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Reference values, intertest correlations, and test-retest repeatability of selected tear film tests in healthy cats

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Objective—To determine reference values, intertest correlations, and test-retest repeatability of Schirmer tear test 1 (STT-1), phenol red thread test (PRTT), tear film breakup time (TFBUT), tear osmolarity, and meibometry in healthy cats.

Design—Evaluation study.

Animals—135 healthy domestic cats aged 0.5 to 12.8 years.

Procedures—Each test was performed once in 120 cats and repeated in 40. Pearson correlation was used to assess correlation among tests. Intraclass correlation coefficients (ICCs) and 95% limits of agreement (LOA) were used to evaluate test-retest repeatability.

Results—Median (95% central range) values were 18 mm/min (9 to 34 mm/min) for STT-1, 29 mm/15 s (15 to 37 mm/15 s) for PRTT, 12.4 seconds (9.1 to 17.7 seconds) for TFBUT, 322 mOsm/L (297 to 364 mOsm/L) for osmolarity, and 32 meibometry units (MU; 11 to 114 MU) for peak meibometry value. The STT-1 and PRTT values were positively correlated. Age was weakly associated with TFBUT and osmolarity. Meibometry measurements were higher for strips that contacted the tear film (285 MU) than for those that touched the eyelid margin only (32 MU). All ICCs were < 0.75, and 95% LOA were wide.

Conclusions and Clinical Relevance—Tear deficiency should be suspected in cats with STT-1 < 9 mm/min, PRTT < 15 mm/15 s, or TFBUT < 9 to 10 seconds. Generally poor correlation among tests suggested that thorough tear film analysis requires performance of multiple tests in concert. Relatively poor test-retest repeatability should be considered when repeated tests are used to monitor tear film dysfunction and response to treatment. (*J Am Vet Med Assoc* 2015;246:426–435)

The surface of the eye is coated by a thin film of tears critical for ocular comfort, optical clarity, and corneoconjunctival health. The tear film keeps the ocular surface moist, provides nutrients and protection against debris and pathogens, helps transport waste away from the ocular surface, and provides a smooth optical surface for refraction.[1,2] The tear film has traditionally been described as a trilaminar structure, consisting of an inner mucinous layer secreted by conjunctival and corneal cells, a middle aqueous layer secreted by the lacrimal glands, and an outer lipid layer composed of meibum and secreted by the meibomian glands.[1] However, the current concept is that the tear film is a bilayered structure, consisting of an inner aqueous-mucinous phase and an outer lipid phase.[3–5] Normal tear film function demands that the volume and physiochemical qualities of all 3 tear film components (individually and relative to each other) remain within an appropriate physiologic range. Disruption of the aqueous component results in quantitative tear film deficiency manifested clinically as KCS (commonly described as dry eye syndrome). Disturbance of the lipid or mucus components of tears (jointly or individually) results in qualitative tear film deficiency, which causes instability and premature evaporation or breakup of the tear film with resultant keratoconjunctival injury.[6]

The diagnosis, treatment, and monitoring of quantitative and qualitative tear film deficiencies require a variety of tear film assays. Ideally, these techniques

Abbreviations

AUC	Area under the curve
CR	Central range
ICC	Intraclass correlation coefficient
KCS	Keratoconjunctivitis sicca
LOA	Limits of agreement
MU	Meibometry unit
PRTT	Phenol red thread test
STT	Schirmer tear test
TFBUT	Tear film breakup time

should be minimally invasive so that the test causes minimal discomfort to the animal and permits data collection from the ocular surface without inducing reflex tearing or otherwise altering the tear film. This is especially important if > 1 test is performed during the same examination. The aqueous component of the tears is commonly assessed on the basis of STTs (STT-1 and STT-2), PRTT, fluorophotometry, meniscometry, and assessment of tear osmolarity.[7,8] Current techniques to characterize meibum include (in order of increasing invasiveness) evaporimetry, interferometry, meibometry, meibum expression with assessment of its physiochemical properties, and meibography.[8,9] Mucins are typically assessed on the basis of TFBUT, biochemical analysis of tears, and cytologic or histologic evaluation of goblet cell density.[8,10–12] Tear assays are constantly evolving, and a diversity of new techniques has recently been described in people, including wavefront aberrometry, corneal topography, advanced imaging (optical coherence tomography and confocal microscopy), and visual acuity testing.[13] Use of these techniques has been increasingly described for people and, to a lesser extent, dogs. However, very few reports exist regarding tear evaluation in cats.

There is increasing evidence that quantitative and qualitative tear film deficiencies are an important cofactor or cause of some of the most common and frustrating ocular diseases of cats, such as KCS,[6,14] chronic nonhealing corneal ulceration,[15] corneal sequestration,[15–17] conjunctivitis,[18–20] and keratoconjunctivitis following feline herpesvirus-1 infection.[19,21] Despite this, reference ranges for many of the tests commonly used to assess the human and canine tear film have not been established for healthy cats. As a result, it is likely that clinically important alterations in tear film health may be undiagnosed or underdiagnosed in cats. Therefore, the primary goal of the study reported here was to establish reference values for diagnostic tests commonly used to assess the tear film, including the STT-1, PRTT, TFBUT, tear osmometry, and meibometry, in healthy cats of various ages and various sex and neuter status combinations. Other aims included evaluation of correlations among these tests and, given that a highly repeatable measurement is essential for evaluating changes attributable to disease or treatment effects, assessment of test-retest repeatability of all 5 tests in a subset of these healthy cats.

Materials and Methods

Study population and design—One hundred thirty-five domestic shorthair cats (270 eyes) were assessed in this study. The study population was composed of 80 female (69 sexually intact and 11 spayed) and 55 male (31 sexually intact and 24 neutered) cats aged between 5 months and 12.8 years, with a median age of 2.6 years (mean ± SD age, 4.2 ± 3.3 years). Animals were included in the study only if they had no known history of ocular or systemic illness and had no signs of adnexal or ocular disease on examination. Cats used in our study were previously involved in various nutritional studies[22,23] that we believe had no impact on ocular surface or tear film health. They were group-housed in cages, with room temperatures maintained between

18° and 24°C and a light-dark cycle of 14 hours of light to 10 hours of darkness. The cats were maintained in a specific pathogen–free facility and were seronegative for feline herpesvirus-1, feline calicivirus, feline coronavirus, FIV, and FeLV. The study was approved by the Institutional Animal Care and Use Committee of the University of California-Davis (protocol No. 17-220).

