Formulation and validation of a predictive model to correct blood glucose concentrations obtained with a veterinary point-of-care glucometer in hemodiluted and hemoconcentrated canine blood samples

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Objective—To determine the effect of PCV on veterinary point-of-care (POC) glucometer measurements in canine blood samples and develop a formula to correct the glucose concentration as measured by a point-of-care glucometer (POCgluc) given a known PCV.

Design—Experimental and prospective study.

Samples—Blood samples from 6 healthy dogs and from 30 hospitalized dogs.

Procedures—60 mL of heparinized blood was obtained from each of 6 healthy dogs. Samples were processed into packed RBCs and plasma. Packed RBCs were resuspended with plasma to achieve a range of PCVs from 0% to 94%. Duplicate POCgluc and PCV measurements were obtained for each dilution; following POCgluc measurements, plasma samples were analyzed for glucose concentration by a clinical laboratory biochemical analyzer (LABgluc). A correction formula for POCgluc was developed. Measurements of POCgluc, PCV, and LABgluc were also determined from blood samples of 30 dogs admitted to the veterinary teaching hospital.

Results—Values of LABgluc for each sample were similar at any PCV. As PCV decreased, POCgluc was falsely increased; as PCV increased, POCgluc was falsely decreased, compared with LABgluc. The absolute difference between POCgluc and LABgluc increased as the PCV changed from 50%. Compared with POCgluc, the corrected POCgluc had a significantly improved correlation with LABgluc, which was also reflected in improvements in Clarke and consensus error grid analyses.

Conclusions and Clinical Relevance—Results indicated that in dogs with hemodilution or hemoconcentration, POCgluc did not reflect actual patient glucose concentrations. Use of a correction formula reduced this error. Corrected POCgluc data had strong, significant correlations with LABgluc data. (*J Am Vet Med Assoc* 2015;246:307–312)

Point-of-care glucometers are commonly used in veterinary hospitals to rapidly measure blood glucose concentrations in whole blood samples obtained from dogs. Point-of-care glucometers designed for humans have variable degrees of accuracy when used with veterinary species.[1–3] A POC glucometer[a] marketed to veterinarians uses an internal, proprietary algorithm, which attempts to improve glucose concentration accuracy by accounting for the species differences between free and hemoglobin-bound glucose.[4] The algorithm generally renders the glucometer more accurate than most human glucometers when used in veterinary species.[1,3,5]

The inaccuracy of POC glucometers for use in hemodiluted and hemoconcentrated samples has been well established in human medicine.[6–12] Compared with the results obtained with serum or plasma in a reference laboratory setting, measured glucose concentrations are falsely decreased in hemoconcentrated samples and glucose concentrations in hemodiluted samples are falsely increased.[6–12] This inaccuracy is thought to be related to the degree of plasma displacement by erythrocytes.[8] An increased number of erythrocytes in a whole blood sample reduces the volume of plasma that penetrates the reagent layer of the glucose test strip; therefore, a hemoconcentrated sample results in a falsely decreased glucose measurement. Alternatively, hemodiluted samples allow penetration of a greater volume of plasma into the test strip reagent layer, resulting in higher measured glucose concentration. There are variations in the degree of inaccuracy among different glucometers, depending on the method by which the glucose con-

Abbreviations

LABgluc	Glucose concentration as measured by a clinical laboratory biochemical analyzer
POC	Point of care
POCgluc	Glucose concentration as measured by a point-of-care glucometer

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centration measurement is obtained.[6] Meters that use coulometric technology to quantify glucose concentrations are least sensitive to Hct interference.[6]

Similar effects of hemodilution and hemoconcentration on blood glucose concentration measured with a POC glucometer have been found in a previous study[5] on dogs. That study[5] was limited by a low number of blood samples from anemic dogs, with most being only mildly anemic (30% to 37%), a bias for higher Hct as a result of overrepresentation of Greyhounds in the study population, and use of multiple operators of the POC glucometers.

