Accuracy of Intraocular Pressure Measurements in New Zealand White Rabbits

K. Sheng Lim,[1,2] *Sanjeewa S. Wickremasinghe,*[1] *M. Francesca Cordeiro,*[3] *Catey Bunce,*[4] *and Peng T. Khaw*[1,2]

Purpose. Rabbits are commonly used for the evaluation of drugs and surgery to lower intraocular pressure (IOP). The accuracy of intraocular pressure measurement is therefore critical in the analysis of data and subsequent extrapolation to humans. The purpose of this study was to establish the most reliable technique, from several currently available methods, of measuring IOP in New Zealand White rabbits.

Methods. The IOPs of 11 healthy New Zealand White rabbits were measured with a fluid-filled pressure transducer system that was connected to the anterior chamber of each animal so that the IOP could be varied by altering the height of a bottle of balanced salt solution. Intraocular pressures were recorded over a range of 50 to 0 mm Hg by the transducer system, and comparative measurements at the same pressures were performed with the Tonopen XL (Mentor, Norwell, MA), the Perkins handheld applanation tonometer (Clement Clarke, Harlow, UK), and the Ocular Blood Flow (OBF) pneumatonometer (OBF Laboratories, Wiltshire, UK).

Results. All three tonometers underestimated the true IOP, especially at higher pressures. Although the mean difference in actual and tonometric IOP was least in the Tonopen XL, there was a high degree of variability through the entire range of IOPs measured. The Perkins tonometer, although slightly less accurate than the Tonopen XL, was more consistent in measurement. The OBF pneumatonometer was the least-reliable estimate of IOP.

Conclusions. All tonometers underestimate IOP, with increasing inaccuracy at higher pressures. The Perkins applanation tonometer was the most reliable for measuring IOP in the range of 0 to 50 mm Hg in New Zealand White rabbits. (*Invest Ophthalmol Vis Sci.* 2005;46:2419–2423) DOI:10.1167/iovs.04-0610

Current methods of intraocular pressure (IOP) measurement have all been calibrated for human use or more specifically for use in white populations.[1] All tonometers provide an estimate of IOP, although Goldmann applanation tonometry is the gold standard of measurement. For research purposes, rabbits are often used in the evaluation of new drugs and surgical procedures for glaucoma. However, the optimal method for measuring IOP in these animals has still not been established.[2–6]

The accuracy of applanation tonometry is dependent on several factors, of which the most important is the use of the correct technique. However, there are other sources of error, including the central corneal thickness[7] and scleral rigidity.[8] In describing applanation tonometry, Goldmann and Schmidt determined that if the diameter of the applanation prism was between 3 and 4 mm, the two opposing forces of resistance to indentation (pushing the tonometer away from the cornea) and surface tension (drawing it toward the cornea) would balance out, allowing accurate measurement of the force necessary to indent the cornea surface adjacent to the prism (IOP). This technique assumed a constant thickness of the central cornea, which they calculated to be 0.52 mm. They acknowledged that variations in central corneal thickness would lead to erroneous measurements. Subsequent studies have quantified the extent of this measurement error with Goldmann applanation. Ehlers et al.[9] demonstrated that for every 10-μm variation there was a 0.7 mm Hg error in measurement, whereas the Rotterdam study[10] found a 0.19-mm Hg error for every 10-μm variation. The Perkins applanation tonometer (Clement Clarke, Harlow, UK) is a hand-held version of the Goldmann instrument.

In normal[11] and scarred corneas, as well as those having undergone previous surgery,[12] studies have shown that errors in IOP measurement due to variations in corneal thickness have been decreased by the introduction of the modified "Mackay-Marg" design of the Tonopen XL (Mentor, Norwell, MA), a handheld microprocessor-controlled tonometer that uses only the central part of the applanation area for pressure measurement, thereby minimizing the effect of differences in central corneal thickness. Even so, Dohadwala et al.[13] have demonstrated that these influences have not been completely eliminated.

The pneumatonometer emits a stream of gas that exerts force against the cornea, depressing the corneal surface against the opposing force of the IOP. The balance of these forces and the resultant effect on the tonometer pressure sensor is used to estimate the IOP.[14,15] Pneumatonometers have been shown to be unaffected by corneal thickness in human[16] and rabbit[17] eyes. The OBF pneumatonometer has a probe that is linked to a computerized control system. The probe may be mounted horizontally in a slit lamp or handheld. The theoretical disadvantage of pneumatonometers is that they are high-displacement tonometers and so may alter the subsequent IOP measurements.