The STT-1, TFBUT, PRTT, tear osmolarity, and meibometry were each performed for 120 cats (240 eyes) selected from the study population (135 cats); not all cats underwent every test, but each cat underwent ≥ 1 test. All of these tests were completed by the same examiner (LS). Each test was performed separately; to permit the ocular surface to recover from the previous test, the interval between any 2 tests was ≥ 4 hours. To evaluate test-retest repeatability, each test was repeated by the same evaluator under identical conditions 1 week later on 40 of these cats (80 eyes) chosen at random. The sample size required to establish reference values and assess repeatability was selected on the basis of published guidelines.[24–26] On each occasion, cats were manually restrained and all tests were performed on both eyes. The order of eyes tested for each animal and each test was determined with random number generation software.[a] The same order was maintained for the sessions in which tests were repeated.

STT-1—The STT-1 was performed by placing a commercially available standardized test strip[b] within the lateral third of the ventral conjunctival fornix of each eye for all cats. A stopwatch was used to ensure a 60-second time lapse, and tear production was recorded in mm/min. When the entire strip was wetted before 60 seconds had elapsed, values were recorded as 35 mm/min. All STT strips used came from a single lot number.

PRTT—Threads used for the PRTT[c] were 75 mm long and had a bend approximately 3 mm from 1 end. The lower eyelid was gently everted, and the bent portion of the thread was placed into the lateral third of the ventral conjunctival fornix with forceps (**Figure 1**). The thread was removed after 15 seconds, with a stopwatch used for timing. The wetted length of the thread (as indicated by color change from yellow to red) was measured in millimeters from the end of the thread (not the bend) and recorded in mm/15 s.

Tear osmometry—Tear film osmolarity was measured with a proprietary osmometer[d] that included single-use test cards containing microchannels to collect tear fluid (Figure 1); these were held by a pen designed to facilitate tear collection and read with a portable reader[d] that measured tear film osmolarity by electrical impedance. At the beginning of each session, quality control procedures were performed according to the manufacturer's recommendations to confirm function and ensure correct calibration of the system. Tear samples were collected by passive capillary action from the inferior tear meniscus near the lateral canthus, without everting the lower eyelid. Osmolarity readings displayed by the reader were recorded in mOsm/L for both eyes. In addition, temperature (°C) and relative humidity (%) of the room were recorded each time a measurement was made.
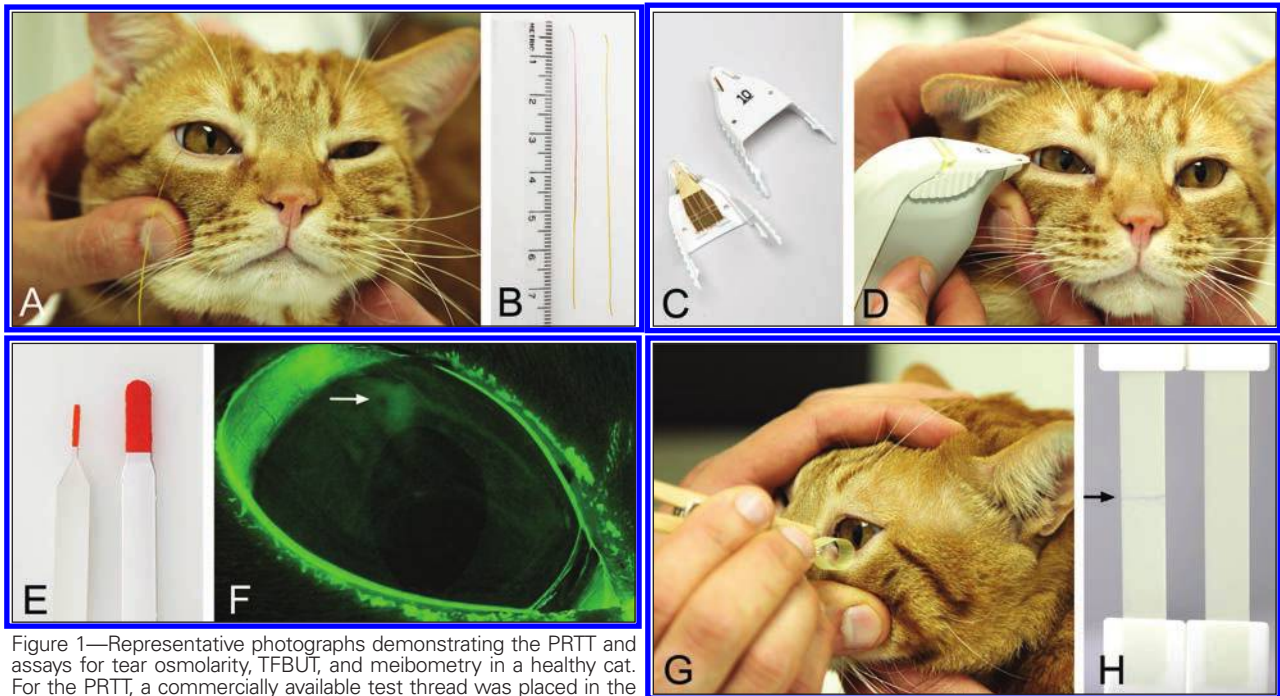
Figure 1—Representative photographs demonstrating the PRTT and assays for tear osmolarity, TFBUT, and meibometry in a healthy cat. For the PRTT, a commercially available test thread was placed in the lateral aspect of the lower conjunctival fornix for 15 seconds (A) and the length of wetted area was measured in millimeters (B). Tear film osmolarity was measured with proprietary, single-use test cards containing microchannels to collect tear fluid (C), and tear samples were collected by passive capillary action from the inferior tear meniscus near the lateral canthus, without everting the lower eyelid (D). For measurement of TFBUT, a small drop of fluorescein was instilled onto the dorsolateral aspect of the bulbar conjunctiva with a proprietary strip (left) that is thinner than conventional fluorescein strips (right; E), and the corneal surface was examined with 16X magnification with light passed through the cobalt blue filter of a slit-lamp biomicroscope to assess the onset of tear film breakup visible as appearance of dark holes (arrow) in the fluorescent tear film (F). For meibometry, a commercially available tape loop was held with a clothespin against the central lower eyelid margin for a few seconds (G) until a translucent lipid line (arrow) was visible on the tape (H). Changes in transparency of the tape resulting from the lipid apposition were then measured photometrically.

