The New Zealand National Eye Bank Study: Trends in the Acquisition and Storage of Corneal Tissue over the Decade 2000 to 2009

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Purpose: To evaluate trends in the acquisition, storage, and utilization of donated corneal tissue in New Zealand, 2000 to 2009.

Methods: The New Zealand National Eye Bank records were analyzed for the decade January 2000 to December 2009. Variables analyzed included donor demographics (age, sex, and ethnicity), donor source, donor cause of death, death-to-preservation interval (DPI), corneal storage time, tissue contamination, endothelial assessment, cornea suitability for transplantation, and corneal tissue utilization.

Results: A total of 1268 eye donors were identified during the 10-year period. Overall, 36% (n = 457) were female and 64% male (n = 813). Median donor age was 67 years, and 23% of donors were younger than 50 years (range, 5–90 years). There was a decrease in donor age over the decade (P = 0.006). The median DPI was 18.5 hours. No relationship was identified between cornea suitability for transplantation and DPI (P = 0.28) or donor gender (P = 0.54). There was a low microbial contamination rate (1%). Human immunodeficiency virus, hepatitis B, or hepatitis C serology was positive in 48 donors (4%). Overall, 90% of corneas were suitable for transplantation with a high utilization rate (88%). A novel association was identified between male sex and lower corneal endothelial cell density (P = 0.03).

Conclusions: This New Zealand National Eye Bank analysis identified trends in the acquisition, storage, and utilization of donated corneal tissue throughout New Zealand over the past decade and provides valuable additional information to the international eye bank data.

Key Words: cornea, corneal transplantation, corneal donation, corneal storage, eye banking, endothelial cell count

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The New Zealand National Eye Bank (NZNEB) is a charitable nonprofit organization responsible for the acquisition, storage, and distribution of donated corneal, scleral, and, more recently, amniotic membrane tissue throughout New Zealand. Established in 1991, the NZNEB has maintained records on all tissue donated to the service. The NZNEB complies with the standards of the Eye Bank Association of Australia and New Zealand (EBAANZ). Since 1993, all corneas acquired by the NZNEB have been preserved by organ culture. The purpose of this study was to analyze the NZNEB data from 2000 to 2009, identifying trends in the acquisition and storage of donated corneal tissue. Results from this study are compared with previous NZNEB and international studies.

MATERIALS AND METHODS

The NZNEB records were analyzed for the decade from January 1, 2000, to December 31, 2009. Variables included donor demographics (age, sex, and ethnicity), donor source, donor cause of death, death-to-preservation interval (DPI), corneal storage, tissue contamination, endothelial cell density (ECD), cornea suitability for transplantation, and corneal tissue utilization.

To investigate change in age or DPI over time, linear regressions were used with year as the explanatory variable and age or DPI as the outcome. Risk factors associated with unsuitability for transplantation were investigated using a generalized mixed model, with suitable or unsuitable for transplantation as the binary outcome, and cause of death, age, sex, ethnicity, year, storage time, and DPI as explanatory variables. Donor was included as a random effect. To investigate factors influencing ECD, a general linear mixed model was used with ECD as the outcome: sex, age, ethnicity, cause of death, storage time, year, and DPI as explanatory variables and donor as a random effect. For statistical analysis, the various causes of death were grouped into 5 categories [(1) asphyxiation/drowning/poisoning/trauma, (2) cancer, (3) cerebrovascular/cardiovascular, (4) respiratory/organ failure/septicemia, and (5) other] and ethnicity was grouped into 3 categories [(1) Asian/Indian, (2) Maori/Pacific, (3) European/other].

Eye donors are screened for medical and social history exclusions according to the Medical Standards of the EBAANZ. These include potentially infectious or transmissible disease, neurodegenerative disorders or symptoms, eye infections, corneal disease, and risk factors for human immunodeficiency virus (HIV) and hepatitis.

The donor blood sample is tested and must be nonreactive for HIV, hepatitis B virus, and hepatitis C virus. Samples of the organ culture storage medium containing each cornea are tested for bacterial and fungal contamination at 2 points during the storage period. Corneas are released for transplant only if there is no reported growth from these tests. Slit-lamp examination is performed before corneoscleral excision to exclude visible corneal pathology.

All corneas acquired during the study period were preserved by organ culture. Corneas were suspended in glass bottles containing 100 mL of presterilized minimal essential medium with Earle salts supplemented with 2% fetal bovine serum. Freshly defrosted L-glutamine and antimicrobial agents (penicillin 100 U/mL, streptomycin 100 μg/mL, and amphotericin B 0.25 μg/mL) were added at the time of corneal storage. Culture bottles were closed and incubated in a dry (non-CO_2) environment, maintained at a temperature of 34°C.

Light microscopy in combination with 0.4% trypan blue staining for viability assessment and addition of 1.8% sucrose to define cell borders was used to assess the viability and ECD of all corneas procured. The NZNEB's endothelial cell counting system has been calibrated, and this calibration subsequently validated. First, for manual cell counting, a grid graticule in the microscope (Leica Laborlux S with 10× and 20× long working distance lenses) has been calibrated by direct observation of a standard micrometer slide. Second, this calibration has been validated by capturing digital images of the micrometer slide through the microscope camera (Sony XC-ST50CE) and image analysis system (Leica Meteor II PCI Frame Grabber, Leica QWin v3 running on Microsoft Windows XP) to precisely calculate the pixel dimensions. ECD counts obtained manually through the microscope eyepieces or via digitally captured images on the computer screen are identical, therefore confirming the calibration accuracy and the accuracy of our ECD determinations.