Methods

Experiments were performed on 11 healthy New Zealand White rabbits (1–2 kg) at the Institute of Ophthalmology in London and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Findings in an initial examination of all animals were normal, and baseline measurements, including IOP, were re-

From the [1]Glaucoma Service and [4]Research and Development, Moorfields Eye Hospital, London, United Kingdom; the [2]Ocular Repair and Regeneration Biology Unit and the [3]Department of Pathology, Institute of Ophthalmology, London, United Kingdom.

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Corresponding author: K. Sheng Lim, Ocular Repair and Regeneration Biology Unit and Glaucoma Service, Institute of Ophthalmology and Moorfields Eye Hospital, Bath Street, London EC1V 2PD, UK; shenglim@gmail.com.

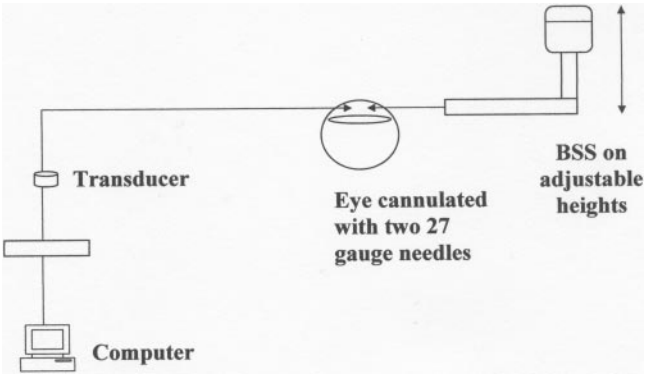

FIGURE 1. Schematic of the experimental setup.

corded. All animals underwent general anesthesia with intramuscular ketamine (50 mg/kg; Pfizer [Parke-Davis], Morris Plains, NJ) and xylazine (10 mg/kg; Bayer, Leverkusen, Germany).

An anterior chamber infusion was established using a 27-gauge needle introduced from the temporal limbus and connected to a bag of balanced salt solution (BSS; Alcon Laboratories, Fort Worth, TX) on a stand with variable height (Fig. 1). Another 27-gauge cannula was inserted into the anterior chamber through the nasal limbus primed with BSS and connected to a pressure transducer system similar to the one described by Foster et al.[1] The system consisted of a pressure transducer (Model P23ID; Gould Inc., Santa Clara, CA) and a bridge amplifier (Model ML110) and recorder (Model ML200; AD Instruments, New South Wales, Australia). Pressure changes were recorded with a commercial software program (MacLab software, ver. 3.5; AD Instruments) on a computer (Performa 630; Apple Computer, Cupertino, CA). Silicone tubing (Silicone High-Strength Tubing; Altec, Alton, UK) was used throughout. The height of the BSS was altered, and the corresponding IOP changes were observed in the pressure transducer system. Each rabbit had at least four sets of IOP readings taken at different bottle heights. The order of measurements with the different methods was randomized, and IOP was recorded at the same time the corresponding IOP in the pressure transducer system was noted by a second masked observer, to reduce bias. IOP was decreased in increments of 5 to 10 mm Hg from 50 to 0 mm Hg and was recorded with each of the instruments—the hand-held Perkins, the Tonopen XL, and the OBF pneumatonometer—in random order.

Each tonometer was first calibrated to the manufacturer's instructions. Measurements by the Tonopen XL were repeated until the coefficient of variation was less than 5%. The same investigator (MFC), who was unaware of the bottle height and transducer measurement (altered by KSL), performed all tonometric measurements. All measurements were taken under open stop-cock conditions.

Statistical Analysis

To assess the accuracy (the absolute difference between the mean IOP recorded by the tonometer and that measured by the transducer) and variability (amount of scatter from the mean value) of each tonometer, we determined the difference between the tonometer and transducer measurements at each bottle height and calculated the mean and SD of these differences. To assess the reliability of each instrument at different IOPs, the differences between tonometer and transducer measurements of IOP (tonometer –transducer) were plotted against actual IOP (transducer). Linear regression analysis was used to determine best-fit lines, to assess the variation in measurement error at the different IOPs. For each tonometer, linear regression analysis was used to evaluate the relationship between true IOP as measured by the transducer and that measured by each tonometer.