TFBUT—A modified fluorescein-impregnated paper strip[e] was used to deliver fluorescein to the ocular surface for the TFBUT (Figure 1). Prior to use, a single drop of eyewash[f] was applied to the fluorescein strip. The strip was then gently shaken until the entire fluorescein strip was moistened and a small droplet of fluorescein was formed at the tip of the strip. The strip then was touched to the dorsolateral bulbar conjunctiva so that the flat side of the strip briefly made contact with the conjunctival surface. After 3 manually controlled blinks, the eyelids were gently held open and the dorsolateral corneal surface was observed with 16X magnification with light passed through the cobalt blue filter of a slit-lamp biomicroscope.[g] The TFBUT was measured as the time from eyelid opening to the first signs of tear film breakup, evident as the appearance of 1 or more dark spots within the fluorescent green tear film. A stopwatch was used to determine 1 measurement/eye, recorded to the nearest tenth of a second.

Meibometry—Samples of meibomian gland secretions were collected and investigated as described by the manufacturer of the meibometer[h] used. First, the lower eyelid was gently everted, and with a clothespin to hold the proprietary meibometer tape, the matte surface of the tape loop was brought into contact with the central lower eyelid margin (Figure 1). The tape was held in place until a translucent lipid line was visible on the tape loop (typically a few seconds). If blinking occurred or if the tape loop came into contact with the lacrimal lake, the measurement was repeated with a new tape loop. How-

ever, a subset of samples contaminated with the more aqueous component of the tears was analyzed, and those meibometry values were compared with results from samples collected without aqueous contamination from the same cats. In all instances, the tape loop was air-dried and changes in transparency of the tape caused by lipid apposition were measured photometrically by the meibometer. The manufacturer recommends air-drying the sample for 5 to 10 minutes before measurement to permit evaporation of the aqueous component of the tear film.[27] To assess the effect of time spent air-drying within this recommended range, a subset of loops was air-dried following sample collection for 5 (n = 45), 6 (31), 7 (27), 8 (10), 9 (2), or 10 minutes (5). In addition, a subset of these tape loops (n = 15) was air-dried for an additional 20 to 25 minutes after being read with the meibometer (so that total time between sample collection and the second reading was 30 minutes). For all analyses, the tape loop was manually withdrawn from the reading window of the meibometer at a steady rate over 8 to 10 seconds (timed with a stopwatch). Results were displayed as peak values and AUCs, in arbitrary units (ie, MUs). To reduce variability, each tape loop was measured 3 times photometrically and the mean of the displayed peak values and AUCs was used for each cat.

Data analysis—Statistical evaluation was performed by use of commercially available software.[a,i,j] For each diagnostic test, results from the right and left eyes were compared by means of a Wilcoxon signed rank test. When no signifi-

cant difference in the distribution of test results was found between eyes, only data from the left eye were used for subsequent analyses because averaging the data from both eyes would add little information and could potentially confound correlation and repeatability analyses.[28] For reference values, normality of the data was assessed with the Shapiro-Wilk test. Nonnormally distributed data are presented as median and 95% CR (2.5th to 97.5th percentile). Joint effects of sex and neuter status (sexually intact male, neutered male, sexually intact female, or spayed female) on test values were evaluated by means of the Kruskal-Wallis test. Effects of age on test values were assessed through linear regression analysis. Pearson correlation coefficients were calculated to analyze linear associations between diagnostic tests. For meibometry, the effect of air-drying time (1-minute intervals from 5 to 10 minutes) on peak meibometry measurements was evaluated by linear regression. The Wilcoxon signed rank test was used to compare meibometry measurements after 5 to 10 minutes and 30 minutes of air-drying and to compare meibometry measurements from strips that contacted the eyelid margin only with those from strips that also came into contact with aqueous tear film.

A comprehensive panel of tests was used to assess the test-retest repeatability of STT-1, PRTT, TFBUT, tear osmometry, and meibometry. To evaluate for systematic bias across both sessions, the paired t test or Wilcoxon signed rank test was used to compare the means or dis-

tributions, respectively, of test and retest measures.[29] To evaluate the relative reliability of each test, ICCs[30,31] were calculated with commercial software.[j] Interpretation of the ICCs was conducted in accordance with suggestions of Portney and Watkins,[32] whereby values > 0.75 indicated good reliability, values between 0.40 and 0.75 implied moderate reliability, and values < 0.40 suggested poor reliability. To evaluate the absolute reliability of each test, 95% LOA were calculated as the mean difference ± 1.96 × SD (differences), and the differences between test-retest measures and their means were graphically displayed by the creation of Bland-Altman plots.[33] For all analyses, values of $P \le 0.05$ were considered significant.

Results

Reference values—No significant (range of P values, 0.08 to 0.63) difference was detected between right and left eyes for any test. Therefore, only values obtained from left eyes were analyzed. Data were not normally distributed for any test ($P < 0.05$), so all results are presented as median (95% CR). Reference values expressed as median (95% CR) were 18 mm/min (9 to 34 mm/min) for STT-1, 29 mm/15 s (15 to 37 mm/15 s) for PRTT, 12.4 seconds (9.1 to 17.7 seconds) for TFBUT, 322 mOsm/L (297 to 364 mOsm/L) for tear osmolarity, and 32 MU (11 to 114 MU) for peak meibometry value (**Table 1**; **Figure 2**). Meibom-

Table 1—Reference values and test-retest repeatability of STT-1, PRTT, TFBUT, tear osmolarity, and meibometry determined in a study of 135 healthy domestic shorthair cats.

