Tear fluid collection in dogs and cats using ophthalmic sponges

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ABSTRACT

Objective - To compare the use of two ophthalmic sponges for tear collection in dogs and cats.

Animals studied - Ten healthy dogs and 10 healthy cats.

Procedures - A strip (4x10 mm) of either cellulose or polyvinyl acetal (PVA) sponge was inserted into the ventral fornix of each eye for either 15, 30 or 60 seconds. The wetted strip was placed into a 0.2-mL tube that was first punctured at its bottom. Tears were eluted through the drainage hole into a 1.5-mL tube via centrifugation. Tear volume absorbed (VA) and tear volume recovered (VR) were calculated as the difference of the post- and pre-collection weight of the 0.2-mL tube and 1.5-mL tube, respectively. Recovery ratio (RR) was determined as the ratio between VR and VA.

Results - Ophthalmic sponges were well tolerated by all subjects. In dogs and cats, median (95% range) VA, VR and RR were: 44 µL (11-106 µL) and 16 µL (2-43 µL); 27 µL (1-84 µL) and 6 µL (0-29 µL); 64 % (7-91%) and 35% (0-86%), respectively. PVA sponges achieved significantly greater VR in cats and RR in both species. All parameters were significantly greater with a collection time of 60 vs. 30 and 15 seconds. Body weight was associated with VA and VR in dogs but not cats.

Conclusions – PVA is better than cellulose for tear collection given its superior recovery. Ophthalmic sponges could facilitate routine analysis of tear fluid in dogs and cats, although further studies are needed to evaluate the quality of tears obtained with this method.

Key Words: Tears, cellulose, PVA, absorption, recovery, canine, feline
INTRODUCTION

Tear fluid is an exciting and growing area for clinical research. In addition to fulfilling multiple functions on the ocular surface (e.g. lubrication, nutrition, and defense against infection), tears can provide critical data for the clinician and scientist. Among many other applications, tear fluid can be examined for drug pharmacokinetics\textsuperscript{1,2} discovery of biomarkers in ocular diseases (e.g. dry eye disease)\textsuperscript{3} and systemic diseases (e.g. cancer).\textsuperscript{4,5}

Microcapillary glass tubes and Schirmer strips, the two most popular tear collection methods in humans,\textsuperscript{6} have also been described in animals,\textsuperscript{1,5,7} but present several drawbacks. Microcapillary tubes require several minutes of collection to obtain small tear volumes and can result in trauma, especially in uncooperative veterinary patients. Schirmer strips entail post-collection elution and additional processing steps that could negatively impact the quality of tear analysis.\textsuperscript{8}

The optimal method for tear collection in animals should be safe, minimally invasive, rapid and cost-effective. Ophthalmic surgical sponges fulfill all of these criteria as they are developed for use on the ocular surface, have a rapid and large absorptive capacity, are relatively inexpensive and, following centrifugation, provide tears that are directly available for analysis. Most ophthalmic sponges are made of either cellulose or polyvinyl acetal (PVA).\textsuperscript{9} Cellulose is a natural hydrophilic material, while PVA is a bioengineered polymer formed by hydrolysis of polyvinyl acetate (hydrophobic) into polyvinyl alcohol (hydrophilic) and subsequent acetalization.\textsuperscript{10,11} The variable affinity of these materials for water impacts their ability to absorb tear fluid, but could also interfere with the subsequent release and recovery of the fluid.\textsuperscript{12}

An efficient and non-invasive method for obtaining tear fluid is an essential prerequisite for tear analysis to be implemented more routinely in veterinary medicine. The present study aims to compare the use of cellulose and PVA ophthalmic sponges for tear collection in dogs and cats.
We hypothesize that this method is safe and efficient in retrieving adequate volume of tears. Further, we assume that cellulose sponges provide greater absorption of tears in situ, but lesser recovery of tear fluid post-centrifugation compared with PVA sponges.

**MATERIAL AND METHODS**

**Animals** - Ten dogs (20 eyes) and 10 cats (20 eyes) were enrolled in the study. Prior to study inclusion, all subjects were confirmed to have normal Schirmer tear test values (≥15 mm/min in dogs, ≥9 mm/min in cats) and no evidence of ocular surface disorder by slit-lamp examination (SL-17; Kowa Company, Ltd., Tokyo, Japan) and fluorescein staining. The study was approved by the Institutional Animal Care and Use Committee of Iowa State University.