Corneal contamination was usually visually obvious by turbidity and pH change; however, samples of culture medium were routinely tested for bacterial or fungal growth after 3 to 7 days of storage and before transplantation.

RESULTS

Donor Demographics

There were a total of 1268 eye donors during the 10-year study period, with the average number per year of 127 (SD = 10). We identified no significant trends in donor numbers over the 10-year period ($P = 0.79$). Donor sex demonstrated a male predominance across the 10-year period. Overall, 36% of donors were female (n = 457) and 64% were male (n = 813). Donor numbers and sex distribution are displayed in Figure 1.

The median donor age was 67 years (10th percentile = 32 years, 90th percentile = 81 years), with the age range of 5 to 90 years. The most common age group for donation was the 70 to 79 age group (n = 367, 28%), followed by the 60 to 69 age group (n = 234, 18%). A significant proportion of donors were younger than 50 years, accounting for 23% of all eye donors (n = 294). There was a significant decrease in donor age over the decade ($P = 0.006$), with the median age decreasing from 71 years in 2000 to 65 years in 2009. The age distribution of eye donors is displayed in Figure 2.

Donor ethnicity data have been collected by the NZNEB since 1993. The majority of donors during the study period were European white (94.2%, n = 1195), followed by Maori (1.5%, n = 19), Polynesian (1.0%, n = 13), Indian (1.0%, n = 13), Asian (0.6%, n = 8), and other ethnicities (1.6%, n = 20).

Donor Source and Donor Cause of Death

Eye donors were acquired via the following sources: the Auckland coroner (47%, n = 581), public hospitals (38%, n = 472), multiorgan donors (11%, n = 137), private hospitals (1%, n = 9), and other locations (4%, n = 49). There was a downward trend in the number of donors procured from the Auckland coroner, with a corresponding increase in those procured from other locations, during the 10-year period, as demonstrated in Figure 3.

The most common cause of donor death was cardiovascular disease (37%, n = 474), followed by cerebrovascular disease (18%, n = 226) and trauma (12%, n = 146). Table 1 illustrates the median age, sex distribution, and contribution to the total number of donors for each cause of death. There were no significant trends in the distribution of the various causes of death over the 10-year period.

Death-to-Preservation Interval

The DPI is defined as the time from donor death to the time when the donor cornea is stored in culture medium. The most common DPI from 2000 to 2009 was 20 to 24 hours (26%), followed by 15 to 19 hours (25%), 10 to 14 hours (20%), 25+ hours (16%), 5 to 9 hours (11%), and 0 to 4 hours (2%). A total of 8 DPIs were not recorded during the decade. Overall, the median DPI was 18.5 hours (10th percentile = 8.5 hours, 90th percentile = 26 hours), with strong statistical evidence of an increase in DPI over the decade ($P < 0.0001$), as illustrated in Figure 4.