The purpose of the study reported here was to determine the effect of a wide range of PCVs on glucose concentration measurements obtained with a POC glucometer marketed specifically for veterinary use. An additional goal was to develop and validate a mathematical formula to more accurately predict canine plasma glucose concentrations, given a POCgluc and a known PCV. The primary hypothesis was that POCgluc would correlate closely with plasma LABgluc when PCVs are within the reference range. We also postulated that hemodiluted samples would yield falsely increased measurements and hemoconcentrated samples would yield falsely decreased measurements with the POC glucometer. Lastly, we hypothesized that a correction formula could be developed that would improve the accuracy of POCgluc measurements obtained for clinical patients with PCVs outside the reference range.

Materials and Methods

Sample collection and processing—Following standard aseptic jugular venipuncture technique with a 19-gauge winged infusion catheter[b] and 60-mL syringe containing 750 U of sodium heparin anticoagulant,[c] 60 mL of blood was obtained from 6 dogs owned by hospital staff and deemed healthy on the basis of history and findings of physical examination and routine laboratory analysis (ie, CBC and serum biochemical analysis). Blood sample collection was approved by the Clinical Research Committee, which serves as the University of Georgia Veterinary Teaching Hospital's internal review board for all clinical research involving client-owned animals, and owner consent was obtained prior to blood collection. The POCgluc, PCV, and total protein concentration were measured in duplicate on each whole blood sample immediately prior to processing. Total protein and PCV measurements were completed for all samples by filling 3 micro-Hct tubes and centrifuging in a micro-Hct centrifuge[d] at 11,800 × *g* for 3 minutes. Then, PCV was read from a micro-Hct capillary tube reader card,[e] and total protein concentration was estimated by refractometry.[f] One individual read all PCV and total protein concentration measurements for all dogs.

Each 60-mL whole blood sample was transferred to a 150-mL disposable blood transfer bag,[g] which was centrifuged[h] at 6,500 × *g* for 6 minutes. The plasma was decanted off the RBCs into a clean beaker with a manual plasma extractor,[i] and the remaining packed RBCs were placed in a separate clean beaker.

Packed RBCs were aliquotted into 17 glass tubes,[j] and with varying quantities of each dog's own plasma, the packed RBCs were resuspended to achieve a wide range of PCVs. All suspensions were made by a single individual. Duplicate measurements of POCgluc, PCV, and total protein concentration were obtained for each suspension by the described procedures. Point-of-care measurements were considered unreadable after 3 failed attempts with 3 test strips. One person performed all POCgluc measurements with a single POC glucometer.[a] This glucometer used glucose dehydrogenase and a coulometric biosensor to measure plasma glucose concentration from a 0.3-µL sample of whole blood. The testing range of the glucometer was 20 to 750 mg/dL (1.1 to 41.7 mmol/L), with a reported accuracy of 0.1% in canine blood samples.[13] All measurements of POCgluc for each dog were done with glucose test strips of the same lot number.

After POCgluc measurements were obtained, samples were centrifuged[d] at 1,500 × *g* for 5 minutes. The plasma was decanted and immediately frozen at –20°C. These plasma samples were batch analyzed for glucose concentration on a clinical pathology laboratory biochemical analyzer[k] within 7 days after collection. During all phases of processing and handling, all samples, including whole blood, plasma, packed RBCs, and subsequent suspensions, were kept at 4°C.

Development of correction formula—Mean POCgluc and LABgluc measurements were identified for each sample. The difference between POCgluc and LABgluc (ie, glucose concentration difference) was calculated for each sample. A correction formula for POCgluc was developed on the basis of a simple linear regression model describing the glucose concentration difference versus PCV.

Validation of correction formula—The correction formula was subsequently tested on clinical canine patients that had been admitted to the University of Georgia Veterinary Teaching Hospital. Convenience samples were obtained from admitted dogs that had heparinized blood taken as part of a routine diagnostic workup and were in compliance with institutional clinical research guidelines. Because data were obtained from diagnostic tests completed in the course of routine standard clinical patient care or with residual blood samples obtained during the course of routine care, specific client consent and approval by the Clinical Research Committee or an institutional animal care and use committee were not required. Blood was obtained with standard venipuncture techniques, and PCV and total protein concentration measurements, single LABgluc measurement, and duplicate POCgluc measurements were obtained for each patient following the already described processing techniques.

Statistical analysis—Measurements of mean PCV and POCgluc were obtained for each sample. Repeated-measures ANOVA was used to test for differences between POCgluc and LABgluc measurements. Glucose concentration difference was calculated for each sample. A linear regression model was used to describe the relationship between glucose concentration difference and PCV. Mean slope and R^2 were obtained from the linear regression line. Values of $P < 0.05$ were considered significant.