**Tear collection** - Three durations (15 seconds, 30 seconds, 60 seconds) and two types of ophthalmic sponges were compared: one cellulose-based (Weck-Cel® Sponge, Points and Strips, Beaver-Visitec catalog # 000866), and one PVA-based (Ultracell® Eye Wick, Beaver-Visitec catalog # 40431). Each eye of each subject underwent 6 experiments, i.e. one from each combination time/sponge. The order of testing was randomized (Excel 2016, Microsoft, Redmond, Washington) and successive collections in the same eye were separated by ≥ 10 minutes to permit return to basal tearing status. This duration between successive tests was chosen based on reports of tear turnover time in other species (3-7 min in humans, 7 minutes in horses) and the knowledge that only 3.39% of fluorescein remained on the ocular surface of control canine eyes 5 minutes after fluorescein instillation.

Weck-Cel® and Ultracell® sponges came prepackaged as 8 x 35 mm and 4 x 170 mm strips, respectively. All sponges were cut into 4 x 10 mm strips using clean surgical blades. The 10-mm
length of the strip was chosen as a compromise between maximizing the absorption potential while taking into account the ease of strip insertion and retention in the ventral conjunctival fornix of dogs and cats.

The bottom of 0.2-mL Eppendorf tubes (Eppendorf® thin-walled PCR tubes, Thermo Fischer Scientific, Pittsburgh, PA) was manually punctured using 18-gauge needle to create a drainage hole, and a single sponge strip was placed in each 0.2-mL tube. For each eye, the sponge strip was inserted in the ventral conjunctival fornix using a disposable plastic tweezer (Evident®, Union Hall, VA) – a process facilitated by manually retracting the lower eyelid and, in some cases, elevating the third eyelid via globe retropulsion (Figure 1). A stopwatch was used to accurately measure the duration of tear collection (15, 30 or 60 seconds), at which time the wetted sponge was removed from the eye and sealed into the corresponding 0.2-mL tube.

For extraction of tear fluid, the 0.2-mL tube containing the wetted sponge was transferred to a 1.5-mL Eppendorf tube (Eppendorf® safe-lock micro test tubes, Eppendorf North America Inc., Westbury, NY) and the combination was spun at 6000 rpm for 1 minute in a centrifuge (MyFuge™ Mini, Benchmark Scientific Inc., Sayreville, New Jersey). The centrifugal force pulls the tear fluid out of the sponge, through the hole in the bottom of the smaller tube and into the larger tube. The duration of centrifugation (1 minute) was selected based on a pilot study showing no further fluid elution after 60 seconds (data not shown).

Assuming tear fluid density equaled 1 g/mL, tear volume absorbed (VA) was calculated as the difference of the post- and pre- collection weight of the 0.2-mL tube containing the sponge, while tear volume recovered (VR) was calculated as the difference of post- and pre- centrifugation weight of the 1.5-mL tube. Weights were determined to the nearest 0.001 g (Gemini-20, American Weight Scales Inc., Norcross GA).
Recovery ratio (RR), expressed as a percentage, was determined as the ratio between volume recovered and volume absorbed. Fluorescein dye was instilled onto the surface of each eye at completion of all tests, and cobalt blue light was used to evaluate for conjunctival or corneal ulceration.

**Data analysis** - The Shapiro-Wilk test was used to assess the normality of distribution of investigated parameters (VA, VR, RR). Differences between the right vs. left eyes, dogs vs. cats, cellulose vs. PVA sponges, 15 vs. 30 seconds, 15 vs. 60 seconds and 30 vs. 60 seconds were evaluated using the Mann-Whitney U test. The Spearman’s rank correlation was used to test the association among the three investigated parameters (VA-VR, VA-RR, VR-RR), as well as the association between each of these parameters with Schirmer test values and body weight. Statistical analysis was performed using Sigmaplot version 13.0 (Systat Software Inc, San Jose, CA). The values $P < 0.05$ were considered statistically significant.

