Donor Criteria

A total of 99 corneas recovered from 58 donors between February 2016 and April 2016 were chosen at random for this study. Donor characteristics are as follow: Age range 11 – 75 years (median of 65 years), 69% male, 31% female, 17% pseudophakic, 31% diabetic history, and death to recovery time for all tissues were between 2 and 24 hours. ECD range for examined tissues were 1,183 cells/mm^2 – 3,484 cells/mm^2. For eye bank prepared tissues, specular images were acquired during post-processing evaluations (imaged on the EB10 first, then immediately imaged on the CellChekD+) and cell measurements were performed by the same technician. For the experiments where the same cells were counted, only research tissues (those that were unsuitable for transplantation due to positive serology results) with research consent were used.

Image Acquisition and cell Measurements

The Konan EKA-10 with the EB10 software package (EB10) and the Konan CD-15 with the CellChekD+ software package (CellchekD+) were used in this analysis (Konan Medical, Irvine, CA. USA). Specular images were collected during routine evaluation on both instruments by trained eye bank technicians. Images were obtained first on the EB10 and then immediately after on the CellChekD+ (with less than 5 minutes of elapsed time between image acquisitions on the two instruments). Endothelial cell densities (ECD), hexagonal ratio (HEX), and coefficient of variation (CV) measurements were taken by the same technician who acquired the images in order to maintain consistency. Cell measurements using the center method were taken manually.

For the experiments where the exact same cells were measured, an S-stamp (Moria, Antony, France) was directly applied to the endothelium at the center of the cornea and the endothelium was stained with Vision Blue (DORC International BV, Zuidland, The Netherlands) for 30 seconds to highlight the 'S' for orientation purposes during imaging. A sample of cells adjacent to the marked areas were imaged and measured. For experiments examining the reproducibility of the center method on each specular microscope, one image (per microscope) was acquired and the ECD was calculated and used as a reference (using 70 selected cells per image). Two eye bank technicians took an additional 10 counts of the same 70 cells, and these ten ECD calculations were compared to the reference. For all other experiments, specular images were obtained on both microscopes per routine protocols where three central regions were imaged, at least 120 cells were measured from each cornea, and the average cell count from the three images defined the ECD.[9,10]

Statistical Analysis

Non-parametric paired Wilcoxon tests[11] and Spearman correlation were used to compare ECD, HEX, and CV measurements between the two instruments. Bland-Altman analysis was performed as previously described to show the mean difference and 95% limits of agreement[12]. Statistical analysis was performed using R Statistical Software[13] (version 3.2.4). Statistical significance is defined by $p<0.05$.

RESULTS

Endothelial cell densities by random sampling

To compare ECD measurements between the two systems, a number of corneas were surveyed by random sampling. For each of the fifty (50) corneas examined, three specular

images were analyzed and at least 120 endothelial cells per cornea were measured. Final cell density calculations from the two systems were used in this comparison. The cell densities measured by the CellChekD+ was higher 56% of the time (28 out of 50), resulting in an average difference of 34 cells/mm^2 overall (range=0-518 cells/mm^2, p=0.14, Table 1). ECD measurements were highly correlated between the two systems (R^2=0.91, p<0.001, Fig. 1A), and Bland-Altman analysis revealed that 95% of the differences in cell counts were within ±303 cells/mm^2 of the mean difference (Fig. 1B). These results provide support that the calculated ECD values are similar between the two instruments.

Endothelial Cell Measurements by a Direct Comparison Method

To directly compare endothelial cell densities (ECD), hexagonal ratio (HEX), and coefficient of variation (CV) between the two instruments, specular images of the same areas (n=20) were acquired on both microscopes and the same cells were counted (Fig. 2A). The overall average difference in cell densities calculated by the two instruments was 18 cells/mm^2 (range=0-140 cells/mm^2, p=0.17, Table 1). When comparing each pair of measurements individually, the average difference in ECD measurements was 1.8%

Table 1. Summary of measurements.

