BRIEF COMMUNICATION

Influence of Schirmer strip wetness on volume absorbed, volume recovered, and total protein content in canine tears

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Abstract

Objective: Determine the protein content and volume of tears sampled by Schirmer strips wetness ranging from 20 to 35 mm.

Animals Studied: Ten healthy beagle dogs.

Procedures: Each dog underwent 20 tear collections per day (10 sessions in each eye, spaced by ≥1 h) for 4 separate days, providing 200 tear samples for each length of wetness evaluated: 20, 25, 30, and 35 mm. A Schirmer strip was placed in each eye until the selected mm-mark was reached, calculating the volume absorbed (VA) as the difference between the post- and pre- collection weight (assuming 1 mg~1 µL for tear fluid), and the volume recovered (VR) as the amount pipetted from the tube following centrifugation. Total protein content (TPC) was measured with infrared spectroscopy. Outcome measures were compared with the Kruskal-Wallis test.

Results: Median values for VA (µL), VR (µL) and TPC (mg/mL) were as follows: 20 mm (18, 10, 5.94), 25 mm (22, 12.5, 5.97), 30 mm (25.5, 16, 5.89), and 35 mm (31, 22.5, 7.13). Both VA and VR were significantly greater (p < .001) for Schirmer strips wetness of 35>30>25>20 mm. TPC was significantly greater (p < .001) for 35 > 20–30 mm, but not among other groups (p = 1.000).

Conclusions: The study established normative data to consider when canine studies use Schirmer strips to collect tears for bioanalytical purposes (eg, proteomics, pharmacokinetics). Although 35 mm yielded higher VA and VR, the higher TPC could be explained by greater disruption of ocular surface homeostasis. Absorption to 20–30 mm is the suggested length of strip wetness for bioanalytical tear collection in dogs.

KEYWORDS

absorbent material, dog, keratoconjunctivitis sicca, schirmer tear test, tear collection, tear film

1 INTRODUCTION

The Schirmer tear test is a routinely performed procedure in dogs and other species, assessing the patients’ aqueous tear production to diagnose and manage dry eye disease.1,2 Schirmer strips are also increasingly used to sample tear fluid and quantify various components in the canine tear film, including proteins3 and xenobiotics.4 Schirmer strips are often considered superior to other collection methods in dogs (eg, capillary tubes, ophthalmic sponges) given

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improved reproducibility of bioanalytical results\textsuperscript{5,6} as well as several other advantages described in a recent review article.\textsuperscript{7} For instance, Schirmer strips are well tolerated, easy to use, and yield a relatively large tear sample in a short duration, while capillary tubes require an experienced operator and cooperative patient, and only provide a small volume of tears over a long collection time.\textsuperscript{7} The main drawback of Schirmer strips, however, is that the volume of tears retrieved is not readily available with Schirmer strips, leading some publications to report tear film concentrations in µg/g of strip rather than µg/µL (ie, mg/mL) of fluid.\textsuperscript{4}

The primary objective of this study was to correlate values from strip wetness (in mm) and volume of tears sampled by Schirmer strips in dogs (in µL), using results from hundreds of in vivo measurements. A secondary goal was to determine whether a selected mm wetness is preferred for future investigations, evaluating total protein content in tear fluid as well as reproducibility of the volume absorbed and volume recovered in canine eyes.

2 | MATERIAL AND METHODS

The study involved 5 male neutered and 5 female spayed beagle dogs (2.5 years old, 8.7–11.3 kg), all confirmed to be healthy based on physical and ophthalmic examinations. Four lengths of Schirmer strip wetness were evaluated, each on a separate day: 20, 25, 30, and 35 mm. Each dog underwent 20 tear collections per day (10 sessions in both eyes, spaced by ≥1 h) for a total of 200 tear samples for each mm wetness evaluated. Without topical anesthetic, a dye-free Schirmer tear strip (Eye Care and Cure Corp.) was placed in the ventrolateral conjunctival fornix until the selected mm wetness was reached. Tear volume absorbed (VA) was calculated as the difference of post- and pre-collection weight of a 2-mL tube containing each Schirmer strip, measuring weights to the nearest 0.001 g (Gemini-20, American Weight Scales Inc.) and assuming tear fluid density equals 1 g/mL. Following centrifugation in 0.2-mL tubes at 3884 g for 2 min, tear volume recovered (VR) was measured to the nearest µL with a micropipette. Tear samples were diluted 1:5 with phosphate-buffered saline (PBS Gibco\textsuperscript{TM}, Thermo Fisher Scientific, Inc.), and total protein content (TPC) was measured with infrared spectroscopy, as previously described.\textsuperscript{3} The study was approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC # 19–210).