Clinical relevance of the POCgluc and corrected POCgluc measurements were examined by use of both Clarke and consensus error grid analyses. A χ^2 analysis was used to evaluate Clarke and consensus error grid analyses when comparing POCgluc and corrected POCgluc measurements against LABgluc. Error grid analysis assigns a specific level of clinical risk to blood glucose concentration errors. The actual (LABgluc; x-axis) and estimated (POCgluc or corrected POCgluc; y-axis) plasma glucose concentrations were plotted on a scattergram, which was then divided into 5 risk zones (zones A to E). To establish the risk boundaries in error grid analysis, the target blood glucose concentration range was assumed to be between 70 and 180 mg/dL. The 5 risk levels for Clarke error grid analysis were labeled: < 20% deviation in estimated blood glucose concentration from LABgluc or both estimated blood glucose concentration and LABgluc < 70 mg/dL (level A), deviation from LABgluc of > 20% but leads to no treatment or benign treatment (level B), overcorrection of acceptable blood glucose concentration or misinterpretation of euglycemia for hyper- or hypoglycemia (level C), dangerous failure to detect and treat because of estimated blood glucose concentration errors (level D), and erroneous treatment (ie, treatment contradictory to that actually required; level E).[14,15] The consensus error grid analysis also divides the plot into 5 zones: no effect on clinical action (zone A), altered clinical action unlikely to affect outcome (zone B), altered clinical action likely to affect clinical outcome (zone C), altered clinical action could have serious medical risk (zone D), and altered clinical action could have dangerous consequences (zone E).[15,16]

Results

Experimental data—Mean baseline PCVs and total protein concentrations for the 6 donor dogs were 50% (range, 46% to 56%) and 6.8 g/dL (range, 6.4 to 7.2 g/dL), respectively. Following processing of packed RBCs and plasma, 17 resuspended samples were generated for each dog. For all suspensions, PCV ranged between 0% (plasma) and 94% (packed RBCs). The POC glucometer failed to read 4 of 7 samples with PCVs > 80%, all of which were undiluted packed RBC samples.

For each dog, all plasma LABgluc were not different from one another regardless of PCV (**Figure 1**). As PCV decreased, POCgluc incrementally increased, and as PCV increased, POCgluc decreased, compared with LABgluc. Even though each dog had a different baseline blood glucose concentration, the slope of the lines generated by POCgluc measurements over the range of PCVs was similar ($P < 0.001$) among dogs (**Figure 2**).

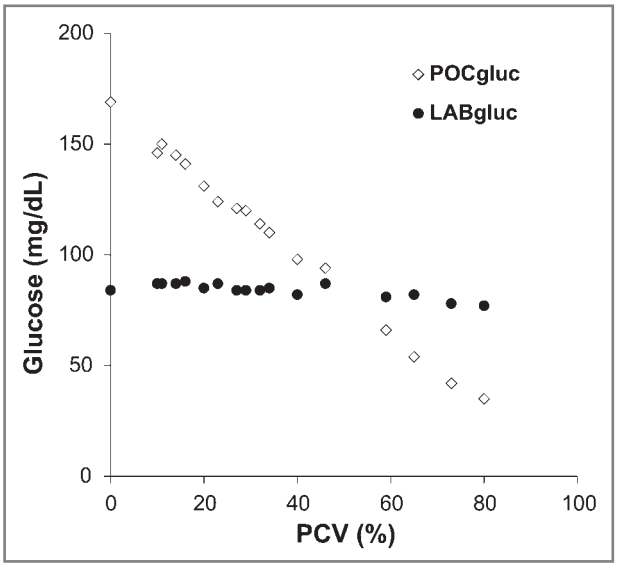

Figure 1—Comparison of measurements of LABgluc and POCgluc at various PCVs for 1 dog; data are representative of findings from blood samples of 6 healthy dogs (experimental data set) and 30 dogs admitted to a veterinary teaching hospital (validation data set) used for formula validation with regard to the effect of increasing or decreasing PCV.