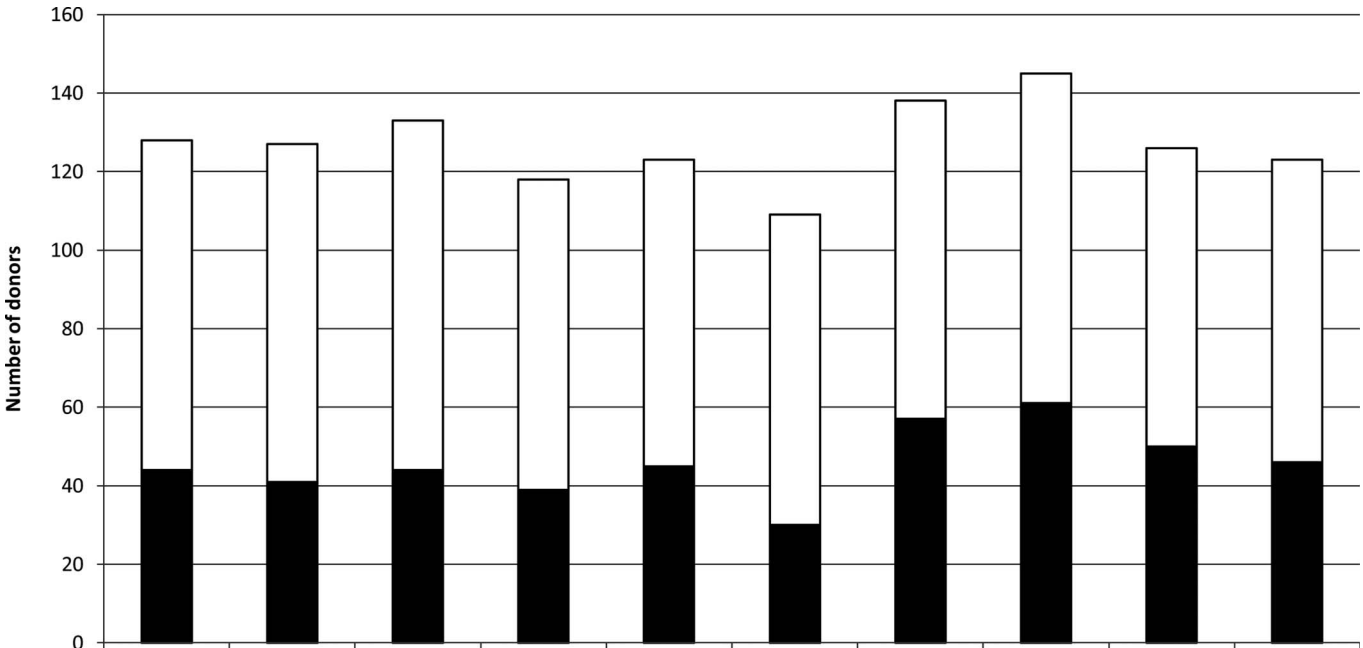
Corneal Acquisition and Utilization

There were 2516 corneas acquired during the decade of the study. Of these, 90% (n = 2252) were suitable for transplantation and 10% (n = 264) deemed unsuitable for transplantation. Overall, 88% (n = 2203) of all corneas procured were used for transplantation (Fig. 5). There were no reports of infectious disease transmission or contaminated tissue leading to endophthalmitis in recipient patients.

There was an association between unsuitability for transplantation and increasing donor age ($P = 0.01$) and corneas that were donated earlier in the decade ($P = 0.001$). There was no relationship between suitability for transplantation and donor ethnicity ($P = 0.52$), donor cause of death ($P = 0.84$), DPI ($P = 0.28$), and donor sex ($P = 0.54$) (Table 2).

Unsuitable Corneas

The various reasons that corneas were unsuitable for transplantation are listed in Table 3. There were a total of 264



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Male	84	86	89	79	78	79	81	84	76	77
Female	44	41	44	39	45	30	57	61	50	46

FIGURE 1. Annual number of eye donors (n = 1268) and sex distribution in the 10-year period 2000 to 2009.

unsuitable corneas over the decade. Of these, the most common cause of unsuitability was poor endothelium that failed quality standards (n = 50), followed by positive serology (n = 48) [hepatitis B (14), hepatitis C (32), and HIV (2)], unsuitable donor history (n = 36), and other, miscellaneous causes (n = 47). Causes included within the "other" category are corneal dystrophies, metastasis to eye, excessive arcus senilis, and previous trauma.

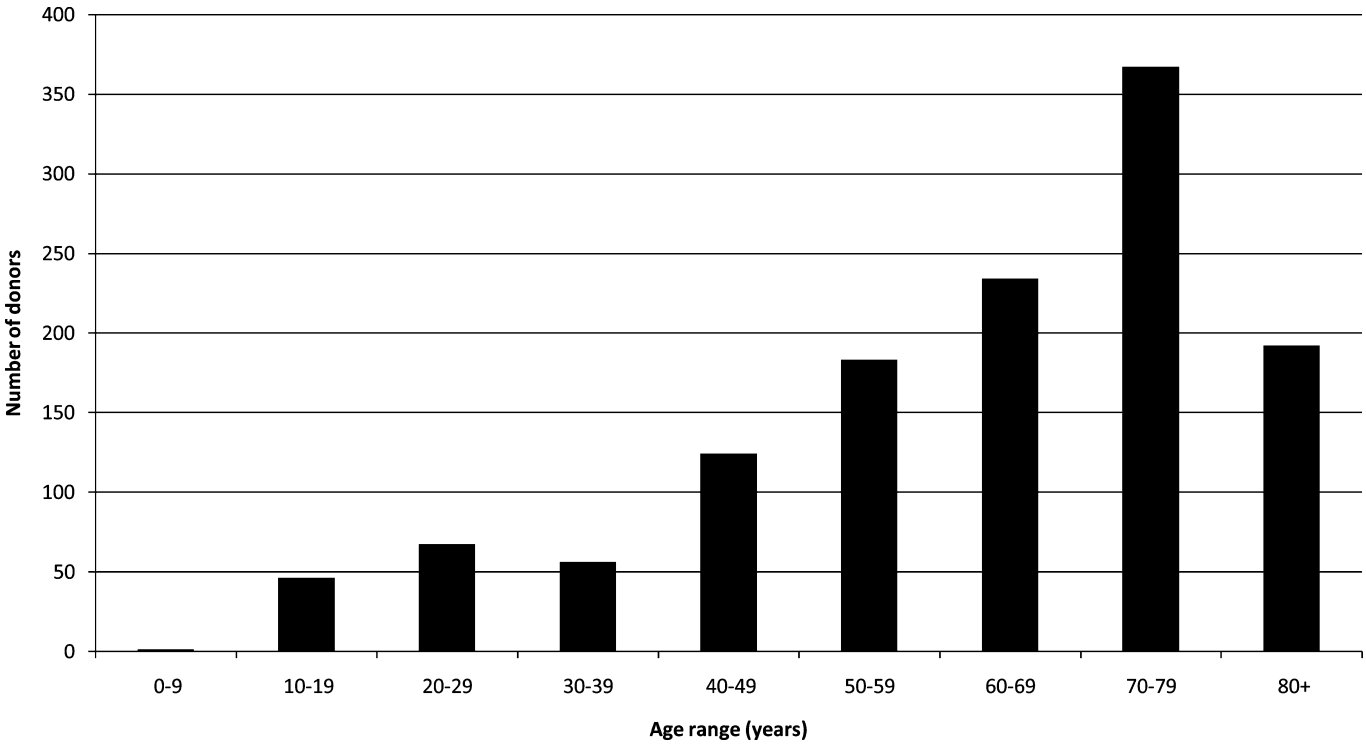


FIGURE 2. Age distribution of eye donors (n = 1268) in the 10-year period 2000 to 2009.