Variable	STT-1 (mm/min)	PRTT (mm/15 s)	TFBUT (s)	Osmolarity (mOsm/L)	Meibometry (MU)
Reference value (n = 120)	18 (9 to 34)	29 (15 to 37)	12.4 (9.1 to 17.7)	322 (297 to 364)	32 (11 to 114)
Test-retest repeatability (n = 40)					
95% LOA	−11 to 11	−10 to 13	−6.2 to 5.9	−39 to 57	−52 to 66
ICC*	0.44 (0.15 to 0.66)	0.19 (−0.13 to 0.47)	0.20 (−0.12 to 0.48)	0.19 (−0.12 to 0.47)	0.51 (0.24 to 0.71)

Each test was performed on 120 of 135 cats without signs of ocular disease (not every cat had every test performed). The interval between any 2 tests was ≥ 4 hours. Reference values are presented as median (95% CR [ie, 2.5th to 97.5th percentiles]). Measurements were repeated 1 week later for 40 randomly selected cats; test-retest repeatability is indicated by the 95% LOA and the ICC (95% confidence interval).
*Intraclass correlation coefficients were interpreted as representing poor (< 0.4), moderate (0.4 to 0.75), or good (> 0.75) test reliability.[32]
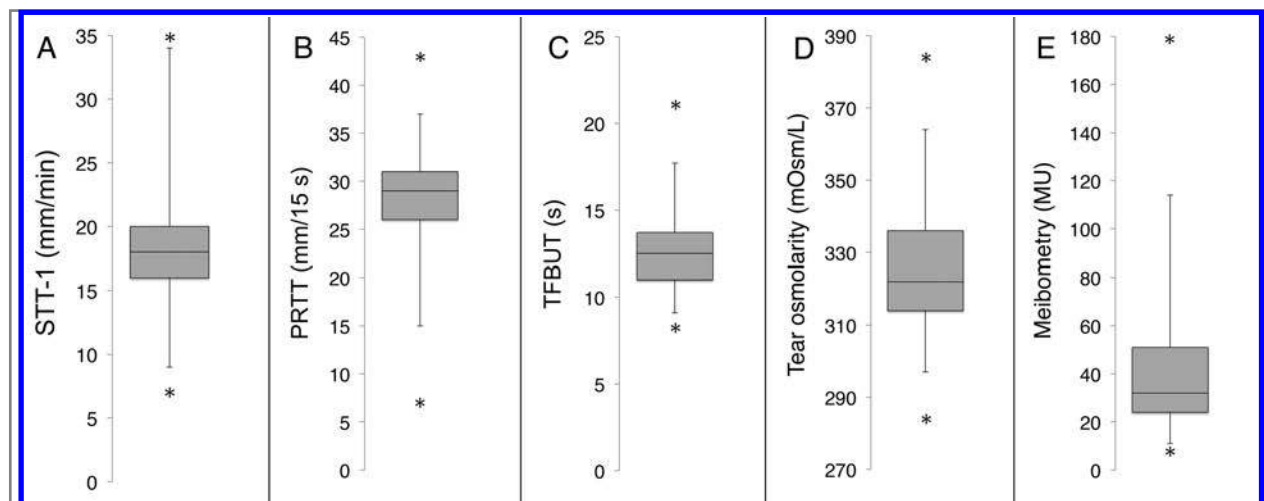

Figure 2—Box-and-whisker plots for the STT-1 (A), PRTT (B), TFBUT (C), tear osmolarity (D), and peak meibometry values (E) in 120 healthy cats. Median values are shown by a horizontal line. First and third quartiles (25th and 75th percentiles) are represented by the lower and upper limits of the box, respectively. The 2.5th and the 97.5th percentiles are shown as the lower and upper whiskers, respectively, and minimum and maximum values are indicated (asterisk).
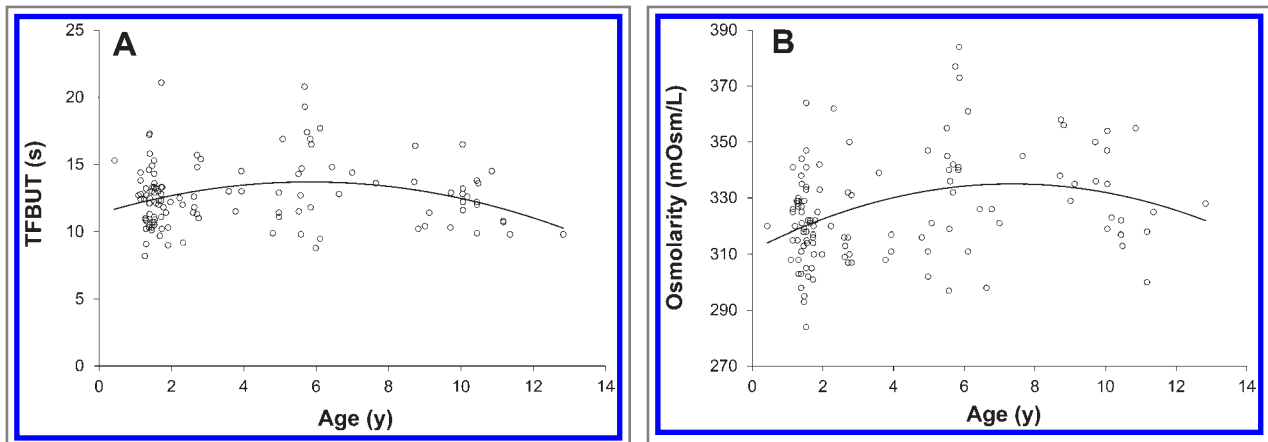
Figure 3—Effect of age on TFBUT (A) and tear osmolarity (B) in 120 healthy cats. The parabolic curve represents a second-order polynomial regression. Measurements for both tests significantly increased until approximately 7 years of age, then decreased with advancing age.

etry AUCs ranged widely (13 to 483 MU) with a median (95% CR) value of 104 MU (21 to 416 MU). A significant association was not detected between peak meibometry value and drying time between 5 and 10 minutes ($r = 0.15$, $P = 0.094$). Similarly, median (95% CR) peak meibometry values obtained from samples remeasured after 30 minutes of air-drying (31 MU; 10 to 150 MU) were not significantly ($P = 0.25$) different from those measured after 5 to 10 minutes (27 MU; 10 to 136 MU). However, peak meibometry measurements were significantly ($P = 0.001$) higher for strips that came in contact with the aqueous component of the tear film (285 MU; 55 to 466 MU) than for strips that touched the eyelid margin only (32 MU; 11 to 114 MU).