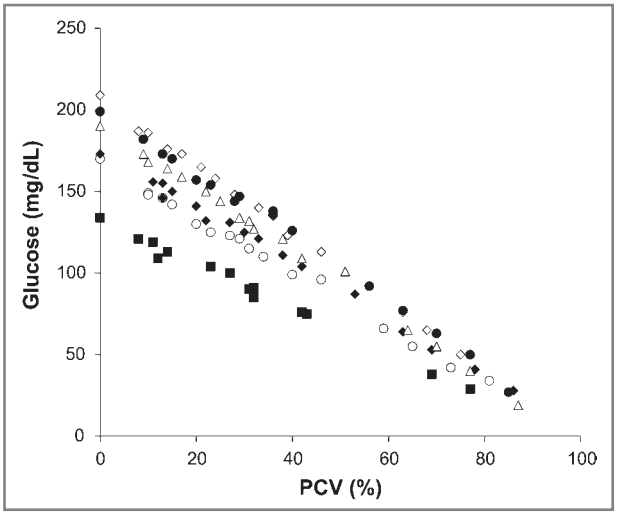

Figure 2—The POCgluc measurements obtained at various PCVs for 6 healthy dogs. Each donor dog is represented by a separate symbol type on the figure. The slope of each line was similar to all other lines ($P < 0.001$).

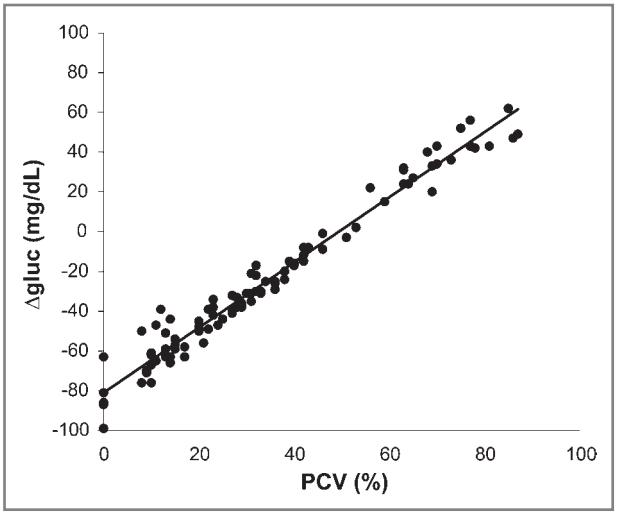

Figure 3—Linear regression model for change in glucose concentration measurements (Δgluc; ie, POCgluc – LABgluc) at various PCVs developed from experimental data from 6 healthy dogs. Mean slope of the model line for all data points was 1.6 with an intercept of –81.3 ($R^2 = 0.97$; $P < 0.001$).