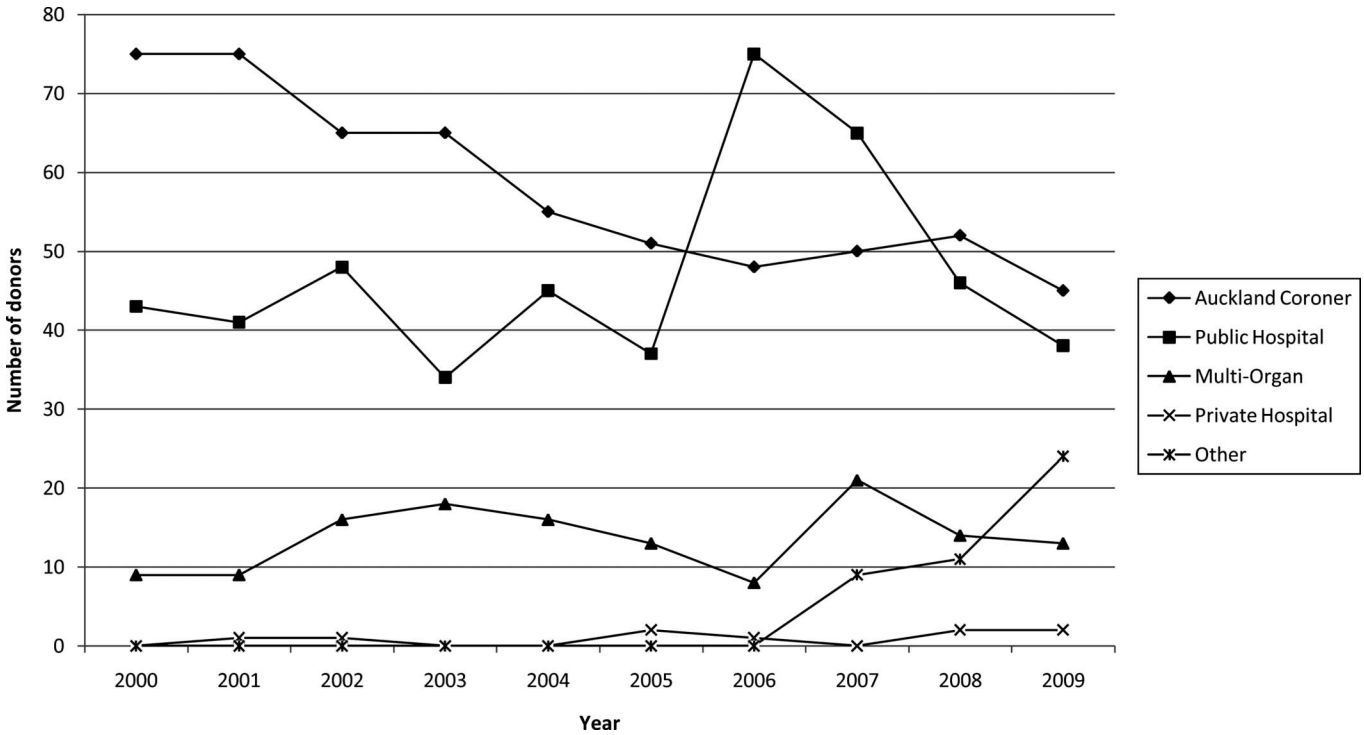


FIGURE 3. Sources of eye donors to the NZNEB in the 10-year period 2000 to 2009.

Corneal Contamination

A total of 31 corneas were contaminated over the decade, accounting for only 1% of all corneas acquired (Table 2). Causes of corneal contamination include coagulase-negative staphylococcus (n = 23), yeast (n = 2), methicillin-resistant *Staphylococcus aureus* (n = 2), *Stenotrophomonas maltophilia* (n = 1), and *Pseudomonas aeruginosa* (n = 3).

Endothelial Cell Density

Corneas with an ECD of less than 2500 cells per square millimeter were deemed unsuitable for transplantation, accounting for 2% (n = 50) of all corneas acquired. The ECD for all corneas acquired during the study period is highlighted in Figure 6. There was a statistical association between increasing donor age and decreased ECD ($P < 0.0001$). There was also an association between male gender and lower ECD ($P = 0.03$). No association was shown between ECD and cause of death ($P = 0.43$), ethnicity ($P = 0.13$), or DPI ($P = 0.34$) (Table 4).

TABLE 1. The Median Age and Sex Distribution and Contribution to the Total Number of Eye Donors for Each Cause of Donor Death in the 10-Year Period 2000 to 2009

Cause of Death	Median Age (10th–90th Percentile)	% of Donors	% Female
Cardiovascular	71 (50–82)	37.4	7.8
Cerebrovascular	62 (42–81)	17.8	48.2
Respiratory/organ failure	72 (46–83)	5.1	46.9
Trauma/injury	40 (18–77)	11.5	26.7
Cancer	70 (52–78)	7.3	46.2
Septicemia/infection	73 (36–82)	1.5	26.3
Poisoning	47 (21–67)	2.7	35.3
Asphyxia	41 (18–56)	3.5	17.8
Drowning	28 (16–69)	1.1	57.1
Others	71 (33–82)	12.1	15.7

Storage Time

The storage times for corneas acquired during the study period are displayed in Figure 7. The majority of corneas were stored for a period of 5 to 9 days (35%), with 30% stored for 10 to 14 days. Only 11 corneas (<1%) were stored longer than 25 days.