Age of cats did not significantly affect the values for STT-1 ($r = 0.16$; $P = 0.083$), PRTT ($r = 0.01$; $P = 0.88$), or peak meibometry ($r = 0.11$; $P = 0.24$). A second-order polynomial regression showed a positive but weak association between age and TFBUT values ($r = 0.25$; $P = 0.006$) and between age and tear osmolarity values ($r = 0.34$; $P = 0.003$). For both tests, values had a tendency to increase until approximately 7 years of age and then decrease with age (**Figure 3**). A significant effect of sex and neuter status on STT-1 and peak meibometry values was not detected. However, neutered males had significantly ($P = 0.03$) longer median (95% CR) TFBUTs (13 seconds; 9.7 to 20.1 seconds), compared with values of sexually intact females (11.9 seconds; 8.9 to 16.5 seconds). Sexually intact males had significantly ($P = 0.02$) higher median (95% CR) PRTT results (31 mm/15 s; 24 to 36 mm/15 s) than did sexually intact females (29 mm/15 s; 12 to 37 mm/15 s) and spayed females (24 mm/15 s; 20 to 31 mm/15 s). Sexually intact males had significantly ($P < 0.001$) lower tear osmolarity (315 mOsm/L; 294 to 339 mOsm/L) than did neutered males (340 mOsm/L; 300 to 380 mOsm/L), spayed females (332 mOsm/L; 317 to 358 mOsm/L), and sexually intact females (325 mOsm/L; 302 to 359 mOsm/L). Median (95% CR) difference in measurements between the left and right eyes of the same cat recorded during the same session was as follows: STT-1, 3 mm/min (0 to 15 mm/min); PRTT, 3 mm/15 s (0 to 13 mm/15 s); TFBUT, 1.6 seconds (0 to 5.6 seconds); tear
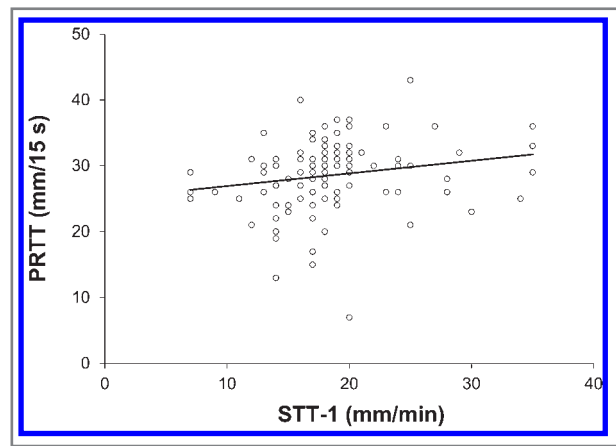

Figure 4—Results of Pearson correlation testing for STT-1 and PRTT results in 120 healthy cats. A weak positive correlation ($r = 0.19$; $P = 0.049$) was found.

osmolarity, 12 mOsm/L (1 to 51 mOsm/L); peak meibometry value, 15 MU (1 to 104 MU); and meibometry AUC, 71 MU (4 to 301 MU). Ambient temperature and humidity during recording sessions ranged from 20° to 23°C and 24% to 34%, respectively.

Intertest correlations—Results of the STT-1 and PRTT had a weak positive correlation ($r = 0.19$; $P = 0.049$; **Figure 4**). Peak and AUC values for meibometry were also positively correlated ($r = 0.68$; $P = 0.001$). However, no significant correlations were detected between any other test pairs (range of P values, 0.11 to 0.91).

Test-retest repeatability—No significant difference was detected between median (95% CR) test values obtained at 2 sessions 1 week apart for STT-1 (19 mm/min [12 to 30 mm/min] and 18 mm/min [15 to 35 mm/min]; $P = 0.44$), PRTT (28 mm/15 s [21 to 37 mm/15 s] and 27 mm/15 s [19 to 34 mm/15 s]; $P = 0.16$), TFBUT (12.3 seconds [9.8 to 17.2 seconds] and 12.6 seconds [8.1 to 16.6 seconds]; $P = 0.75$), or peak meibometry (36 MU [13 to 164 MU] and 35 MU [10 to 86 MU]; $P = 0.41$). However, tear osmolarity recorded
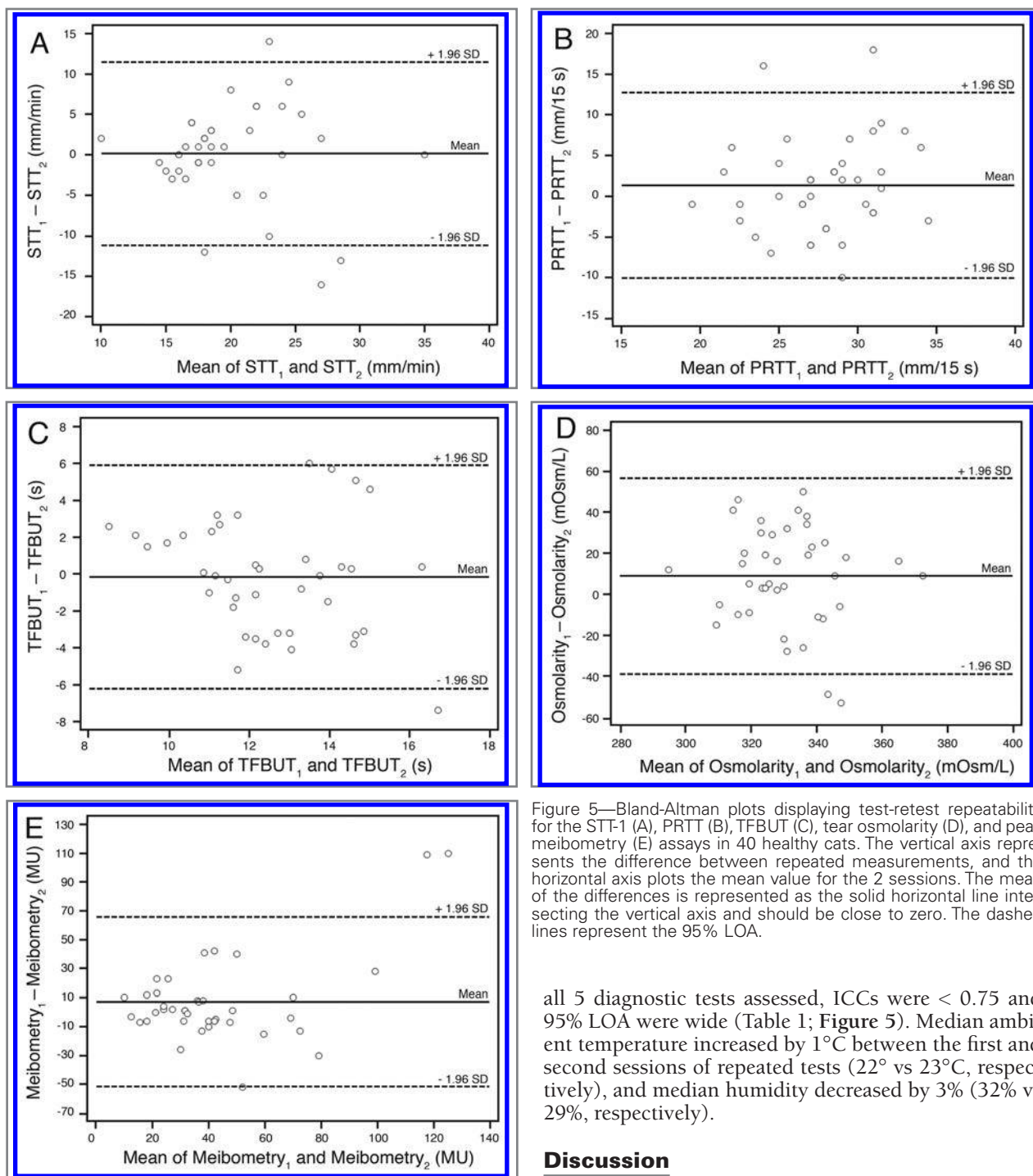
Figure 5—Bland-Altman plots displaying test-retest repeatability for the STT-1 (A), PRTT (B), TFBUT (C), tear osmolarity (D), and peak meibometry (E) assays in 40 healthy cats. The vertical axis represents the difference between repeated measurements, and the horizontal axis plots the mean value for the 2 sessions. The mean of the differences is represented as the solid horizontal line intersecting the vertical axis and should be close to zero. The dashed lines represent the 95% LOA.