RESULTS

The Pearson correlation coefficient (r) and gradient of regression lines (m) were similar in each rabbit for each tonometer. As such, the data from all rabbits were pooled for further analysis. (Tonopen XL: $r = 0.98 \pm 0.02$, $m = 0.84 \pm 0.15$; Perkins: $r = 0.98 \pm 0.03$, $m = 0.98 \pm 0.17$; and OBF: $r = 0.97 \pm 0.03$, $m = 0.50 \pm 0.11$).

All tonometers underestimated the actual IOP. Through the entire range of IOPs measured, on average, the Tonopen XL

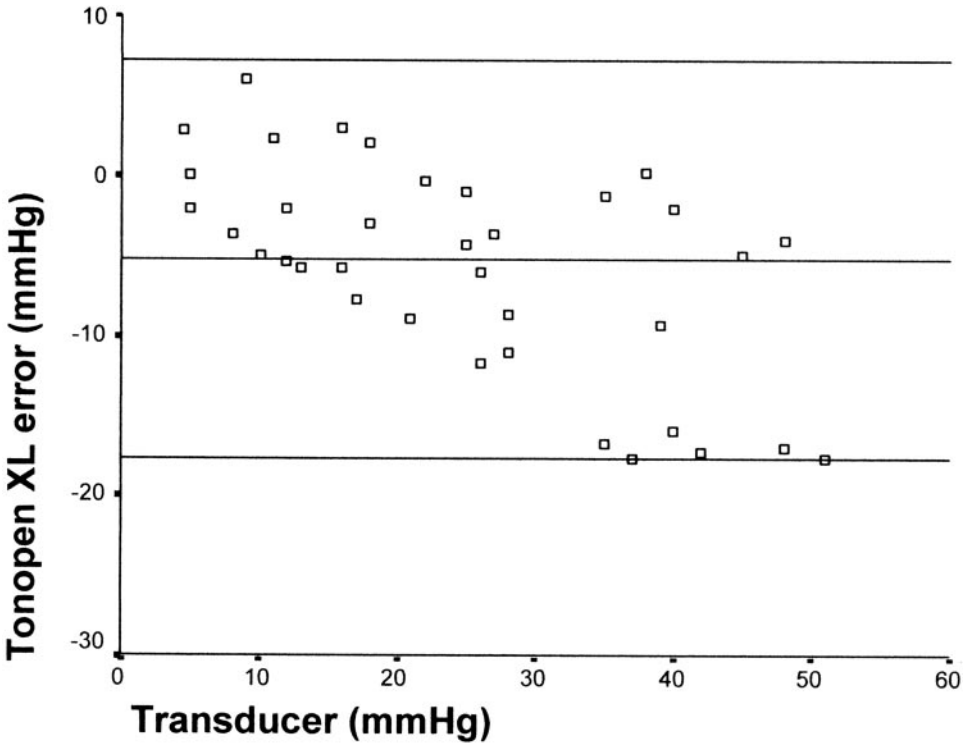

FIGURE 2. Scatterplot of actual IOP (transducer measured) against measurement error for the Tonopen XL. *Horizontal lines*: the mean error ± 2 SD.