	EB10 Averages			CellChekD+ Averages					
	ECD (cells/mm^2)	HEX	CV	ECD (cells/mm^2)	HEX	CV	Difference in ECD (cells/mm^2)	p-value (ECD)	n
Random Sampling	2413 ± 464	55	37	2447 ± 509	59	36	34	0.14	50
Direct Comparison	2865 ± 412	56	36	2883 ± 434	55	36	18	0.17	20
Processed DMEK	2520 ± 414	55	36	2506 ± 375	55	36	14	0.65	20
Processed DSAEK	2505 ± 256	57	35	2541 ± 243	58	35	36	0.29	20

ECD = Endothelial cell densities, mean ± standard deviation.
n = number of corneas measured
DMEK = Descemet membrane endothelial keratoplasty
DSAEK = Descemet stripping automated endothelial keratoplasty

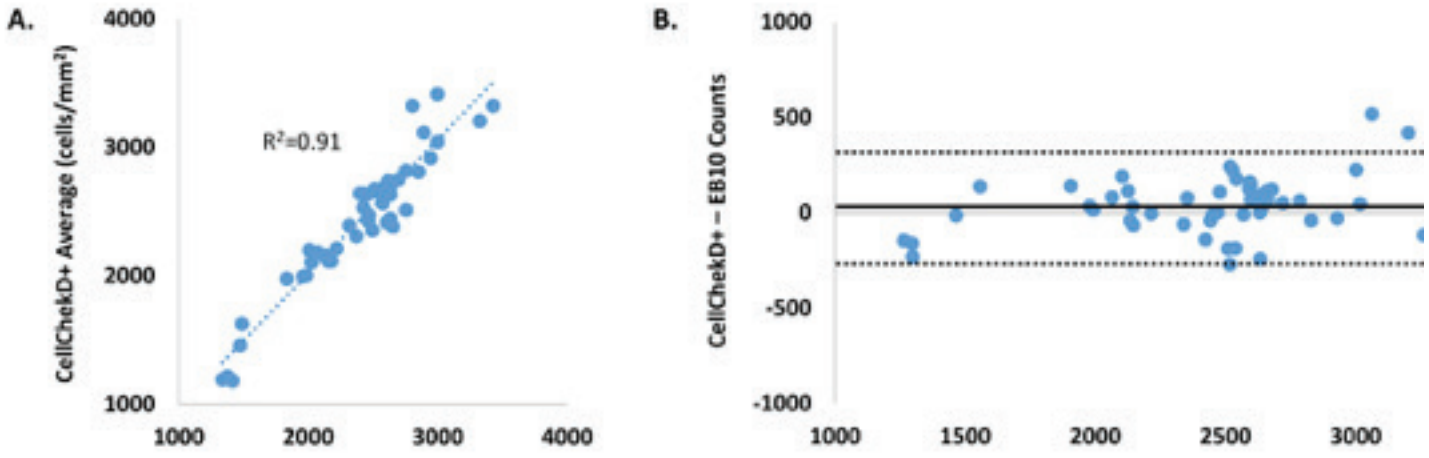

Fig. 1: Comparison of endothelial cell measurements by random sampling.
A) Correlation of ECD calculations by the EB10 and CellChekD+ through random sampling for 50 corneas (p<0.001). ECD values were calculated by software accompanying each specular microscope system.
B) Bland-Altman analysis of differences in ECD calculations between the two systems. The overall mean difference was 34 cells/mm2 (solid line), and the 95% limits of agreement are shown as dotted lines (±303 cells/mm2 from the mean difference).