DISCUSSION

Since its establishment in 1991, the NZNEB has been the major supplier of donated corneal, scleral, and, more recently, amniotic membrane tissue throughout New Zealand. Serving a current estimated population of more than 4.3 million, the NZNEB supplies donor corneas to approximately 19 corneal surgeons (of 103 ophthalmologists in New Zealand) performing more than 200 corneal transplants per year nationwide. The NZNEB complies with the standards of the EBAANZ[1,2] and ensures that donated eye tissue is distributed in an unbiased, needs-based manner throughout New Zealand. Currently, most routine corneal transplants can be booked with a corneal tissue availability time of approximately 3 to 6 months, and emergency graft tissue is generally available within 24 to 48 hours.

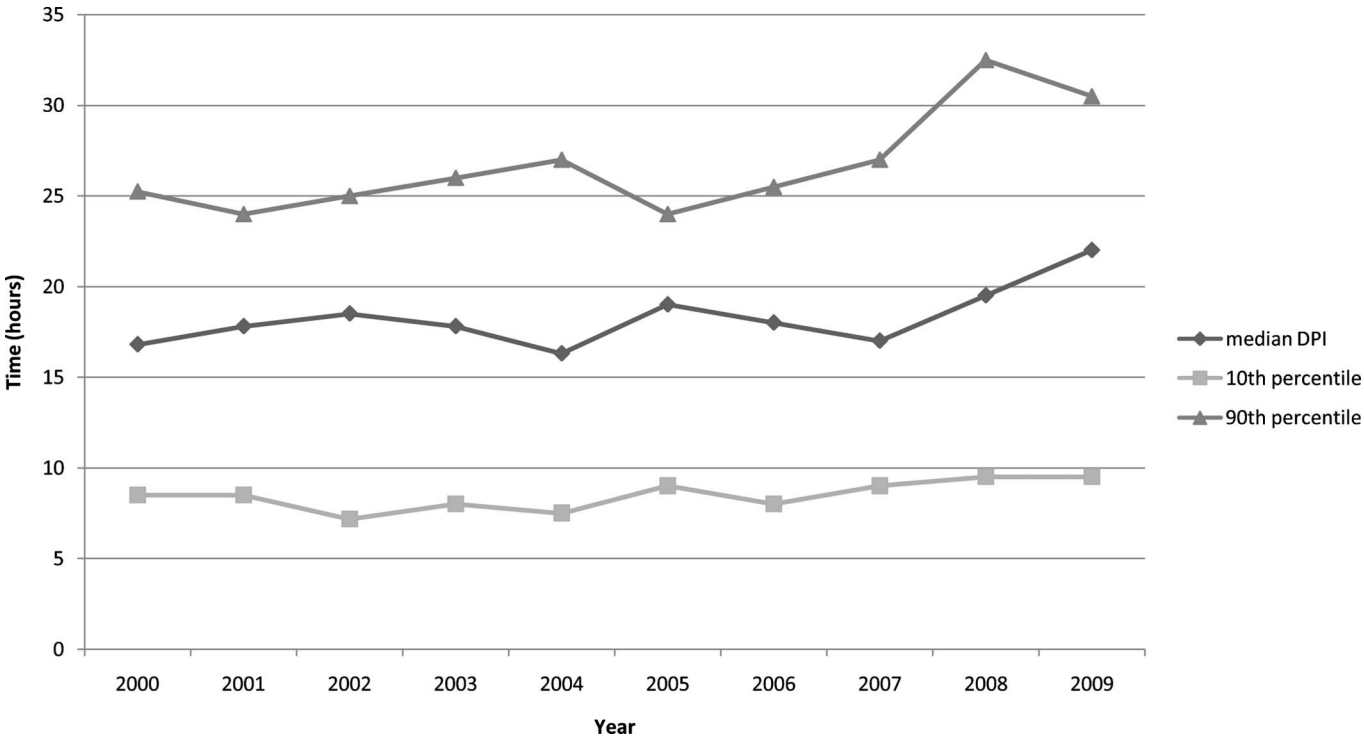


FIGURE 4. Changes in median DPI in corneas acquired by the NZNEB (2000–2009).

There were a total of 1268 eye donors in the 10-year period from January 2000 through December 2009, which is comparable to NZNEB data published from the previous decade.[3,4] Fortunately, donor numbers remained stable over the 10-year period, sufficient to meet local demands, compared with reports of decreased numbers of corneal donations elsewhere.[5,6]

Corneas donated earlier in the decade were more likely to be unsuitable for transplantation. One explanation for this is the reduction in the rate of corneal contamination over the decade, with an overall corneal contamination rate of 1%. This rate was significantly reduced compared with the previous decade at 5%[3] and is almost certainly explained by improvements in donor tissue storage, with exclusive use of organ culture preservation and the addition of antimicrobial agents at the time of storage. Donor rim swab testing was also eliminated midway through the decade, which may have contributed to false-positive results in the past. It was found that positive rim swab testing of the donor eye after decontamination procedures did not correlate with subsequent growth in the corneal culture medium.