Normality of data was assessed with the Shapiro-Wilk test. Outcomes of interest (VA, VR, TPC) did not differ between right vs. left eyes (Wilcoxon signed-rank test, \( p \geq .129 \)), thereby statistical analyses were conducted on values averaged from both eyes: (i) Spearman’s correlation to evaluate the relationship between VA, VR, and TPC; (ii) Kruskal-Wallis test to compare each outcome among the 4 groups; and (iii) One-way ANOVA to compare the coefficients of variability (CV\%) of each outcome among the 4 groups. Statistical analyses were performed with SigmaPlot 14.0 (Systat Software, Inc.), and \( p \) values <.05 were considered as statistically significant.

3 | RESULTS

Figure 1 summarizes VA and VR results for the four lengths of strip wetness tested. Median (95% range) VA and VR were 18 µL (13–22 µL) and 10 µL (7–13 µL) for tear samples collected until the 20-mm mark, 22 µL (17–26 µL) and 13 µL (10–15 µL) for 25-mm, 26 µL (20–30 µL) and 16 µL (13–19 µL) for 30-mm, 31 µL (25–38 µL) and 23 µL (18–28 µL) for 35-mm (Figure 1). Both outcomes increased significantly from one length to the next (\( p \leq .001 \)), that is, 35 mm» 30 mm» 25 mm» 20 mm.

The variability in VA and VR among repeated collections (200 samples for each length of wetness) is summarized in

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Box-and-whisker plots depicting tear volume absorbed (A) and tear volume recovered (B) following tear collection in 10 dogs using Schirmer strips placed in the ventral conjunctival fornix until the 20-mm, 25-mm, 30-mm, or 35-mm lengths of wetness were reached. Median and mean values are shown by the horizontal solid and dashed line, respectively. 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. Asterisks (*) depict statistical differences (\( p \leq .001 \)) among lengths of strip wetness.}
\end{figure}
Table 1. The highest variability (ie, highest CV%) was noted for VR in samples collected until the 35-mm mark (11.4%), a finding that was significantly greater than CV% of 30-mm (7.0%, \( p = .008 \)).

A weak positive association was noted between TPC and VA (Spearman’s rho = 0.23, \( p < .001 \)) as well as TPC and VR (Spearman’s rho = 0.21, \( p < .001 \)). Median TPC was relatively similar (\( p = 1.000 \)) whether collecting canine tears with Schirmer strips until the 20-mm mark (5.94 mg/mL), 25-mm (5.97 mg/mL) or 30-mm (5.89 mg/mL). In contrast, TPC in tears collected until the 35-mm mark (7.13 mg/mL) was significantly higher (\( p < .001 \); Figure 2).

### DISCUSSION

Determination of the volume sampled following tear collection is essential, allowing investigators to assess whether sufficient sample was collected for the desired bioanalytical assay, and provide accurate quantification (in \( \mu g/\mu L \)) of the analyte(s) of interest. Such information is relatively easy to obtain when using microcapillary tubes (eg, 2- \( \mu L \) or 5- \( \mu L \) tubes); however, this tear collection method presents several drawbacks that have prompted researchers to consider indirect tear sampling as a suitable alternative.¹