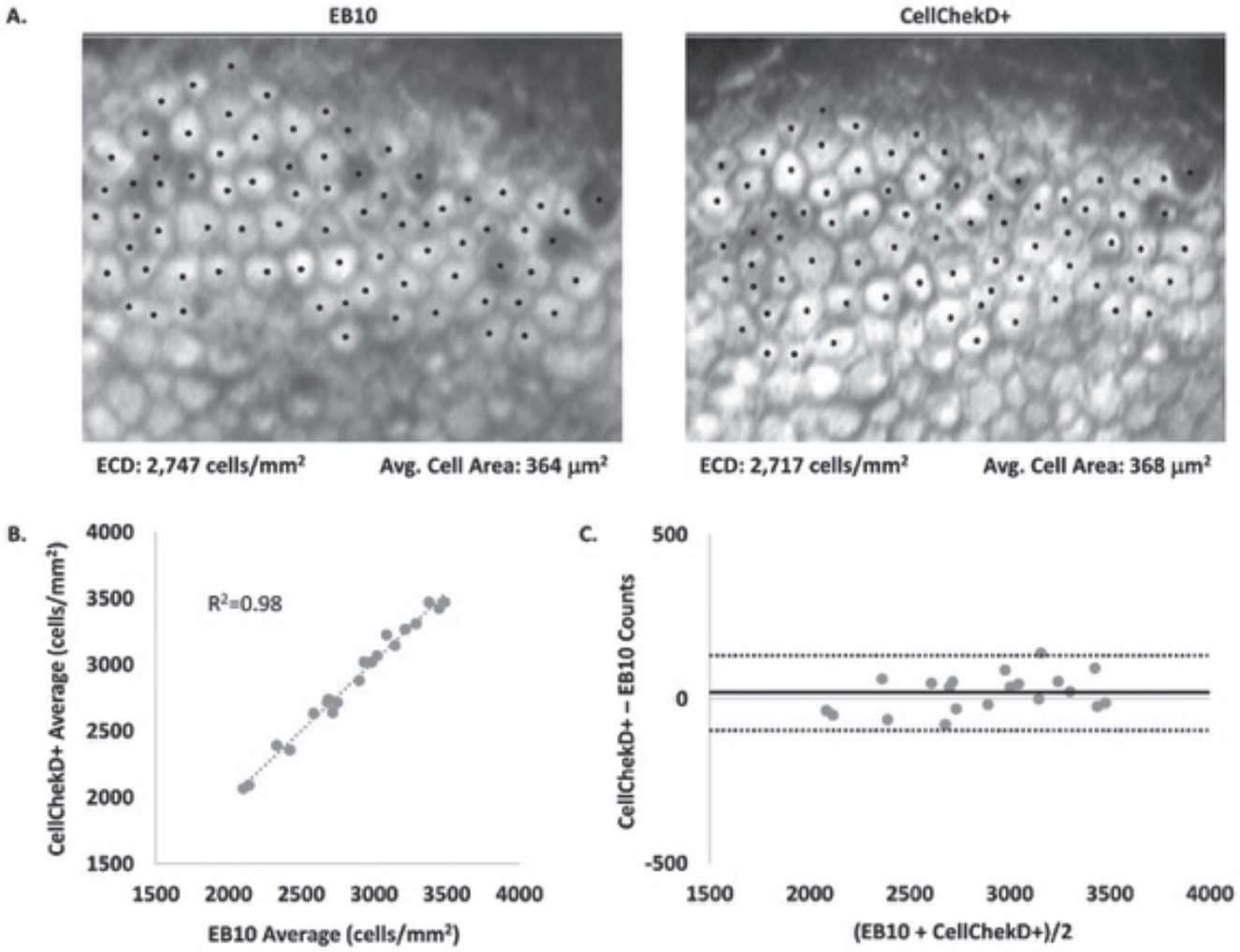

Fig. 2: Direct comparison of endothelial cell measurements.
A) An example of endothelial cell measurements between the EB10 and CellChekD+. Specular images were taken on both microscopes and the same exact cells were counted. ECD and average cell size are given under each image.
B) Correlation of ECD calculations between the two systems ($p<0.001$).
C) Bland-Altman analysis showed that ECD calculations between the two systems differed by less than ±100 cells/mm2 for 19 out of the 20 corneas examined. The overall mean difference was 18 cells/mm2 (solid line), and the 95% limits of agreement were ±132 cells/mm2 from the mean difference (dotted lines).

(range=0-4.5%). Furthermore, the cell counts were highly correlated between the two systems (R^2=0.98, $p<0.001$, Fig. 2B). For 19 of the 20 samples examined, the differences in measurements were within ±100 cells/mm^2 of the mean difference (Fig. 2C). The average HEX (EB10=56, CellChekD+=55) and CV (36 on both systems) values measured by the two instruments were also not significantly different (HEX: p=0.87, CV: p=0.49). The results from this direct comparison further show that both instruments yielded similar ECD, HEX, and CV calculations when the same cell population is measured.