**RESULTS**

In dogs, mean ± SD (range) weight, age and Schirmer test values were 19.3 ± 14.4 kg (2.2-58 kg), 6.5 ± 3.6 years (2-13 years) and 21.7 ± 2.9 mm/min (18-28 mm/min), respectively. There were 7 spayed females and 3 male neutered dogs, and various breeds were represented: Border Collie cross (n=2), Greyhound, Great Dane, Australian Shepherd, Pitbull terrier, Yorkshire terrier, Pembroke Welsh Corgi, West Highland white terrier and Labrador Retriever. In cats, mean ± SD (range) weight, age and Schirmer test values were 4.9 ± 1.1 kg (3.7-7 kg), 5.0 ± 4.0 years (0.7-13 years) and 18.6 ± 3.3 mm/min (15-25 mm/min), respectively. There were 2 spayed females and 9
male neutered cats, and breeds included Domestic Short Hair (n=7), Domestic Medium Hair (n=2), Domestic Long Hair (n=1).

The collection method was well tolerated by all subjects. Although slight irritation was noted in a few eyes during tear collection, it was mild and transient. No subject developed a corneal or conjunctival ulcer, despite a total of 240 tear collections performed throughout the study.

No significant difference (range of $P$ values, 0.43 to 0.95) was detected between right and left eyes for any parameter evaluated (VA, VR, RR) in both species. Therefore, the average of values obtained from both eyes was used for further analysis. Data were not normally distributed for any test ($P < 0.05$), so all results are presented as median and 95% range (2.5-97.5th percentiles).

Overall, irrespective of the sponge type or collection duration, median (95% range) of the parameters evaluated were as follows: VA = 44 µL (11-106 µL) in dogs and 16 µL (2-43 µL) in cats; VR = 27 µL (1-84 µL) in dogs and 6 µL (0-29 µL) in cats; RR = 64 % (7-91%) in dogs and 35% (0-86%) in cats. Dogs had a significantly greater VA, VR and RR compared to cats ($P < 0.001$), although values varied widely between one dog to another. For instance, the lightest dog examined (Yorkshire terrier, 2.2 kg) had a median VA, VR and VR of 16 µL, 4 µL and 36%, respectively, while the heaviest dog examiner (Great Dane, 58 kg) had a median VA, VR and RR of 71 µL, 58 µL and 79%, respectively.

The effects on sponge type on VA, VR and RR are shown in Figure 2. In dogs (Figure 2A), VA and VR ranged widely but were not statistically different between PVA and cellulose groups ($P = 0.191$ and $P = 0.802$, respectively); in contrast, RR was statistically greater ($P = 0.004$) with PVA sponges (79%, 22-91 %) compared with cellulose sponges (55%, 9-86%). In cats (Figure 2B), no difference in VA was noted between the two sponge types ($P = 0.464$). In contrast, VR was
statistically greater \((P = 0.040)\) with PVA sponges (8 µL, 1-27 µL) compared with cellulose sponges (3 µL, 0-28 µL), and RR was statistically greater \((P < 0.001)\) with PVA sponges (49%, 6-90%) compared with cellulose sponges (22%, 0-53%). These findings were particularly pronounced when the volume of tears absorbed was small: median RR of cases with VA ≤ 20 µL was much lower with cellulose sponges compared to PVA sponges in dogs (13% vs. 48%, respectively, \(P = 0.03\)) and in cats (12% vs. 38%, respectively, \(P < 0.001\)).

The effects of collection duration on VA, VR and RR are shown in Figure 3. In both species, a collection of 60 seconds achieved significantly greater VA, VR and RR when compared to 30 seconds and 15 seconds (range of \(P\) values, 0.001 to 0.049). Also, a collection of 30 seconds achieved significantly greater VA and VR compared to 15 seconds (range of \(P\) values, 0.002 to 0.045), but no difference was noted with RR \((P = 0.054\) in dogs, \(P = 0.064\) in cats).