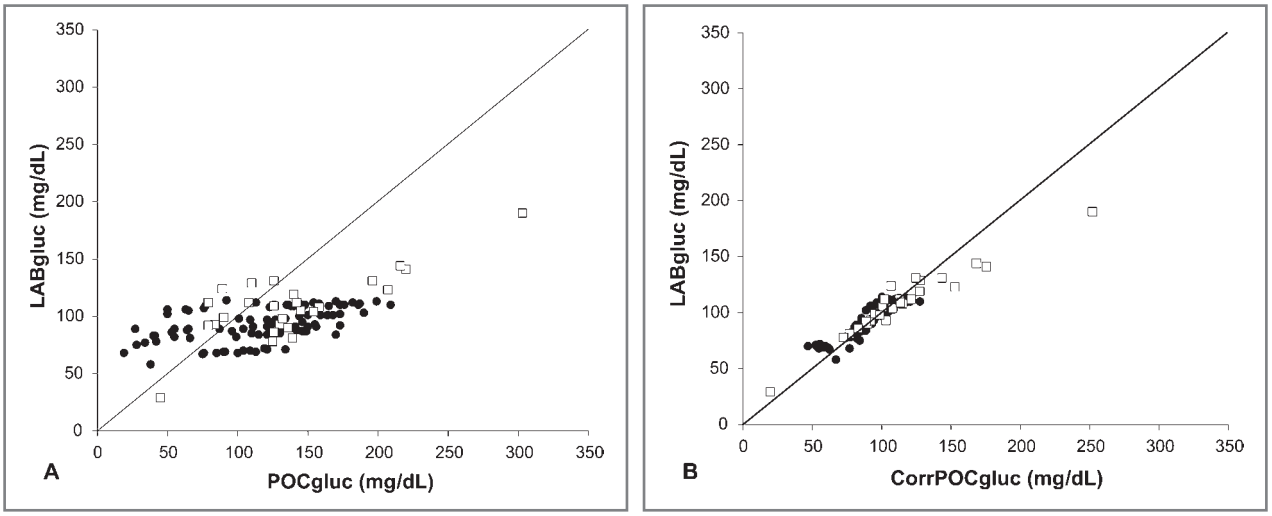

Figure 4—Agreement between measurements of LABgluc and POCgluc (A) and corrected POCgluc (CorrPOCgluc; B) for experimental (black circles; 6 healthy dogs) and validation (open squares; 30 dogs admitted to the veterinary teaching hospital) data. The line of perfect agreement between the 2 methods is depicted. A wide distribution of data points away from the line is evident when comparing POCgluc with LABgluc. Agreement between glucose measurements is significantly ($P < 0.001$) improved when applying the correction formula to POCgluc measurements.