Reproducibility of the Center Method by Manual Dot Placement

A portion of the 1.8% overall difference in paired-ECD measurements between the two systems may be due to input error when using the center method (Fig. 3). To quantify this input error, a specular image was acquired on the EB10, and 70 endothelial cells were counted and used as a reference for comparison. The same 70 cells were counted 10 more times by the same technician to determine the reproducibility of the ECD measurements. This experiment was repeated by a second technician as an experimental replicate. The resulting differences in ECD calculations

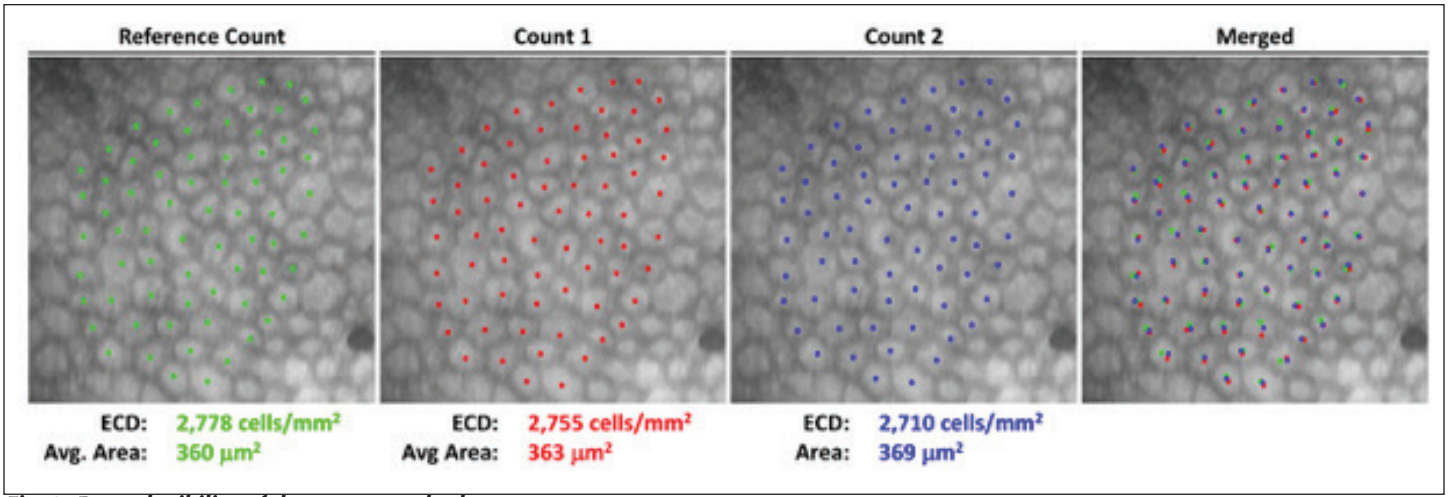

Fig. 3: Reproducibility of the center method.
Examples of ECD measurements from the reproducibility experiment. The reference and example of two counts taken on the CellChekD+ are shown. The merged image (far right) demonstrates the variability of the dots placed on the same cells.

ranged from 0-83 cells/mm^2 from the reference for technician one and 15-123 cells/mm^2 for technician two (Table 2). This experiment was repeated on the CellChekD+ using a new specular image acquired on that system as a reference. On the CellChekD+, the replicate counts differed by 16-75 cells/mm^2 for technician one and 0-24 cells/mm^2 for technician two (Table 2). In this test, the average error due to the center method was approximately 1.6% on the EB10 and 1.1% on the CellChekD+ (Table 2). These results suggest that much of the 1.8% difference in ECD measurements described in the previous section may be attributed to input error of the center method.