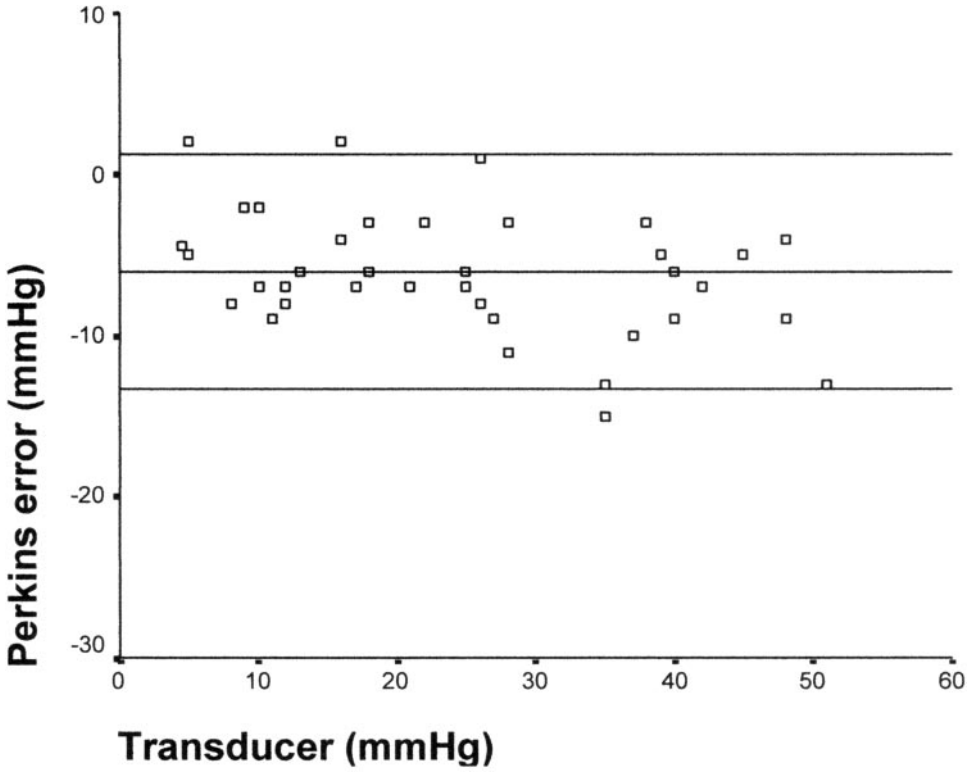

FIGURE 3. Scatterplot of actual IOP (transducer measured) against measurement error for the Perkins. *Horizontal lines*: the mean error ± 2 SD.

was the most accurate, although there was a high degree of variability (−5.59 ± 5.81 mm Hg). The Perkins instrument was slightly less accurate, although there was less variability in measurement (−6.05 ± 3.65 mm Hg). The OBF pneumatonometer was the least accurate and most variable (−11.50 ± 7.10 mm Hg).

Figures 2, 3, and 4 illustrate the measurement error of each instrument (transducer IOP − tonometer-measured IOP) at different transducer-determined IOPs. As the actual IOP increased, all the instruments became less accurate, and the degree of underestimation increased. Although underestimation occurred with all the tonometers, the extent to which this

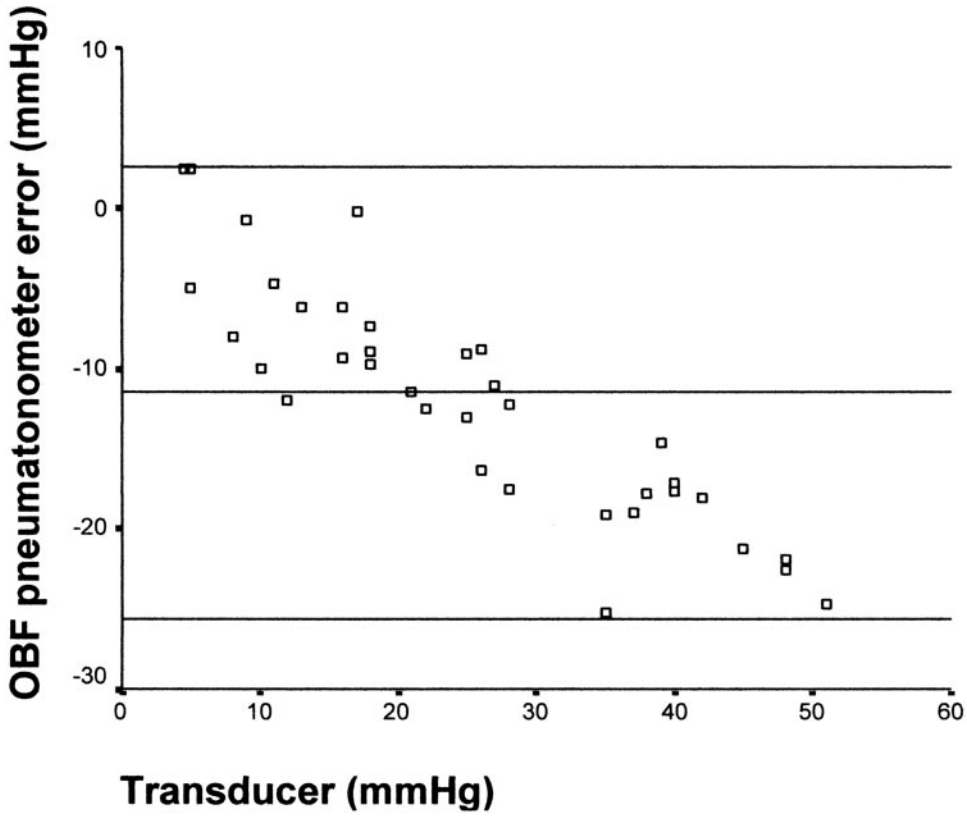

FIGURE 4. Scatterplot of actual IOP (transducer measured) against measurement error for the OBF pneumatonometer. *Horizontal lines*: the mean error ± 2 SD.