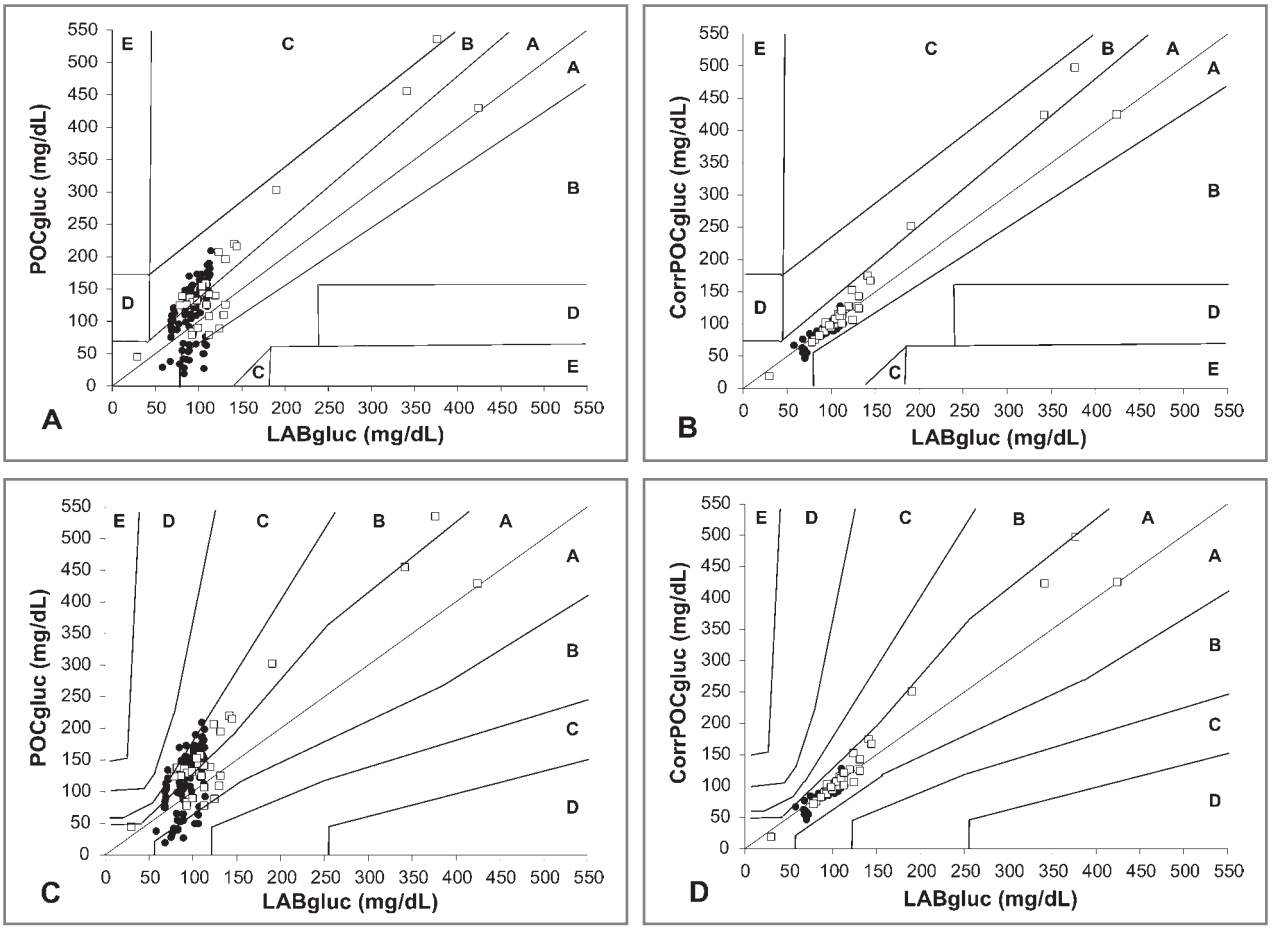

Figure 5—Error grid analysis for POCgluc and CorrPOCgluc (y-axis), compared with LABgluc (x-axis). Results of Clarke error grid analysis (panels A and B) and consensus error grid analysis (panels C and D) of the experimental (black circles; 6 healthy dogs) and validation (open squares; 30 dogs admitted to the veterinary teaching hospital) data are shown. The 5 risk levels for Clarke error grid analysis are as follows: A = < 20% deviation in estimated blood glucose concentration from LABgluc or both estimated glucose concentration and LABgluc < 70 mg/dL; B = deviation from LABgluc measurement > 20% but leads to no treatment or benign treatment; C = overcorrection of acceptable blood glucose concentration or misinterpretation of euglycemia for hyper- or hypoglycemia; D = dangerous failure to detect and treat because of estimated blood glucose concentration errors; and E = erroneous treatment (ie, treatment contradictory to that actually required). The 5 interpretation zones for consensus error grid analysis are as follows: A = no effect on clinical action; B = altered clinical action unlikely to affect outcome; C = altered clinical action likely to affect clinical outcome; D = altered clinical action could have serious medical risks; and E = altered clinical action could have dangerous consequences.

Mean glucose concentration difference was 41 mg/dL (range, –62 to 99 mg/dL), and glucose concentration difference increased as PCV changed from 50% (**Figure 3**). On the basis of the slope and intercept of the model line obtained by linear regression, a predictive formula was developed to correct the POCgluc given a known PCV to more accurately predict canine plasma glucose concentrations:

$$\text{CorrPOCgluc} = \text{POCgluc} + ([1.6 \times \text{PCV}] - 81.3)$$

where CorrPOCgluc is corrected POCgluc. After applying the correction formula to POCgluc measurements, the mean difference between corrected POCgluc and LABgluc measurements decreased to 5.4 mg/dL, with a maximum absolute difference of 23 mg/dL (**Figure 4**). Corrected POCgluc more closely approximated LABgluc than did POCgluc ($P < 0.001$).

Validation data—Thirty samples were obtained from 30 dogs for the validation study. The PCVs and total protein concentrations ranged between 12% and 72% and 4.2 and 9.0 g/dL, respectively. The POCgluc ranged between 45 and 694 mg/dL, and LABgluc ranged between 29 and 874 mg/dL. The mean difference between POCgluc and LABgluc was 29 mg/dL, with a maximum difference of 180 mg/dL. The mean difference between corrected POCgluc and LABgluc was 5.5 mg/dL, with a maximum difference of 181.3 mg/dL (**Figure 4**). Corrected POCgluc measurements were significantly ($P < 0.001$) closer to LABgluc measurements than were POCgluc measurements.

Clarke and consensus error grid analyses were plotted (**Figure 5**). Clarke ($P < 0.001$) and consensus ($P < 0.001$) error grid analyses differed significantly between POCgluc and corrected POCgluc for the experimental data. Clarke ($P < 0.001$) and consensus ($P = 0.008$) error grid analyses also differed significantly between POCgluc and corrected POCgluc for the validation data. These results confirmed that use of corrected POCgluc significantly reduced clinical risk, compared with the use of POCgluc alone to guide therapeutic decisions, given that almost all values fell within zone A.