The age distribution of eye donors was similar to that reported from European, American, and Australian eye banks,[7–9] where the majority of donors were older than 50 years. The most common age for eye donation came from the 70 to 79 age group. We found a significant decrease in median donor age over the decade (71 years in 2000 vs 65 years in 2009) and a strong association between advancing age and corneas that were unsuitable for transplantation. Interestingly, this association was not identified in our previous NZNEB studies[3]; however, these data are comparable to other eye bank reports of reduced rates of corneal tissue utilization with advancing donor age.[10,11]

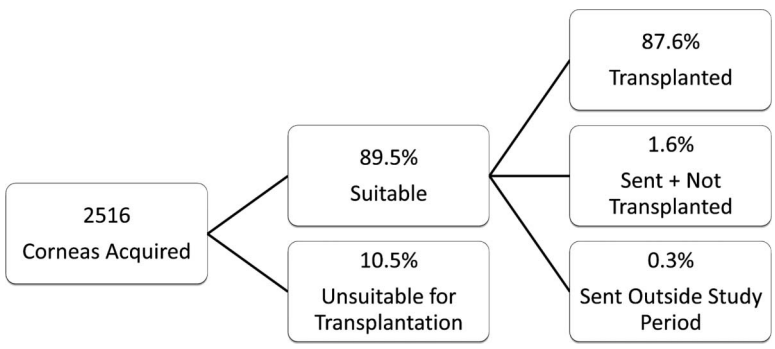


FIGURE 5. Corneal utilization for the NZNEB (2000–2009).

TABLE 2. Statistical Summary of Unsuitable Corneas (2000–2009)

	Unsuitable Corneas: Statistical Summary Table				
Effect	**Category**	**Odds Ratio**	**95% Confidence Limits**		***P***
Cause of death					0.84
	Cancer vs respiratory/organ failure/septicemia	1.6	0.6	4.4	
	Cerebrovascular/cardiovascular vs respiratory/organ failure/septicemia	1.1	0.5	2.3	
	Other vs respiratory/organ failure/septicemia	1.1	0.3	3.3	
	Asphyxiation/drowning/poisoning/trauma vs respiratory/organ failure/septicemia	1.4	0.5	3.5	
Age	—	1.02	1.00	1.03	0.01
Sex	Female vs male	0.8	0.5	1.5	0.54
Ethnicity					0.52
	Asian/Indian vs Maori/Pacific	3.1	0.3	32.1	
	European/other vs Maori/Pacific	1.2	0.2	6.6	
Year	—	0.82	0.73	0.92	0.001
DPI	—	0.98	0.95	1.01	0.28

Donor gender demonstrated a male preponderance across each of the 10 years, and for the 10-year study period, a total of 64% of corneal donors were male. This ratio was similar to other published eye bank data.[7,9] We found no relationship between donor gender and cornea suitability for transplantation. However, somewhat surprisingly, we identified an association between male gender and lower ECD. We are unsure of the cause, if any, of this association, and this was not evident in our previous studies. To our knowledge, such an association has not previously been reported in other eye bank studies, and currently, we believe this to be a potentially anomalous association that remains a topic for further investigation in ongoing NZNEB studies.

Perhaps unsurprisingly, the most common donor cause of death was cardiovascular disease, followed by cerebrovascular disease, which is similar to reports from other large studies.[7,9] There was no relationship between donor cause of death and cornea suitability for transplantation, whereas Patel et al[3] reported from New Zealand that those who died of cardiovascular or cerebrovascular disease were more likely to have corneas that were suitable for transplantation.

TABLE 3. The Various Causes of Unsuitable Corneas for Transplantation (2000–2009) (n = 264)

Causes of Unsuitable Cornea	No. Corneas	% of Total No. Corneas Acquired (N = 2516)
Inadequate "failed" endothelium	50	2.0
Serology positive	48	1.9
Other	47	1.9
Unsuitable donor history	36	1.4
Bacterial positive	24	1.0
Expired storage maximum	22	0.9
Gross defect	16	0.6
Undetermined serology	14	0.5
Mycology positive	7	0.3
Total	264	10.5

The median DPI was 18.5 hours, with strong evidence of an increase in DPI over the decade. This can be explained by changes in DPI criteria at the NZNEB. Before 2000, the NZNEB generally did not procure tissue with an anticipated DPI more than 24 hours. However, international data showing good results with donor corneal DPIs more than 24 hours[12] led to a change in practice at the NZNEB. Therefore, in the decade reported, donor corneas with a DPI of 25 hours or more accounted for 16% of all corneas acquired, and there was no relationship between the DPI and corneas that were deemed unsuitable for transplantation.

Earlier studies reported that donor corneas must have an ECD of 2500 or more cells per square millimeter to be suitable for transplantation.[3] However, with the recent advent of changes in technique for corneal transplantation and lamellar keratoplasty, the NZNEB no longer rejects corneas with an ECD of less than 2500 cells per square millimeter because they may be used for nonendothelial lamellar transplantation. As reported in other eye bank studies,[9,10,13,14] we noted an association between increasing age and decreased ECD; however, no association was noted between ECD and donor cause of death, ethnicity, or DPI.