**Correlations** – Volume absorbed was positively correlated with volume recovered in both dogs and cats \((r = 0.98\) and \(r = 0.80\), respectively; \(P < 0.001\)). A weak positive correlation was found between Schirmer values and tear collection parameters (VA, VR, RR) in dogs \((r = 0.38, r = 0.41, r = 0.36, \text{respectively}; \ P \leq 0.004)\), but not in cats (range of \(P\) values, 0.054 to 0.41). Also, a moderate correlation was found between body weight, VA and VR in dogs \((r = 0.62, r = 0.61, \text{respectively}; \ P < 0.001)\) but not in cats (range of \(P\) values, 0.37 to 0.64).

**DISCUSSION**

The present study describes a method of tear collection that is minimally invasive, safe, rapid and user-friendly. Although the use of ophthalmic sponges is not novel in veterinary species,\(^{19-22}\) the study allows for technique standardization and provides key information about the quantity of tears recovered relative to sponge type and collection duration. Relative to collection with
microcapillary tubes, the method most commonly used in humans,\textsuperscript{23} ophthalmic sponges allow for a greater and faster retrieval of tears in companion animals. Indeed, up to 84 μL and 29 μL (97.5\textsuperscript{th} percentile) of tears can be recovered within 60 seconds in dogs and cats, respectively, and the sample obtained is undiluted and readily available for analysis. In contrast, it is slow and difficult to obtain adequate volumes of tear fluid using microcapillary tubes. In dogs, the use of microcapillary tube did not yield any tear sample in over half the subjects tested, while only 2-10 μL were collected in the remaining dogs.\textsuperscript{24} In cats, over 6 minutes of collection with microcapillary tube followed by 20 minutes of centrifugation were required to collect ≥ 6-8 μL of non-stimulated tear fluid.\textsuperscript{25}

Tear collection with ophthalmic sponges was well tolerated by all dogs and cats. Although not critically evaluated, the animal’s reaction to having an absorbent strip in the ventral conjunctival fornix was subjectively similar to the one seen with the Schirmer tear test in clinical patients – that is, either no noticeable reaction or mild and transient irritation. Further, no animal developed corneal or conjunctival ulceration despite six consecutive collections per eye and per subject. However, repeated sampling did induce localized conjunctival hyperemia in a few eyes, which could theoretically affect the tear fluid composition by increasing leakage of serum proteins.\textsuperscript{26} Whenever possible, it is therefore preferred to avoid successive tear collections in the same eye.

Cellulose and PVA sponges have different properties that reflect on their ability to absorb and release tear fluid. Cellulose sponges are highly hydrophilic and, as such, generally absorbed greater volumes of tears but retained a greater portion of the fluid following centrifugation. This finding was particularly pronounced when the volume of tears absorbed was small (less than 20 μL). Taken together, our data suggest that PVA sponges are superior to cellulose sponges for tear collection in companion animals, as volume recovered is ultimately more relevant than volume absorbed,
and cellulose sponges would retain too much fluid when absorption volume is low. This preference is further supported by the fact that PVA sponges became softer and remained thin post collection, and therefore were easier to manipulate and insert into small 0.2-mL Eppendorf tubes; in contrast, cellulose sponges maintained their rigidity and became thicker after absorption of tear fluid.

Our study demonstrated that VA, VR and RR were significantly greater with a test duration of 60 seconds compared to 30 seconds and 15 seconds. Beckwith-Cohen and colleagues have collected tears in dogs using an eye spear in the ventral conjunctival fornix for 120 seconds,\(^\text{19}\) which resulted in greater volume of tears (median = 150 μL) relative to our study. This is consistent with our findings that longer collection duration yield greater volume of tears, although the eye spear technique (with intact spear handle) is likely to induce more reflex tearing as compared to the technique described herein. Ultimately, the test duration selected by the investigator depends on the volume of tears needed for the experiment. With recent advances in laboratory techniques, 5-10 μL of tear sample may be sufficient for selected analyses. Li and colleagues were able to identify with high confidence a total of 54 proteins using less than 5 μL of reflex tear fluid.\(^\text{27}\) Similarly, simultaneous measurement of six cytokines was reported in merely 5-10 μL of tear fluid using optimized flow cytometry.\(^\text{28}\) However, this small sample volume does not take into account possible losses (e.g. transfer, storage) or the need to repeat certain experiments in duplicates. If one requires 10 μL of tear fluid for an experiment in companion animals, our data suggest that a duration of 15 seconds may be enough in dogs whereas 60 seconds are necessary in cats (median VR = 9.5 μL and 10.5 μL, respectively).