TABLE 1. Statistical Analysis of the Error in IOP Measurement

Tonometer	Accuracy* (mm Hg)	Variance (mm Hg)	Pearson Correlation Coefficient (*r*)	Gradient of Regression Line** (*m*)	*P*
Tonopen XL	−5.69	5.81	0.62	−0.28	<0.001
Perkins	−6.05	3.65	0.38	−0.10	0.014
OBF pneumatonometer	−11.5	7.10	0.88	−0.44	<0.001

* Accuracy is expressed as the mean (transducer − tonometer).
† Gradient of regression line and its significance were derived from linear regression analysis

occurred varied widely. From linear regression analysis, best-fit lines were constructed to ascertain the degree of error in estimation of each instrument at increasing IOPs. Table 1 describes the variation in measurement error.

As with accuracy, the OBF pneumatonometer was most prone to error at higher IOPs, with a 0.44-mm Hg underestimation for every 1-mm Hg increase in actual IOP. Despite being superficially the most accurate, the Tonopen XL was also prone to error at greater IOPs (0.28 mm Hg error for every 1 mm Hg increase). The Perkins tonometer was the most consistent in the degree of underestimation (only 0.1 mm Hg of underestimation for 1 mm Hg increase in IOP).

Figure 5 illustrates the overall relationship between the different tonometers and actual IOP. Despite the high correlation (*r*) of each tonometer with actual IOP in all cases, the gradients of the regression lines were very different. Table 2 describes the relationship of tonometer measurement to actual IOP. The Perkins tonometer gave the highest level of correlation with actual IOP, with a gradient of 0.90. (A 1.0-mm Hg increase in actual IOP corresponded to a 0.9-mm Hg increase in IOP measured by the Perkins.)

To correct tonometry estimates to give a closer approximation of actual IOP in rabbits, the following equations derived from linear regression can be used:

$$\text{Actual IOP (mm Hg)} = 1.12\ (\text{Tonopen XL}) + 3.07,$$

$$\text{Actual IOP (mm Hg)} = 1.04\ (\text{Perkins}) + 5.42, \text{ or}$$

$$\text{Actual IOP (mm Hg)} = 1.51\ (\text{OBF}) + 5.64.$$

DISCUSSION

In glaucoma research, animal studies play an important role in the development of new treatment modalities. Although accuracy of IOP measurement is desirable, it is often the trend of change in IOP that we are most interested in. Many different tonometers are currently used in animal experimentation, the accuracy of which, including the three tonometers evaluated in this study, has already been established in human eyes. However, when tonometers designed for human eyes are used in other species, their suitability is limited by the differences in corneal anatomy (lack of Bowman's membrane in rabbits), corneal rigidity, and tear film composition. The ideal tonometer in animal research should be accurate, but also should be consistent, with low variability in measurements.

All three techniques of IOP measurement underestimate actual IOP. There was a trend for each instrument to underes-