all 5 diagnostic tests assessed, ICCs were < 0.75 and 95% LOA were wide (Table 1; **Figure 5**). Median ambient temperature increased by 1°C between the first and second sessions of repeated tests (22° vs 23°C, respectively), and median humidity decreased by 3% (32% vs 29%, respectively).

Discussion

To our knowledge, the present study describes for the first time the results of 5 common tear film tests performed by 1 operator for > 120 healthy domestic cats of various ages and sex and neuter status combinations; in addition to establishing reference values, the present study assessed test-retest repeatability of these same 5 tests in healthy cats. Throughout the study, several precautions were taken to reduce bias and increase the reliability of the data obtained: only 1 investigator (LS) performed all measurements to eliminate interexaminer variability, measurements were performed in the same controlled environment, and sufficient time was

during the second session (323 mOsm/L; 293 to 368 mOsm/L) was significantly (P = 0.027) lower than that recorded in the first session (335 mOsm/L; 302 to 373 mOsm/L), indicating a systematic bias.

Median difference in median (95% CR) measurements from the same eye at different sessions was as follows: STT-1, 3 mm/min (0 to 14 mm/min); PRTT, 3 mm/15 s (0 to 16 mm/15 s); TFBUT, 2.2 seconds (0.1 to 6.0 seconds); tear osmolarity, 19 mOsm/L (3 to 50 mOsm/L); peak meibometry value, 10 MU (1 to 95 MU); and meibometry AUC, 52 MU (8 to 318 MU). For

allowed between tests to ensure that the ocular surface had recovered from any effects of the previous test. In addition, the STT-1 was performed with 1 lot number of commercially available strips for all cats, given that results can be affected by absorptive capacity of the test strip used.[34,35] Although this controlled environment likely enhanced repeatability of the values generated within this population, data may differ for other test conditions and different feline populations, especially brachycephalic cats.

The median STT-1 value in the present study (18 mm/ min; 95% CR, 9 to 34 mm/min) is similar to previously reported values (14.3 to 20.2 mm/min) for cats.[20,36–40] Unlike dogs, in which tear production decreases with increasing age[41] but is unchanged by sex,[41,42] we found no effect of age or sex on this measure in the feline population tested. Despite the relative consistency of results among studies reporting mean STT-1 values of cats, it is unclear what the lower limit for STT-1 values should be in healthy cats. Applying a cutoff of 2 SDs below the reported mean (16.92 mm/min) for 50 cats, Veith et al[36] suggested that STT-1 values < 6 mm/min should be considered abnormal. Data from the present study suggested that STT-1 values < 9 mm/min (the 2.5th percentile) together with clinical signs of KCS would be supportive of aqueous tear deficiency. The diagnosis of KCS in cats is further complicated by textbook claims that healthy cats can have STT-1 values as low as 0 mm/ min.[43–45] However, no healthy cat in the present study had an STT-1 value < 7 mm/min. Additionally, it has been asserted that low STT values in cats may result from stress-induced increased sympathetic tone causing a temporary reduction in tearing during testing.[19,20] However, although not quantified in the present study, it was our impression that cats that were anxious or more resistant to restraint had a relatively rapid wetting of the STT strip (and not a decrease), which would not support that speculation.