Overall, our ECD measurements are higher compared with other international eye bank studies possibly because of the way ECD is assessed. There are in fact no internationally agreed methods of assessing ECD, and factors such as the sampled area of the endothelium, the imaging equipment used, and the degree of observer bias will all affect the ECD obtained. Furthermore, most techniques have been developed to assess the ECD in cold-stored corneas rather than in corneas stored in the organ culture system that the NZNEB has used since 1991. From this time, the NZNEB has always assessed the entire corneal endothelium, not just the central cornea as seems to have more recently become standard practice, and this may contribute to higher mean values.

Similar to many other eye banks that use an organ culture system, the NZNEB uses a fixed-frame "naked-eye" counting method that averages the cell counts of several fields after intravital trypan blue staining to assess corneal viability for

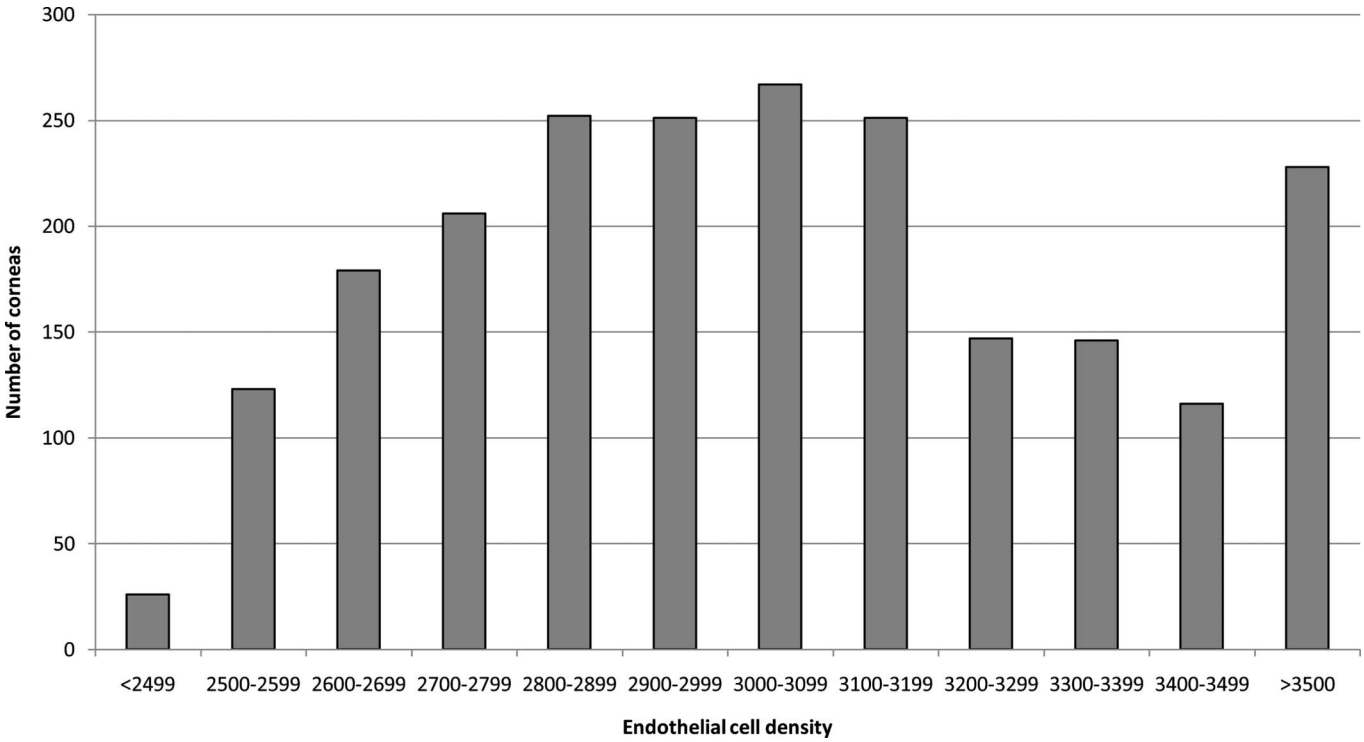


FIGURE 6. ECD for corneas assessed during the study period.

transplantation. The NZNEB uses 3 ECDs (central, paracentral, and peripheral) of 300 to 400 cells in total. Cells are counted directly through the microscope using a grid graticule, and the average count is multiplied by a calibrated constant to produce a final estimate expressed in cells per square millimeter.

Because our assessment includes the peripheral endothelium, which is known to have a higher ECD,[15] our mean ECD is consequently higher and thus cannot be readily compared with ECDs from other eye banks obtained using different techniques. However, for comparative purposes, we have previously published ECD data from our eye bank including methodology.[16] Because the accuracy of manual counting is well known to be prone to intra- and interobserver error,[3] we are currently developing a protocol that aligns more closely with international current practice in ECD determination.

