

Intra- and inter-operator variability of refractometric total proteins measurement of canine plasma

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Refractometric total proteins are commonly used in practice as a quick and inexpensive way to measure total protein concentration in bodily fluids. Little information is available about how the operator performing the measurement affects the results.

The aim of our study was to determine the inter- and intra-operator variability of refractometric total proteins measured on canine plasma using a temperature-compensated handheld refractometer.

A pooled sample of canine lithium-heparin plasma was created using leftover samples from dogs presented to our hospital. The sample was then divided into three aliquots. Total proteins of these aliquots were measured by veterinary nurses, interns, residents and specialists working at our hospital.

Statistical analysis revealed excellent inter-operator (ICC 0.99, CI 95% 0.971–1.00) and intra-operator (ICC 0.997, CI 95% 0.990–0.999) variability.

Having different operators measuring refractometric total plasma proteins in practice should not affect the results. This suggests different operators can be used when monitoring total plasma proteins of a patient over time and when designing a study that involves this test.

Keywords: dog, plasma, refractometer, total proteins, variability

Introduction

Handheld temperature-compensated refractometers were introduced into the medical field in the 1960s. They allow measurement of the total proteins dissolved in a solution by measuring the angle of refraction between room air and the sample of interest. Because proteins are the main solute of plasma and body fluids other than urine, refractometers are commonly used as a quick and inexpensive way to measure total protein concentration in these bodily fluids in both humans and veterinary patients (George 2001). They are also commonly used for the determination of urine-specific gravity (USG). Studies investigating the effect on the refractometer model and sample storage on measurement of USG are available in the literature (Du Preez et al. 2020; Mösch et al. 2020; Rowe et al. 2021; Tvedten & Norén 2014). Other studies have focused on refractometric measurement of total proteins and investigated how the results are affected by serum bilirubin (Gupta & Stockham 2014) and urea (Legendre et al. 2017). Information about the effect of the operator on refractometric measurements is less defined. Studies have assessed interobserver variability of USG. Du Preez et al. (2020) and Rowe et al. (2021) found good agreement between different operators when assessing USG with a refractometer. A study by Mösch et al. (2020) also found good inter-operator variability and intra-operator variability was acceptable. There are currently no similar studies investigating refractometric measurement of plasma proteins.

The aim of our study was to determine inter- and intra-operator variability of plasma proteins measured using a handheld refractometer. We also investigated the effect of the operator's

job role (veterinary nurse, intern and clinicians). We hypothesised that operators that did the measurement more frequently (nurses and interns), would show less inter-operator variability compared to people that used the refractometer less often.

Material and methods

Leftover samples of canine whole blood in lithium-heparin-coated tubes were centrifuged at 2 500 g (Clinispin Horizon 842 Vet) for five minutes. Plasma was then used to create a pooled sample that was then divided in three identical aliquots (A, B and C) on the first day of the data collection and then stored at 4 °C until completion of the study. Data were collected over six days. Canine total proteins are stable in heparinised plasma for three days when stored at +4 °C (Thoresen et al. 1992), but information about the stability of this analyte for longer storage time is not available. Two of the researchers (FV and WJ) evaluated the refractometric total proteins of the three aliquots at the beginning of the data collection, after 24 hours and at the end of the data collection. This was done to assess stability of total proteins during the data collection period. All measurements were performed using a handheld temperature-compensated refractometer (RHCN-200ACT Combined Refractometer).

Qualified veterinary nurses, interns and clinicians working at our hospital were enrolled in the study. Participants were asked to measure refractometric total proteins of samples A, B and C. All participants were provided with the three samples, the refractometer, disposable transfer pipettes (drop size 50 µL), distilled water and a standard form to record their results. Participants were all blinded to the composition of the samples and to the results obtained by other people enrolled in the study

and they were asked not to share their results with others. The number of participants was limited to the number of colleagues available during the period of data collection. Calibration of the refractometer was performed by each participant, and it was checked by one of the researchers (WJ) after each measurement. The researcher also calibrated the refractometer again after each participant completed the study.

All statistical analysis was carried out using R 4.1.3, (R Core Team, 2022). Data was tested for normality using the Kolmogorov–Smirnov Test in the “stats” package (R Core Team, 2022). Data on job role was not normally distributed (nurse: $D = 1, p < 0.01$; intern: $D = 1, p < 0.01$; resident $D = 1, p < 0.01$), nor was aliquot (sample A: $D = 1, p < 0.01$; sample B: $D = 1, p < 0.01$; sample C: $D = 1, p < 0.01$) according to the Kolmogorov test. Time was also not normally distributed (0h: $D = 1, p < 0.01$; 24 h: $D = 1, p < 0.01$; 144 h: $D = 1, p < 0.01$). Levene’s test for homogeneity of variance using the “car” package (Fox & Weisberg 2019) showed that the data for the operators job role was non-significant ($F = 2.271_{(2,150)}, p = 0.763$; nurse: $F = 1.54_{(2,90)}, p = 0.221$; intern: $F = 0.231_{(2,9)}, p = 0.80$; resident: $F = 0.473_{(2,45)}, p = 0.626$), as was the data for aliquot ($F = 2.203_{(2,150)}, p = 0.114$), sample A: $F = 0.503_{(2,48)}, p = 0.61$; sample B: $F = 0.269_{(2,48)}, p = 0.765$; sample C: $F = 0.194_{(2,48)}, p = 0.825$). To account for any observer effects across the three aliquots, a repeated measures mixed effects model using maximum likelihood to account for the non-normality, but non-significant variability of the data (package “nlme”, Pinheiro et al. 2022) was used to determine if the job role or aliquot sample had a significant effect on the measurement of total proteins. To assess if any change in the concentration of total proteins occurred in the samples over time, a repeated measures one-way ANOVA was carried out using the “stats” package (R Core Team, 2022) followed by pairwise comparison using a Bonferroni-corrected p -value. Levene’s test for time was also non-significant ($F = 0.031_{(2,15)}, p = 0.97$). Inter- and intra-operator variability was determined by calculating the Intraclass Correlation Coefficient (ICC). Inter-operator variability was assessed using a two-way random effects model for absolute agreement using mean of multiple raters via the “irr” package in R (Gamer et al. 2019). Intra-operator variability was assessed using a two-way mixed effects model for absolute agreement, of the average score. The ICC was interpreted following recommendations by Koo and Li (2016): < 0.50 poor correlation; $0.50–0.75$ moderate correlation; $0.75–0.90$ good correlation; > 0.90 excellent correlation. Statistical significance was set at $p < 0.05$.