The median (95% CR) PRTT value for cats in the present study was 29 mm/15 s (15 to 37 mm/15 s), with significantly higher values for sexually intact males than for sexually intact and spayed females. Brown et al[37] reported approximately similar PRTT values for healthy cats (median, 23 mm/15 s; 95% CR, 18 to 28 mm/15 s) but did not analyze differences attributable to sex or neuter status. In people, there is extensive evidence that androgenic hormones promote lacrimal function.[46] Our PRTT data, but not our STT-1 data, suggest this may also be the case in cats. In the present study, the PRTT was easily and rapidly performed, and subjectively, it appeared to be better tolerated than the STT-1. Further, the PRTT is believed not to cause tear instability and therefore may interfere minimally with subsequent tear film tests performed during the same examination.[47] Finally, assessment of in vitro variation in absorptive capacity of phenol red threads suggests that they are more reliable than STT strips.[35,48] Taken together, these data and findings from the present study suggest that the PRTT may be a useful alternative to STT-1 for measurement of aqueous tear production in healthy cats, as has been reported for humans. However, the present study revealed only a weak correlation between PRTT and STT-1 values, similar to results described for hu-

mans.[49,50] Although both tests are believed to measure the aqueous component of the tear film, the PRTT is believed principally to measure residual tear film volume of the inferior conjunctival fornix, whereas STT-1 is thought to measure residual tear volume and reflex tear production.[7] Therefore, despite evidence in humans that reflex tearing persists even when topical anesthesia is used (as measured by use of the STT-2),[51] it would be interesting to compare the correlation between PRTT and STT-2 values in cats. Although the PRTT classically relies on yellow cotton thread turning to orange or light red when wetted by alkaline tears,[52] threads in the present study did not always change color. Regardless, the length of the wetted thread could still easily be determined when this occurred.

The TFBUT is recommended as a noninvasive test to aid in the diagnosis of qualitative tear film abnormalities.[6] Rapid TFBUT suggests an unstable tear film and has been reported for cats with conjunctivitis,[18–20] chronic nonhealing ulcers,[15] and corneal sequestra[15,16] as well as those previously infected with FHV-1.[19] In the present study, TFBUT (median, 12.4 seconds; 95% CR, 9.1 to 17.7 seconds) was shorter than TFBUTs reported in other studies[16,18,19,38] (mean, 13.5 to 21 seconds). This may be attributable to differences in the number of eyes tested, age range of cats tested, interexaminer variation (especially given the subjectivity of this assay), number of measurements made each time, the source and volume of fluorescein used, and method of application. In the present study, we used a fluorescein strip modified specifically for this purpose in humans,[e] which has a notably smaller surface area (10 mm^2) than do standard fluorescein strips (50 to 75 mm^2). Results of studies in humans suggest that TFBUTs are dependent on the volume of fluorescein solution instilled on the ocular surface. For example, the amount of fluorescein delivered to the ocular surface when a 10- or 75-mm^2 fluorescein strip is used is approximately 1 or 3 μL of fluorescein 2% solution, respectively,[53] and TFBUT is significantly lengthened by this increase in volume delivered with the larger strip.[54] In addition, the test strips used in our study[e] improve measurement reliability and enhance measurement precision, compared with a conventional fluorescein strip,[55] thus eliminating the need to average the results of multiple, consecutive TFBUT measurements.[16,18,38] Differences in study methodology notwithstanding, most healthy cats have been reported to have TFBUTs > 10 seconds,[16,18,19,38] which is comparable to findings from the present study, in which only 2.5% of this healthy population had TFBUTs < 9.1 seconds. On the basis of data from the present and previous studies, tear film instability should be suspected in cats with TFBUTs < 9 or 10 seconds.

Tear osmolarity is principally determined by the concentration of solutes in the aqueous component of the tear film; electrolytes play a major role, and proteins and sugars are minor contributors.[56] Imbalances between tear production and elimination cause tear film instability and hyperosmolarity in humans[57,58] and rabbits.[59] As a result, tear osmolarity has been recommended as an objective clinical measurement of the balance among these complex tear film dynamics.[57] In humans, tear film hyperosmolarity is considered a piv-

otal pathophysiologic factor in dry eye disease,[46] with healthy subjects having low and stable tear osmolarity values and subjects with dry eyes having high and less stable values.[60,61] Healthy humans have a median tear osmolarity of 301 mOsm/L, with a 50% CR (25th to 75th percentile) of 298 to 304 mOsm/L.[62] Repeated measurements in healthy human subjects have little variability.[60] Results of the present study revealed that tear osmolarity in healthy cats (median, 322 mOsm/L; 95% CR, 297 to 364 mOsm/L) is higher than that in humans but similar to that reported in a study[20] of 74 eyes from healthy cats (mean ± SD, 328.5 ± 17.94 mOsm/L). However, in the present study, difference in osmolarity was as high as 74 mOsm/L between the left and right eyes of the same cat recorded at the same session and as high as 53 mOsm/L for the same eye at separate sessions 7 days apart. Differences in tear osmolarity recorded between both sessions (systematic bias) may be related to instrument or individual variations, as changes in room temperature and relative humidity were minimal, and an in vivo study[63] in people failed to establish a significant correlation between environmental factors and tear osmolarity. Furthermore, tear osmolarity has not been shown to differ between cats with (mean ± SD, 328.5 ± 17.94 mOsm/L) and cats without (mean ± SD, 325.0 ± 24.84 mOsm/L) conjunctivitis.[20] Taken together, these data suggest that inherent variability in feline tear osmolarity or in this assay reduces its diagnostic benefit in cats.

Meibometry was assessed in the present study as a novel and noninvasive approach to quantify lipid content at the lid margins in a population of healthy cats. To the authors' knowledge, this is the first peer-reviewed report of meibometry in cats, although a conference proceedings described data from 16 cats.[k] The median peak meibometry value in our healthy feline population was 32 MU (95% CR, 11 to 114 MU), which is notably lower than reported mean values in cats (67.6 MU),[k] dogs (179 to 211 MU),[64,65] and humans (250 to 268 MU).[66,67] Similar to findings in dogs and humans, meibometry results in the present study did not vary significantly between male and female cats. The variability among reported meibometry values may be explained by differences in species investigated, instrument used, and methodology among studies.[64–67,k] Given the novelty of this technique in veterinary medicine, the present study was designed to assess some of these variations in methodology. Our data suggest that it is critical to determine whether the tape strip is contaminated by contact with the aqueous component of the tear film. In the present study, median peak meibometry values for strips that touched the lacrimal lake (285 MU) were more similar to normative data described for dogs and humans. Avoidance of aqueous tear contamination was best achieved by tilting the cat's head slightly downward (approx 20° from horizontal plane), gently everting the lower eyelid and applying sufficient pressure to obtain an imprint across the width of the tape without bending the handle of the loop, and holding the tape loop in place for a short period of time (generally < 5 seconds). Although time the tape was allowed to air-dry ranged widely in other studies (none,[67] 1 minute,[65] 3 minutes,[66] or 30 minutes[64]), our

data suggest that this is unlikely to have contributed to data variability among studies. A final likely source of variation among and within studies is the rate at which the tape loop is withdrawn from the meibometer during the reading; per the user guide recommendation, this should be done at slow and similar speeds to obtain reproducible and comparable measurements.[27] Unfortunately, this process is not automated and presumably introduces another source of variability.