The reducing numbers of donors sourced from the Auckland coroner continue the trend observed in the previous decade[3] and is secondary to reducing numbers of deceased people requiring autopsy in New Zealand. In response to this trend, donor programs were established at some public hospitals, and there was an increase in donors sourced from

TABLE 4. Statistical Summary of ECD (2000–2009)

	ECD: Statistical Summary Table			
Effect	**Category**	**Beta Coefficient Estimate**	**Standard Error**	***P***
Cause of death				0.43
	Cancer vs respiratory/organ failure/septicemia	43.3	44.0	
	Cerebrovascular/cardiovascular vs respiratory/organ failure/septicemia	19.8	29.2	
	Other vs respiratory/organ failure/septicemia	71.6	48.7	
	Asphyxiation/drowning/poisoning/trauma vs respiratory/organ failure/septicemia	54.7	37.5	
Age	—	−9.9	0.6	<0.0001
Sex	—			0.03
	Female vs male	56.0	25.2	
Ethnicity				0.13
	Asian/Indian vs Maori/Pacific	−15.0	86.5	
	European/other vs Maori/Pacific	−94.5	56.4	
Storage time	—	−7.4	1.4	<0.0001
DPI	—	−1.2	1.2	0.34
Year	—	8.7	4.4	0.05

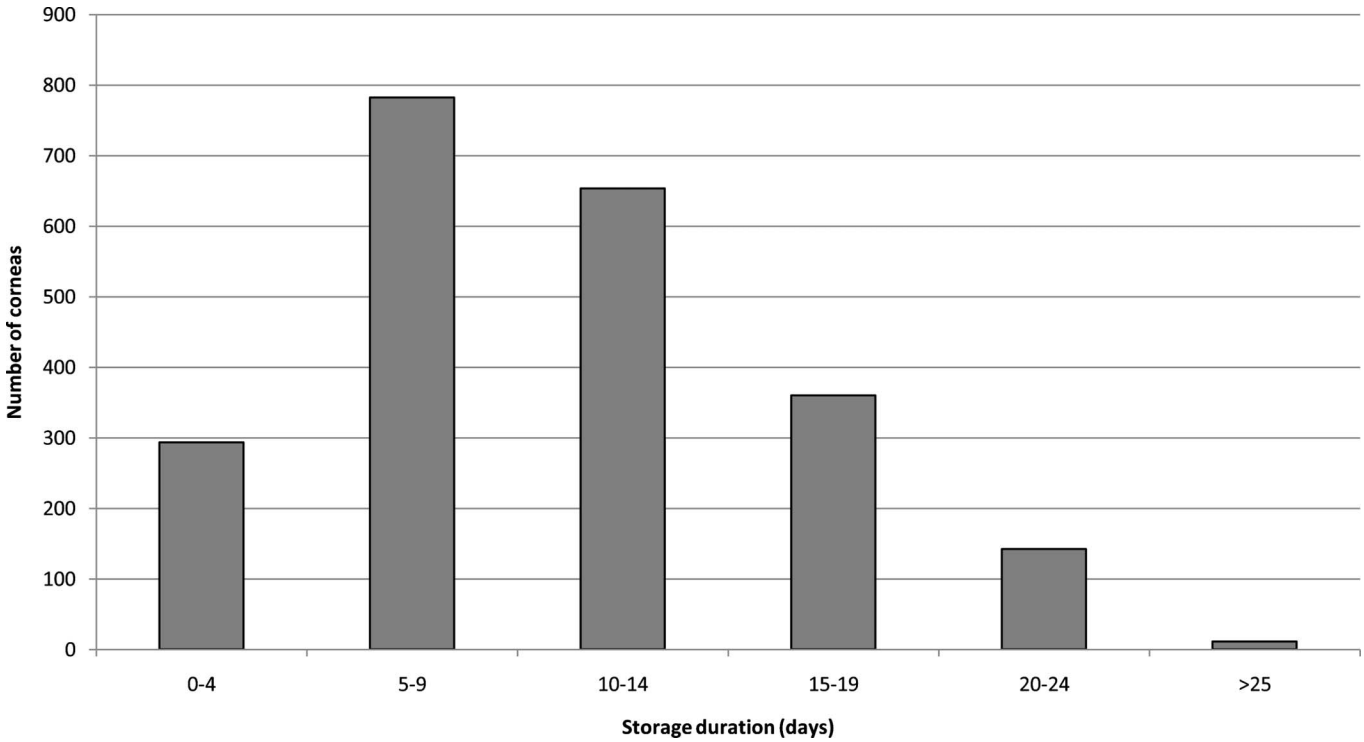


FIGURE 7. Storage period for 2516 corneas during the study period.

other locations including hospices, funeral homes, and family referrals. The increased supply from other locations reflects increased efforts from eye bank staff in encouraging referral of potential donors from these sources. During occasional periods of tissue shortage, corneas have been sourced from Australian eye banks.

Compared with previous studies analyzing tissue utilization,[3,10,17] the NZNEB utilized a high 88% of all corneas acquired. This is likely a result of the NZNEB's detailed prescreening of all potential donors, where by contraindicated donors are eliminated before tissue acquisition. This, in combination with effective corneal storage techniques and low rates of corneal contamination as discussed above, coalesce to result in an exceptionally high rate of tissue utilization.

The NZNEB provides a valuable clinical service and maintains a detailed database that is crucial to documenting the progress and management of donated corneal tissue in New Zealand. During the 10-year study period, we identified trends in donor source, reducing donor age, and improved tissue storage with decreased rates of contamination. We also witnessed high tissue utilization rates, increasing DPIs not affecting tissue suitability for transplantation, and a novel association between gender and corneal ECD.

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