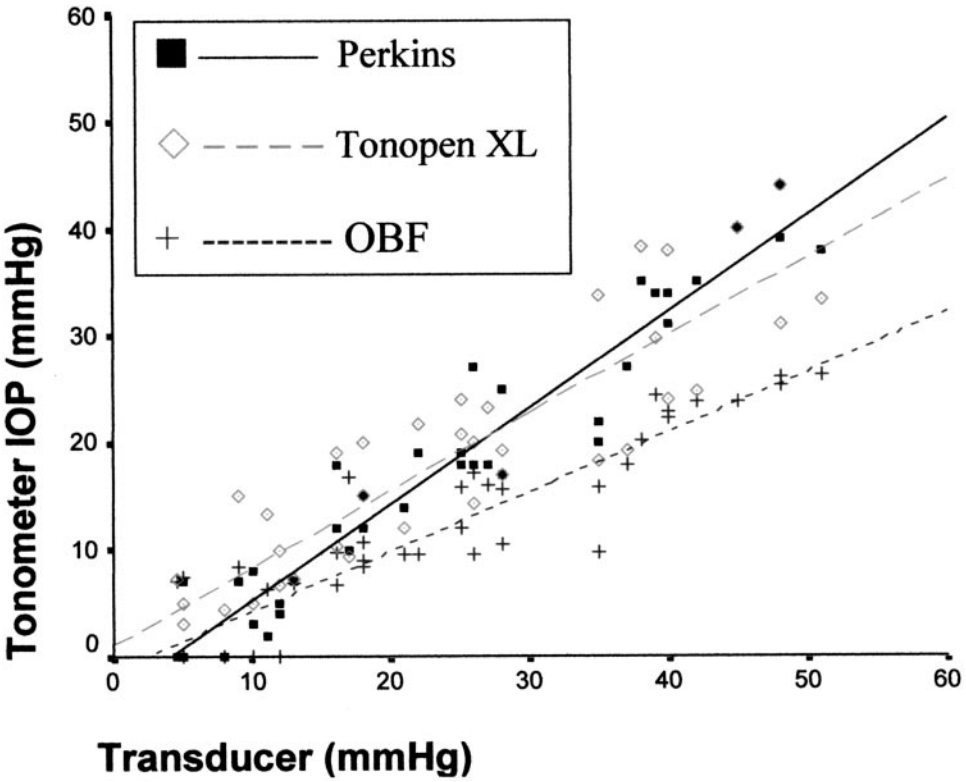

FIGURE 5. Scatterplot of actual IOP (transducer measured) against IOP estimates measured by tonometers. *Lines*: univariate regression summarizing the relationship between the two variables.

TABLE 2. Statistical Analysis of the Relationship between Tonometer Measurements and Transducer-Measured IOP

Tonometer	Pearson Correlation Coefficient (*r*)	Gradient of Regression Line* (*m*)	*P*
Tonopen XL	0.90	0.73	<0.001
Perkins	0.97	0.90	<0.001
OBF pneumatonometer	0.92	0.56	<0.001

* The gradient of the regression line and its significance were derived by linear regression analysis.

timate the transducer-measured IOP by greater amounts at higher IOPs.

The Tonopen XL showed the least error in estimation of true pressure; however, there was a high degree of variability in the measurements, especially at higher IOPs, where it significantly undervalued the IOP. The OBF pneumatonometer was the least-accurate method of measurement and had the most variability. As the actual IOP was raised, the degree of underestimation increased markedly. The Perkins applanation tonometer, although not quite as accurate overall as the Tonopen XL, had significantly less variability of measurement. It revealed a highly significant association between the level of IOP measured and the transducer pressure within the range measured. In addition, although it underreported the actual IOP, it was certainly the most consistent as to the extent of measurement error through the measured range of IOPs.

Other groups have reported variable and inconsistent findings. Neault et al.[6] reported a finding similar to ours, in that they found the pneumatonometer consistently underreports the IOP. Abrams et al.[2] reported that the pneumatonometer gave a sinusoidal error in IOP measurement, overestimating the IOP at lower pressures and underestimating at higher pressures. They also concluded that the Tonopen XL was the most accurate at predicting true IOP (-4.3 ± 7.29 mm Hg). The Goldmann applanation tonometer and pneumatonometer had accuracies of -5.9 ± 11.56 and $+4.2 \pm 12.25$ mm Hg, respectively). They, however, used a different applanation tonometer (Kowa Optimed, Torrance, CA) and pneumatonometer (Bio-Rad, Santa Ana, CA). Other reasons for the difference in findings could be the small number of rabbits used in their study (four eyes of four rabbits). They also point out that each rabbit had very different regression coefficients and so combining the data from all the rabbits may not be appropriate. In addition, they did not randomize the order of measurements with the different tonometers within each rabbit, although they did change the sequence between rabbits.

Limitations in our study are also secondary to the small population of subjects. However, our rabbits had similar correlation coefficients and gradients of the regression lines, allowing us to pool the data.

Although all four instruments are not as reliable as desired, they do play a role in animal research, where the main objective is to report a change in IOP rather than an absolute measurement. In this circumstance, of the tonometers we tested, the Perkins handheld applanation tonometer was the most reliable in measuring IOP.

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