Endothelial Cell Measurements of Eye Bank Prepared DMEK and DSAEK Tissues

ECD measurements of eye bank processed Descemet membrane endothelial keratoplasty (DMEK) and Descemet stripping automated endothelial keratoplasty (DSAEK) tissues were examined on the two systems. For DMEK tissues, pre-peeled corneas for transplant and research

Table 2. Input error of the center method.

	EB10				CellChekD+			
Counts	ECD 1 (cells/mm²)	ECD 1 Differences	ECD 2 (cells/mm²)	ECD 2 Differences	ECD 1 (cells/mm²)	ECD 1 Differences	ECD 2 (cells/mm²)	ECD 2 Differences
Reference	**2793**	-	**2710**		**2778**	-	**2762**	-
1	2732	2.2%	2762	1.9%	2755	0.8%	2770	0.3%
2	2755	1.4%	2762	1.9%	2710	2.4%	2740	0.8%
3	2770	0.8%	2725	0.6%	2762	0.6%	2762	0.0%
4	2801	0.3%	2778	2.5%	2703	2.7%	2762	0.0%
5	2747	1.6%	2833	4.5%	2747	1.1%	2786	0.9%
6	2817	0.9%	2747	1.4%	2725	1.9%	2755	0.3%
7	2793	0.0%	2762	1.9%	2732	1.7%	2786	0.9%
8	2786	0.3%	2778	2.5%	2755	0.8%	2786	0.9%
9	2762	1.1%	2770	2.2%	2849	2.6%	2747	0.5%
10	2710	3.0%	2762	1.9%	2740	1.4%	2770	0.3%
Avg. (1-10)	**2767**	**1.1%**	**2768**	**2.1%**	**2748**	**1.6%**	**2766**	**0.5%**
Range:	**0-83**		**15-123**		**16-75**		**0-24**	

Differences in ECD measurements from the reference count by two eye bank technicians (ECD 1 and ECD 2).
Average (Avg) ECD calculations include counts 1-10 and excludes the reference.
Range shows the minimum and maximum differences in ECD measurements.

were examined to capture a wide range of ECD. Overall, the average ECD calculated by the two systems differed by 14 cells/mm^2 (range=29-176 cells/mm^2, p=0.65, Table 1) with a high degree of correlation (R^2=0.88, p<0.001, Fig. 4A-B). Both the HEX (average=55 on both systems, p=0.95) and CV (average=36 on both systems, p=0.95) values measured by both systems were essentially identical on this subset of tissues.

Measurements of processed DSAEK tissue were also not significantly different between the two systems (Table 1). ECD measurements differ by 36 cells/mm^2 overall (range=10-342 cells/mm^2, p=0.29, Table 1) with good correlation (R^2=0.70, p<0.001, Fig. 4C-D). HEX and CV values were also not significantly different (HEX average=57 (EB10) and 58 (CellChekD+), p=0.74; CV average=35 on both systems, p=0.55). Bland-Altman analysis revealed that 95% of the differences in cell counts for processed DMEK and DSAEK tissues were within ±287 and ±284 cells/mm^2 of the mean difference, respectively (Fig. 3B, D). Together, these results suggest that both systems yield similar endothelial cell measurements for eye bank prepared DMEK and DSAEK tissues.

DISCUSSION

The incorporation of new and improved technologies allow eye banks to continue serving our communities by providing quality tissues and services to fulfill transplant and research needs. However, indiscriminately applying new technologies without careful consideration of its potential impact may lead to the opposite outcome.

In addition to regular maintenance and calibration checks of existing equipment, the EBAA Medical Standards require that eye banks qualify each piece of new equipment and document the qualification process.[8] In addition to meeting the minimum requirements set by the EBAA for new equipment qualification, we designed a protocol to examine morphometric parameters calculated by the CellChekD+ compared to our existing equipment. We chose to perform this additional step during equipment qualification because our eye bank works with clinicians and researchers who report results that contains a comparison of pre-operative vs. post-operative ECD. We wanted to determine whether implementation of this new system would result in an unintended shift in reported ECD, which it did not. The protocols presented here is in no way presented as a 'standard' for onboarding a new specular microscope, but we hope that our study design and results may aid others in developing their own qualification procedures.