Perhaps one of the most clinically important findings from the present study was the poor test-retest repeatability for all of the diagnostic assays assessed, as has been reported in humans.[68–70] A repeated measurement can vary from the initial value for a variety of reasons, including variation in the diagnostic test or instrument, environmental factors (eg, ambient temperature, humidity, and noise), psychological factors (eg, patient anxiety), and examiner consistency. However, repeatable measurements are a prerequisite for evaluating changes in clinical patients due to disease progression or therapeutic effects. Test-retest repeatability defines the extent to which a measurement is free from random error and is expressed as relative reliability or absolute reliability.[71] Relative reliability indicates to what extent repeated measurements from the same individuals will be consistently positioned or ranked within the test group and was evaluated in the present study by ICC. Absolute reliability evaluates to what degree repeated measurements vary for individuals and was evaluated on the basis of 95% LOA and Bland-Altman plots. In general, the higher the ICC and the narrower the LOA, the more reliable the measurement, with ICC > 0.75 reported to indicate good reliability.[32] The ICCs for the 5 tear film tests performed in this study were all below this preferred value. The relatively wide 95% LOA we found for all tear assays in the present study also suggests that the tests were of low repeatability in the feline population tested. The 95% LOA for the STT-1 from the present study (–11 to +11 mm/min) can be used to exemplify this. Assuming that a value of 20 mm/min is obtained for the STT-1 in a healthy cat, a repeated measurement in the same cat would be predicted by our data to lie between 9 and 31 mm/min for 95% of measurements. Any value included in this interval should be considered measurement error and not represent a true change in STT-1 measurements caused by a change in a cat's condition. Whether the same LOAs apply in diseased animals is not known; however, such information is critical to interpreting test results when they are used to monitor disease progression or response to treatment.

Data from the present study were used to develop reference values in healthy cats for the STT-1, PRTT, TFBUT, tear osmometry, and meibometry. Poor correlation among results of these 5 assays in healthy cats suggests that a complete assessment of tear film function requires that results of > 1 test be assessed in concert in cats. However, poor test-retest repeatability for all assays tested in healthy cats suggests that the use of any of these assays for the monitoring of disease progression or efficacy of therapeutic interventions may not be reliable. Therefore, evaluation of test values, correlation, and test-retest reliability in cats with ocular surface disease is warranted because, in humans, tear film diag-

nostic tests are often more reliable in individuals with quantitative or qualitative tear film deficiencies.[72,73]

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a. Excel, Microsoft Corp, Redmond, Wash.
b. Merck Animal Health, Millsboro, Del.
c. Zone-Quick, FCI Ophthalmics Inc, Pembroke, Mass.
d. TearLab system, OcuSense Inc, San Diego, Calif.
e. DET, Akorn Inc, Lake Forest, Ill.
f. Eye Wash, OCuSOFT Inc, Richmond, Tex.
g. Kowa SL15, Kowa Corp, Naka-ku, Nagoya, Japan.
h. Meibometer MB 560, Courage-Khazaka Electronic GmbH, Cologne, Germany.
i. SigmaPlot, version 11.0, Systat Software Inc, San Jose, Calif.
j. MedCalc, version 12.5, MedCalc Software, Ostend, Belgium.
k. Wenzel A, Mueller JK, Eule C. Meibometry: a reliable tool for feline ophthalmology? (poster presentation). European College of Veterinary Ophthalmologists Meeting, Versailles, France, 2008.

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From this month's *AJVR*

Effects of fentanyl on isoflurane minimum alveolar concentration in New Zealand White rabbits (*Oryctolagus cuniculus*)

Linda S. Barter et al

Objective—To determine effects of increasing plasma fentanyl concentrations on the minimum alveolar concentration (MAC) of isoflurane in rabbits.

Animals—6 adult female New Zealand White rabbits (*Oryctolagus cuniculus*).

Procedures—Rabbits were anesthetized with isoflurane in oxygen; ventilation was controlled and body temperature maintained between 38.5° and 39.5°C. Fentanyl was administered IV by use of a computer-controlled infusion system to achieve 6 target plasma concentrations. Isoflurane MAC was determined in duplicate by use of the bracketing technique with a supramaximal electrical stimulus. Blood samples were collected for measurement of plasma fentanyl concentration at each MAC determination. The MAC values were analyzed with a repeated-measures ANOVA followed by Holm-Sidak pairwise comparisons.

Results—Mean ± SD plasma fentanyl concentrations were 0 ± 0 ng/mL (baseline), 1.2 ± 0.1 ng/mL, 2.2 ± 0.3 ng/mL, 4.4 ± 0.4 ng/mL, 9.2 ± 0.4 ng/mL, 17.5 ± 2.6 ng/mL, and 36.8 ± 2.4 ng/mL. Corresponding mean values for isoflurane MAC were 1.92 ± 0.16%, 1.80 ± 0.16%, 1.60 ± 0.23%, 1.46 ± 0.22%, 1.12 ± 0.19%, 0.89 ± 0.14%, and 0.70 ± 0.15%, respectively. Isoflurane MAC for plasma fentanyl concentrations ≥ 2.2 ng/mL differed significantly from the baseline value. In 3 rabbits, excessive spontaneous movement prevented MAC determination at the highest targeted plasma fentanyl concentration.

Conclusions and Clinical Relevance—Fentanyl reduced isoflurane MAC by approximately 60% in New Zealand White rabbits. Further studies will be needed to investigate the cardiorespiratory effects of isoflurane and fentanyl combinations in rabbits; however, fentanyl may prove to be a useful adjunct to inhalation anesthesia in this species. (*Am J Vet Res* 2015;76:111–115)

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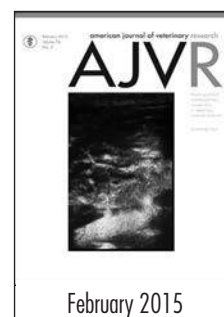
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