The present study assessed several outcomes (VA, VR, and TPC) for selected lengths of Schirmer strips’ wetness in dogs. Of note, wetness lengths <20 mm were purposely not evaluated due to the “concentrating effect” from the absorbent material at low volumes.³ In other words, the protein content retrieved from Schirmer strips wetted with <20-mm of tears is often artificially higher than the true protein content, a finding related to water retention in the hydrophilic cellulose-based Schirmer strip.³ As expected, VA and VR increased significantly with increasing strip wetness. The median VR with Schirmer strips (10–23 \( \mu L \)) is inferior to cellulose or polyvinyl acetal sponges placed in the ventral conjunctival fornix for 1-min (53–60 \( \mu L \))³ but is superior to the volume retrieved with capillary tubes (generally ≤5 \( \mu L \)).⁷ As such, the tear volume obtained following centrifugation of Schirmer strips (≥10 \( \mu L \)) is deemed sufficient for most bioanalytical purposes (eg, cytokines, proteins, lipids, xenobiotics).⁷

Importantly, TPC remained stable for Schirmer’s length of 20 to 30-mm (5.89–5.97 mg/mL) although TPC increased significantly when Schirmer strips were left in the conjunctival fornix until the 35-mm mark was reached (7.13 mg/mL). This finding is likely related to greater irritation and disruption of ocular surface homeostasis when the Schirmer strip is left in the conjunctival fornix for a longer duration, although further studies are needed to support this hypothesis. Thus, Schirmer tear collection limited to 20–30 mm of wetness is preferable, a recommendation further supported by the lower variability in VR compared to 35-mm. The choice between 20-mm, 25-mm, and 30-mm should be based on the amount of tears required for the specific bioanalysis, accounting for possible losses (eg, transfer and storage) or the need to repeat certain assays in duplicates.

A strength of our study is the large sample size—200 samples for each length of strip wetness—providing normative data for VA, VR, and TPC that can be used in future investigations. However, it is possible the frequent collection of tears (10 sessions/day) might have affected the aforementioned outcomes; sessions were separated by \( \geq 1 \) h, providing ample time for tear fluid dynamics to be restored,⁹ albeit the time it takes for baseline protein composition to be restored in canine tears is unknown. Another limitation is the focus on a single manufacturer of Schirmer strips; in a recent study, Lewin et al showed variability in wetness lengths among different manufacturers of Schirmer strips in dogs,¹⁰ and the same may be true for the outcomes evaluated in the present study. The study also focused on a single canine breed (beagles) with

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**TABLE 1**  
Mean ± standard deviation of the coefficient of variability (%) for tear volume absorbed and tear volume recovered in dogs undergoing repeated tear collection with selected lengths of Schirmer strip wetness (20, 25, 30, and 35 mm)

| Length (mm) | Volume absorbed (mg/mL) | Volume recovered (mg/mL) |
|------------|--------------------------|--------------------------|
| 20         | 8.5 ± 2.0                | 10.2 ± 2.3               |
| 25         | 8.1 ± 2.9                | 9.4 ± 2.9                |
| 30         | 7.0 ± 2.2                | 7.0 ± 2.6                |
| 35         | 7.7 ± 3.0                | 11.4 ± 3.4               |

\( p \)-values depict results of one-way ANOVA testing. The asterisk depicts a statistical difference between 30-mm and 35-mm (\( p = .008 \)).
healthy eyes. Disease-induced disruption in blood-tear barrier and ocular surface homeostasis can affect tear film dynamics and composition. Ocular disease likely affects the flow rate captured by Schirmer strips (µL/min) due to changes in tear film dynamics (tear volume, turnover rate), but the impact on VA and VR is likely minimal (to absent) as long as the mm-mark wetness is kept constant (eg, same VA whether 20-mm is reached within 40 s or 70 s). As for TPC, a recent study showed that Schirmer strips may actually be superior to capillary glass tubes for tear collection in inflamed eyes. In fact, disease state did not exacerbate differences between Schirmer strips and capillary tubes in canine eyes, in contrast to the speculation that Schirmer strips might capture larger protein amounts by causing micro-trauma to the hyperemic and more vulnerable conjunctiva.

In summary, although 35 mm yielded higher VA and VR, the higher TPC could be explained by greater disruption of ocular surface homeostasis. Absorption to 20–30 mm is the suggested length of Schirmer strip wetness for bioanalytical tear collection in dogs.

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CONFLICT OF INTERESTS
The authors declare no conflicts of interest.

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