To the best of our knowledge, this is the first reported comparison of endothelial cell measurements where specular images of the same cells were acquired and analyzed on two different instruments. In the direct comparison assay, the endothelium of research corneas were marked using an S-stamp, and the areas surrounding the 'S' were imaged so that the same exact cells can be selected for analysis. This method not only reduces variables introduced by counting different cells during ECD measurements, but also offers a powerful way to identify differences in HEX and CV calculations, which proved to be insignificant in our study. In this comparison, we found only minor differences in ECD measurements between the two systems (18 cells/mm^2 overall, Fig. 2, Table 1). Paired measurements between the two systems differed by an average of 1.8%. Because the same cells were measured manually by using the center method, we further investigated the amount of input error that may be introduced by manual dot placement in our experiments.

The center method relies on the user to define a cell and measurement area by placing dots in the middle of cells within a contiguous group. The distance between the dots are used to calculate cell average size, ECD, HEX, and CV values.[5,14,15] Therefore, dot placement by an operator may add an element of variability to the cell measurements. This small difference in calculated cell area can lead to slightly higher or lower calculated cell counts (Fig. 2-3, Table 2). We found that the average input error of the center method by one technician to be 1.1% on the EB10 and 1.6% on the CellChekD+ (Table 2). For a second technician, the input error was 2.1% on the EB10 and 0.5% on the CellChekD+ (Table 2). These results are similar to those previously described by the Corneal Donor Study Group where a median 2-4% difference (dependent on image quality) in ECD measurements between technicians who read the same images[6,16]. Our results suggest that manual dot placement can introduce approximately 1-1.5% error to this type of analysis. Accounting for this variability, the differences in ECD measurements between the EB10 and CellChekD+ may be reduced to less than 1%. Thus, the results from our direct comparison experiments support the conclusion that calculated ECD values from both instruments can be used interchangeably.

When examining eye bank processed DMEK tissues (Fig. 4A-B), we found very similar results to our direct comparison assay where we imaged and counted the same cells. Because it is common to have folds on the Descemet membrane and endothelium complex post-DMEK processing, only certain areas will yield a proper specular reflection. As a result, we found that we often, inadvertently, imaged and counted the same areas on both microscopes, resulting in an overall difference of 14 cells/mm^2 between the two systems. Thus, endothelial cell measurements from