The present study has a series of limitations worth mentioning. First, VR was generally low in cats, albeit greater compared to microcapillary tubes.\(^\text{25}\) Using ophthalmic sponges, VR was ≤ 10 μL in 86/120 of all feline samples and 20/40 of feline samples collected with a 60-seconds duration.
We suspect that lower VA and VR in cats compared to dogs are likely due to body weight differences rather than species-specific variations, as the lightest dog examined had results similar to the average cat. Low VR may also occur in canine patients with diseased ocular surface (e.g. keratoconjunctivitis sicca). Several options could be considered to increase the amount of tears collected: i) pool samples from both eyes or from repeated collections, ii) stimulate tear production with the nasolacrimal reflex, i.e. provide a noxious stimulus to the nasal mucosa (nasociliary nerve, branch of trigeminal nerve) in order to increase reflex tearing; or iii) use the eye-flush technique, i.e. instill saline on the ocular surface to dilute the tears and increase tear collection rate. Second, this work was limited to quantitative assessment of tear collection, and did not examine the impact of ophthalmic sponges on the quality of the sample obtained. Tear fluid composition could be affected by two sponge-related factors: (1) stimulated tearing could result in greater amounts of plasma-derived proteins (e.g. albumin, IgG) in the sample collected, as shown with Schirmer strips; (2) ophthalmic sponges have both absorbent and adsorbent properties, meaning that the absorbed tear components may not be 100% recovered following extraction. Although protein recovery from ophthalmic sponges is generally excellent, additional studies are warranted to evaluate the interaction of the selected sponge with the agent(s) of interest before any conclusions can be drawn. Last, the technique described herein provides “stimulated” tears, which may differ in composition from “non-stimulated” tears. However, it is the author’s opinion that adequate amount of ‘true’ basal non-stimulated tear fluid cannot be obtained with non-invasive and reproducible technique, especially in companion animals that are often less cooperative than humans. As suggested by Berta, “it is better to use well-controlled (stimulation) methods than to try to cause as little irritation as possible”.

In conclusion, the reported results demonstrate that ophthalmic sponges are an excellent method of tear collection in companion animals: the technique is well tolerated and allows retrieval of adequate amounts of tears in a short duration. The collection duration depends on the amount of tear volume required for subsequent bioanalysis (e.g. metabolomics or drug quantification). Ultimately, the choice of the sponge depends on the nature of the test to be performed,\textsuperscript{33} although a slight preference goes to PVA given its greater recovery ratio (especially with small volumes), easier manipulability, and improved release of certain compounds such as cytokines.\textsuperscript{33}

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FIGURE LEGENDS

Figure 1. Photographs showing the equipment necessary (A) and the procedure of tear collection with ophthalmic sponges (B-D). A 4 x 10 mm strip of ophthalmic sponge (PVA or cellulose) is inserted in the ventral conjunctival fornix using a disposable plastic tweezer. The wetted sponge is removed from the eye and sealed into a 0.2-mL tube that was first punctured at its bottom with 18-gauge needle. The 0.2-mL tube containing the wetted sponge is transferred to a 1.5-mL tube and the combination was spun at 6000 rpm for 1 minute in a centrifuge.

Figure 2. Box-and-whisker plots depicting the effect of sponge type (PVA or cellulose) on the tear volume absorbed (dark grey), tear volume recovered (light grey) and recovery ratio (RR) in 10 healthy dogs (A) and 10 healthy cats (B). 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. For each parameter evaluated, statistically significant differences (P < 0.05) are indicated by an asterix (*).

Figure 3. Box-and-whisker plots depicting the effect of test duration (15 seconds, 30 seconds or 60 seconds) on the tear volume absorbed (dark grey), tear volume recovered (light grey) and recovery ratio (RR) in 10 healthy dogs (A) and 10 healthy cats (B). 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. For each parameter evaluated, statistically significant
differences (P < 0.05) are indicated by the following symbols: #, greater than 30 seconds; †, greater than 15 seconds.
REFERENCES


B

Volume absorbed
Volume recovered

RR = 22 %
RR = 49 %

Tear volume (µL)

PVA
Cellulose