Results

Fifty-one people were enrolled in the study. These included 31 nurses, four interns and 16 clinicians (residents or specialists). The data collection was completed over six days to allow enrolment of an adequate number of participants. Measurements were performed by 28 participants on day 0, 18 participants on day 1 and by 5 participants on day 6.

Repeated reassessment of the total protein concentration of aliquots A, B and C, showed a statistically significant difference over time ($F = 5.466_{(2,15)}, p = 0.02$), with differences between 24 h and 144 h being significant ($p = 0.024$) and differences between 0 h and 24 h ($p > 0.05$) and 0 h and 144 h ($p = 0.058$) not being

significantly different. The difference between total protein concentration at the start of the study and at its end was 3 g/L for all samples (5% increase).

A significant difference was found between the total proteins concentration of sample C compared to sample A and B ($p < 0.05$), with measurements of sample C being 4% lower. Intraclass correlation coefficient showed excellent correlation between operators when data from nurses, clinicians and interns were considered together (ICC 0.99) with a small confidence interval (CI 95% 0.971–1.00, $p < 0.01$). The ICC for intra-operator variability was also excellent (ICC = 0.997, $p < 0.01$), with a small confidence interval (CI 95% 0.990–0.999).

The measurements were not affected by the job role of the operator ($p = 0.302$) (Table I).

Table I: Median and range of the refractometric total proteins (g/L) obtained for each aliquot by the operators grouped by job role – aliquots A, B and C were obtained by dividing a pooled sample created from leftover plasma from canine patients presented at our hospital

Aliquot	A	B	C
Nurses	68 (66–70)	68 (66–70)	64 (62–66)
Interns	68 (68–70)	68 (67–70)	64 (62–66)
Clinicians	68 (66–70)	68 (66–70)	64 (60–69)

Discussion

In our study, excellent correlation was found between repeated measurements of total proteins on lithium-heparin plasma samples using a handheld temperature-compensated refractometer, performed by the same operator on different aliquots obtained from a pooled sample, suggesting measurements using this technique are repeatable. This information is relevant in clinical practice where total proteins may be monitored over time on the same subject to determine progression of disease or response to treatment.

Our results also show excellent correlation between different operators measuring refractometric total proteins. These results are relevant in clinical practice, where it is unlikely that the same operator will be performing all the measurements. They are also relevant for research because they suggest having different operators measuring refractometric total proteins should not affect the data collected. This is especially relevant for retrospective studies, where information about who performed the measurements may not be available. Total protein measurements were not affected by the job role of the operator, suggesting that experience and familiarity with the technique didn’t have a significant impact on the results. Participants to our study included qualified veterinary nurses, interns (qualified veterinarians with at least one year of experience) and clinicians (residents, post-residency clinicians and boarded specialists). This was a convenience sample limited to the staff working in our hospital. Veterinary students, nurse students and laboratory technicians may also be asked to measure refractometric total proteins in veterinary practice. Further studies are needed to determine if our results can also be applied to these professional

figures as their familiarity with the test is likely to be different from our study sample.

Analytical, pre-analytical and post-analytical factors can all affect the result of a test. Our study was designed to determine variability of total proteins concentration secondary to the operator performing the test and other sources of variability were not assessed.

Total proteins concentration in sample C was 4% lower than samples A and B. Since all three samples were created from the same pooled sample, this result was unexpected. This could have been due to inadequate mixing of the pooled sample before dividing it into the three aliquots. There is no guideline that defines the allowable total error for refractometric total proteins, however current guidelines define the allowable total error for total proteins to be 10% (Harr et al. 2013). Since the difference between aliquot C and the other aliquots was within this limit, the discrepancy between the readings was deemed acceptable.

The concentration of total proteins in our pooled sample increased by 5% during the data collection. Dehydration of the sample is a possible explanation. The increase was not considered clinically relevant because it was lower than the allowable total error for total proteins (Harr et al. 2013).

Conclusion

Inter- and intra-operator variability of refractometric measurement of total proteins is low. Low inter-operator variability suggests that having different operators measuring total proteins on canine plasma using a refractometer should not affect the results. The job role of the operator did not affect the variability of the results in our study, but our study sample included only qualified veterinarians and nurses. Further studies are needed to determine if our findings can also be applied to other professional figures that may be required to measure total proteins in clinical practice.

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Conflict of interest

The author(s) declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

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Ethical approval

The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010. Prior to the commencement of the study, ethical approval was obtained from the following ethical review board, the Ethics Review Panel of the Royal College of Veterinary surgeons (Ref 2020-31-Venier). All participants gave written consent to be enrolled in the study.

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