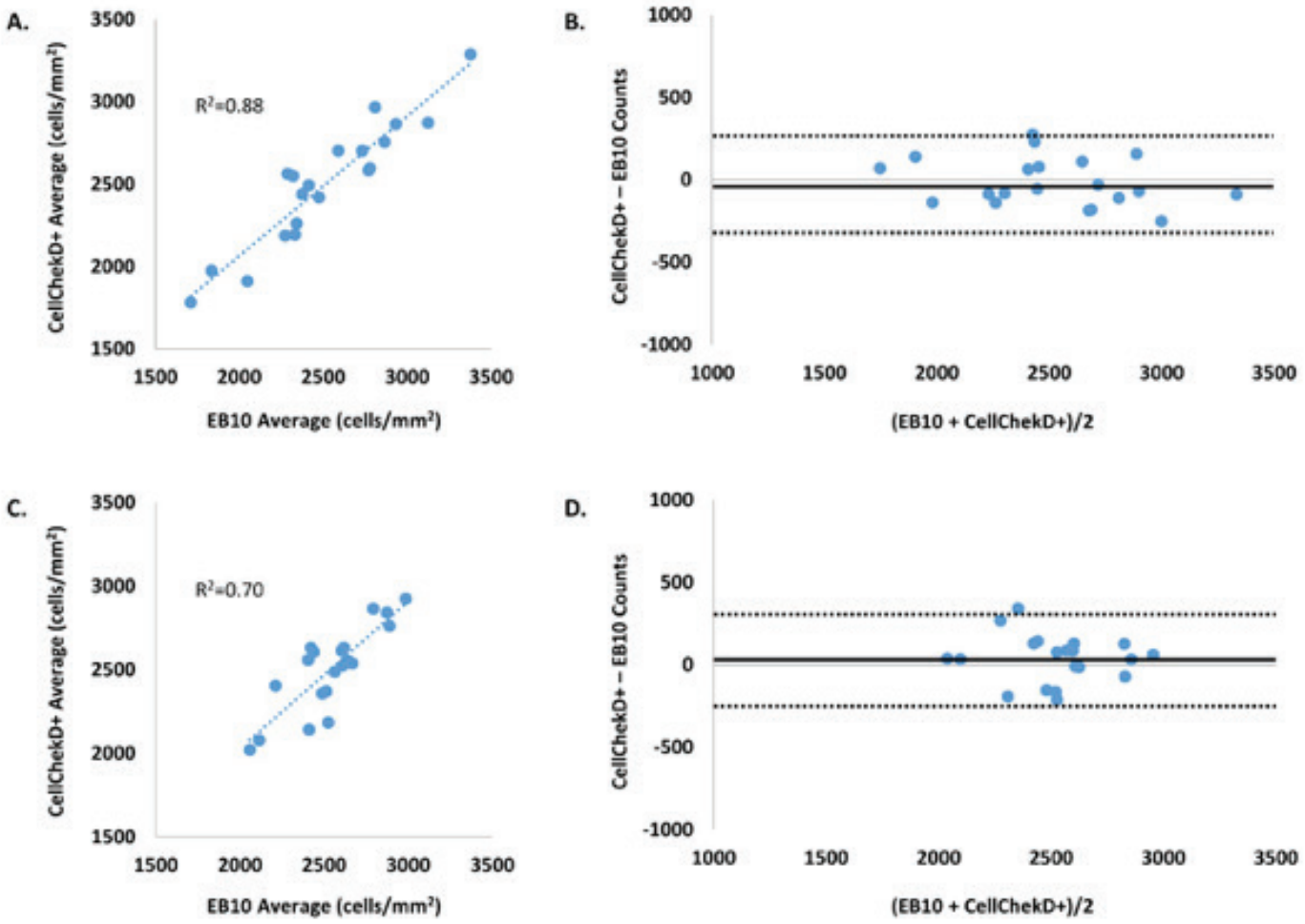

Fig. 4: Comparison of endothelial cell measurements of eye bank prepared tissues.
A) Correlation of ECD for DMEK-processed tissues (p<0.001).
B) Bland-Altman analysis showing the mean difference in cell measurements for DMEK tissues between the two systems (14 cells/mm2, solid line). Calculations by the CellChekD+ were higher 40% of the time. The 95% limits of agreement are shown as dotted lines (±287 cells/mm2 from the mean difference).
C) Correlation of ECD for DSAEK-processed tissues (p<0.001).
D) Bland-Altman analysis showing the average difference in cell measurements for DSAEK tissues between the two systems (36 cells/mm2, solid line). Calculations by the CellChekD+ were higher 65% of the time. The 95% limits of agreement are shown as dotted lines (±284 cells/mm2 from the mean difference).

processed DMEK tissues differed only marginally from the results of the direct comparison experiment (Fig. 2). Once again, we found ECD, HEX, and CV measurements to be essentially identical on both systems.

A greater difference in cell counts between the two systems was observed when we examined ECD by random sampling of unprocessed and post DSAEK-processed tissues. In these experiments, we did not aim to count the same cells or areas of cells. As a result, we saw a slightly larger overall difference in ECD measurements of 34 cells/mm^2 for unprocessed tissue and 36 cells/mm^2 DSAEK-processed tissues compared to the direct comparison above. However, when examining the reproducibility of the center method by manual dot placement, we found that it is possible to get measurement differences ranging from 0-85 cells/mm^2 (and in one extreme case, 123 cells/mm^2) when counting the same cells on the same image. Taking this into consideration, we believe that an overall difference of 34-36 cells/mm^2 for unprocessed and DSAEK-processed fall within an acceptable margin of error.