Discussion

The information gathered from initial POC tests (PCV, total protein concentration, and glucose concentration) frequently contributes to early therapeutic decisions made for critically ill patients or those being evaluated on an emergency basis. According to manufacturer instructions, POC glucometers are not recommended for use in critically ill patients. Despite these recommendations, POC glucometers are often used in intensive care unit or emergency department settings because of their ease of use, availability, and rapid results.

The present study showed that sample PCV had a significant effect on the accuracy of a veterinary POC glucometer. The POC glucometer provided reliable results when the sample to be tested had a PCV within the reference range; glucose concentration measurements obtained by the POC glucometer at PCVs of 42% to 56% generally had ≤ 10 mg/dL deviation from the LABgluc. In hemodiluted samples, however, the POC glucometer yielded falsely high glucose concentration measurements. In hemoconcentrated samples, the POC yielded falsely low glucose concentration measurements. These findings are consistent with previously reported effects of Hct on the accuracy of POC glucometers in canine samples[5]; however, in the present study, the effects of a wider range of PCVs in experimental and clinical populations of dogs were evaluated.

Overall, calculation of corrected POCgluc may reduce the risk of making inappropriate clinical decisions when evaluating hemodiluted or hemoconcentrated samples. Most POCgluc measurements were within zones A and B, indicating that there would have been nominal clinical risk expected without mathematical correction for most samples collected in the present study. However, corrected POCgluc measurements primarily fell within zone A of the Clarke and consensus error grids, thus minimizing clinical risk. In the clinical setting, corrected POCgluc measurements were obtained over a wide range of PCVs, but there were limited numbers of blood samples from dogs with marked hypoglycemia or hyperglycemia. Generally speaking, the corrective equation was less accurate at predicting plasma glucose concentration at both glycemic extremes. Ideally, evaluation of more clinical samples at extremes of glucose concentrations and PCVs may help refine the corrective formula and improve its accuracy.

The PCV and glucose concentration may change greatly and unexpectedly in critically ill patients, and clinicians must interpret glucose readings with caution when POC glucometers are used in patients with PCVs outside the reference range. We used PCV instead of Hct because it is a commonly used POC reflection of RBC mass. Considering that measuring PCV relies on some subjective evaluation, we attempted to reduce this variability by having a single operator perform the test. Several additional variables have been shown to contribute to the inaccuracy of results obtained from a POC glucometer, including Pa_{O_2}, Pa_{CO_2}, and the pH of arterial blood samples as well as the presence of certain drugs, such as mannitol, dopamine, acetaminophen, and ascorbic acid.[17–20] The effect of these variables was not examined in the present study, and interference from 1 or more of these factors may have been present in samples obtained from the clinical population.

Finding a solution to the effect of Hct on POC glucometer measurements is of great importance in the practice of human and veterinary medicine. Reduction of error rates in glucose concentration measurements resulting from anemia has been achieved in humans by the use of correction formulas for several POC glucometers and by use of multichannel glucometers.[10,21,22]

In the study presented here, we developed a predictive equation to limit the effects of PCV on POCgluc. Specific correction formulas would need to be developed for other brands and models of POC glucometers. Although the use of correction formulas is quick and simple, an ideal clinical POC device would simultaneously measure Hct or hemoglobin with glucose concentration and incorporate a corrective formula into the intrinsic POC glucometer algorithm prior to display.

a. AlphaTRAK 2, Abbott Laboratories, Abbott Park, Ill.
b. Exel International Scalp Vein Butterfly Set, Thermo Fisher Scientific, Waltham, Mass.

c. Heparin sodium injection USP, 1,000 U/1 mL, Hospira, Lake Forest, Ill.
d. Triac Centrifuge, BD, Franklin Lakes, NJ.
e. Micro-Hct capillary tube reader, Veterinary Information Network, Davis, Calif.
f. JorVet J-351 Refractometer, Jorgenson Laboratories Inc, Loveland, Colo.
g. Teruflex transfer bag, Terumo Medical Corp, Somerset, NJ.
h. Sorvall RC-3, Thermo Fisher Scientific Inc, Waltham, Mass.
i. Manual Plasma Extractor, Fenwal Inc, Lake Zurich, Ill.
j. BD Vacutainer glass blood tube, Becton, Dickinson and Co, Franklin Lakes, NJ.
k. Hitachi P-Modular 800, Roche Diagnostics, Indianapolis, Ind.

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