One limitation in our study design was that we consistently acquired images first on the EB10 and then on the CellChekD+. Previous studies have linked the differences in tissue warming time to specular image quality and the

ability to obtain accurate ECD measurements.[16,17] Thus, we were diligent in executing our protocol to ensure that there was no more than 5 minutes of elapsed time between the acquisitions of specular images on both systems. We believe that any minute changes that may occur during this short period of time would not be enough to significantly impact the outcome of this study. A possible way to eliminate this small limitation in future studies would be to randomize the order of image acquisition.

It is well known that better specular image quality and counting more cells will yield a more accurate cell count.[5,16] The CellChekD+ not only offers superior images compared to its predecessor, but also offers a larger specular reflection area where more cells can be counted. Thus, the CellChekD+ may offer a more accurate cell count than the older EB10. Because we did not test additional new microscopes from other manufacturers, we must emphasize that we make no claims regarding the superiority of one manufacture's equipment over another. In the current study, we used a standard evaluation protocol (counting approximately 50-80 cells per image and measuring >120 cells per cornea) and found only small differences in ECD calculations between the two systems. Therefore, we conclude that the numbers obtained on the EB10 and CellChekD+ can be used interchangeably; and the small differences in ECD measurements should have no negative impact on standard eye bank evaluation procedures or on-going basic and clinical research.

REFERENCES

1. Matsuda M, Yee RW, Glasser DB, et al. Specular microscopic evaluation of donor corneal endothelium. *Arch Ophthalmol.* 1986;104:259-262.
2. Schroeter J, Rieck P. Endothelial evaluation in the cornea bank. *Dev Ophthalmol.* 2009;43:47-62.
3. Price MO, Knight OJ, Benetz BA, et al. Randomized, prospective, single-masked clinical trial of endothelial keratoplasty performance with 2 donor cornea 4 degrees c storage solutions and associated chambers. *Cornea.* 2015;34:253-256.
4. Sugar A, Gal RL, Beck r W, et al. Baseline donor characteristics in the Cornea Donor Study. *Cornea.* 2005;24:389-396.
5. McCarey BE, Edelhauser HF, Lynn MJ. Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices, and new intraocular drugs and solutions. *Cornea.* 2008;27:1-16.
6. Benetz BA, Gal RL, Ruedy KJ, et al. Specular microscopy ancillary study methods for donor endothelial cell density determination of Cornea Donor Study images. *Curr Eye Res.* 2006;31:319-327.
7. Lass JH, Szczotka-Flynn LB, Ayala AR, et al. Cornea preservation time study: methods and potential impact on the cornea donor pool in the United States. *Cornea.* 2015;34:601-608.
8. Eye Bank Association of America. *2016 Medical Standards.* Washington D.C.: Eye Bank Association of America;2016.
9. Doughty MJ, Muller A, Zaman ML. Assessment of the reliability of human corneal endothelial cell-density estimates using a noncontact specular microscope. *Cornea.* 2000;19:148-158.
10. Binder PS, Akers P, Zavala EY. Endothelial cell density determined by specular microscopy and scanning electron microscopy. *Ophthalmology.* 1979;86:1831-1847.
11. Wilcoxon F. Individual comparisons of grouped data by ranking methods. *J Econ Entomol.* 1946;39:269.
12. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307-310.
13. *R: A language and environment for statistical computing.* [computer program]. Vienna, Austria: R Foundation for Statistical Computing; 2010.
14. Benetz BA, Diaconu E, Bowlin SJ, et al. Comparison of corneal endothelial image analysis by Konan SP8000 noncontact and Bio-Optics Bambi systems. *Cornea.* 1999;18:67-72.
15. Patel SV, McLaren JW, Bachman LA, et al. Comparison of flex-center, center, and corner methods of corneal endothelial cell analysis. *Cornea.* 2010;29:1042-1047.
16. Lass JH, Gal RL, Ruedy KJ, et al. An evaluation of image quality and accuracy of eye bank measurement of donor cornea endothelial cell density in the Specular Microscopy Ancillary Study. *Ophthalmology.* 2005;112:431-440.
17. Pham C, Hellier E, Vo M, et al. Donor Endothelial Specular Image Quality in Optisol GS and Life4˚C. *International Journal of Eye Banking.* 2013;1:doi:10.7706/ijeb.v7701i7702.7752.