Anemia is common in critically ill patients and can substantially decrease oxygen-carrying capacity.1–5 As such, Hgb concentration and Hct values are routinely measured with a cytometer as part of a CBC. This measurement requires specialized equipment and trained personnel, is costly, and can be associated with a prolonged turnaround time.6,7 An alternative method for quantifying RBC mass is determination of the PCV. This method requires a centrifuge, enough blood to fill an Hct tube, and trained personnel who can read the results, which are subject to interpretation.8,9

The use of POC analyzers in the intensive care and emergency setting has several benefits, including short turnaround time, small sample size, minimal personnel training, and reduced costs.10 A commercial veterinary POC Hct meter11 is available to evaluate Hct values and Hgb concentrations in blood samples from cats and dogs; it measures the Hgb concentration through optical reflectance and then calculates the Hct. The meter requires only a single drop of blood (approx 50 µL), which is particularly useful in small animals with limited blood volume; the results are displayed within seconds. The meter is reportedly accurate and precise in evaluation of blood samples of cats and dogs over a wide range of Hct values.11

Endogenous compounds circulating in blood may alter the results from analyzers that rely on optical reflectance methodologies.12,13 Potential confounding compounds (ie, lipids and bilirubin) and conditions (ie, autoagglutination and hemolysis) can change light absorbance and cause light scattering, resulting in potential interference in any assay that uses the transmission of light as part of the detection scheme.14,15

,Objective

To assess the agreement in measurements of Hct values and hemoglobin (Hgb) concentrations in blood samples from dogs and cats between a commercially available veterinary point-of-care (POC) Hct meter and a laboratory-based (LAB) analyzer and to determine the effects of various conditions (ie, lipemia, hyperbilirubinemia, hemolysis, autoagglutination, and reticulocytosis) on the accuracy of the POC meter.

Samples

Blood samples from 86 dogs and 18 cats

Procedures

Blood samples were run in duplicate on the POC meter, which reported Hgb concentration, measured via optical reflectance, and a calculated Hct value. The POC meter results were compared with results from a LAB analyzer. Blood samples with grossly visible lipemia, icterus, hemolysis, and autoagglutination were noted.

Results

Mean ± SD values for LAB Hct were 33.9 ± 15.73% (range, 3.9% to 75.8%), and for LAB Hgb were 11.2 ± 5.4 g/dL (range, 1 to 24.6 g/dL). Mean bias between POC Hct and LAB Hct values was −1.8% with 95% limits of agreement (LOAs) of −11.1% to 7.5% and between POC Hgb and LAB Hgb concentrations was −0.5 g/dL with 95% LOAs of −3.8 to 2.8 g/dL. There was no influence of lipemia (14 samples), icterus (23), autoagglutination (14), hemolysis (12), or high reticulocyte count (15) on the accuracy of the POC meter. The POC meter was unable to read 13 blood samples; 9 had a LAB Hct ≤ 12%, and 4 had a LAB Hct concentration between 13% and 17%.

Conclusions and Clinical Relevance

Overall, measurements from the POC meter had good agreement with those from the LAB analyzer. However, LOAs were fairly wide, indicating that there may be clinically important differences between measurements from the POC meter and LAB analyzer. (J Am Vet Med Assoc 2021;259:49–55)
The objectives of the present study were to assess the agreement in measurements of Hct values and Hgb concentrations in blood samples from dogs and cats between a commercially available veterinary POC meter and a LAB analyzer and to determine the effects of various conditions (ie, lipemia, hyperbilirubinemia, hemolysis, autoagglutination, and reticulocytosis) on the accuracy of the POC meter. Our hypotheses were that the POC meter would be accurate over a wide range of Hct values and that lipemia, hyperbilirubinemia, hemolysis, autoagglutination, and reticulocytosis would not affect meter performance.

Materials and Methods

Animals

Convenience blood samples were obtained from dogs and cats presented to the Veterinary Teaching Hospital of the University of Georgia. On the basis of described methods for test validation by use of a comparison experiment method, the goal was to acquire a minimum of 40 blood samples. Any animal that had an EDTA-anticoagulated blood sample submitted for CBC analysis as part of a diagnostic workup was eligible to participate. Because data were obtained from diagnostic testing completed in the course of routine standard clinical patient workups or by use of residual blood samples obtained during the course of diagnostic investigation, the clinical research committee did not require specific client consent or further approval for this research.

POC Hct meter

All readings from the POC meter were obtained by the same investigator. The POC meter used optical reflectance for determination of the total Hgb concentration and calculated Hct. Single-use test strips were inserted into the meter, and a background blank reading was automatically determined. A hanging drop (approx 50 µL) of EDTA-anticoagulated blood was applied to cover the entire test spot on the strip where it was dispersed within the membrane, and the meter’s optical detector measured the change in membrane reflectance. The intensity of the reflectance was inversely proportional to the Hgb concentration (displayed in g/dL). The meter then calculated the Hct on the basis of a mathematical conversion and reported the result within seconds. Two meters were used, one dedicated for blood samples from cats and the other for blood samples from dogs. Species-specific meter code chips were inserted before use. The code chips were lot specific and were changed, per the manufacturer’s instruction, with every new box of test strips. Quality control solutions were tested at least once between boxes of test strips.

Blood sample collection and analysis

Canine and feline venous blood samples were acquired by direct venipuncture or through an IV catheter, placed into EDTA-containing blood tubes, and submitted to the in-house American Association of Veterinary Laboratory Diagnosticians–accredited laboratory for CBC analysis. Under- or overfilled EDTA-containing blood tubes were not evaluated. Residual EDTA-anticoagulated blood samples were obtained from the laboratory and processed within 24 hours after venipuncture. These blood samples were stored at 4°C (39.2°F) and allowed to equilibrate to room temperature (20°C to 25°C [68°F to 77°F]) for 10 minutes before testing. Grossly visible lipemia, icterus, hemolysis, and agglutination were noted. Additionally, records were retrospectively reviewed to obtain the patient’s serum total bilirubin concentrations (as determined by a LAB chemistry analyzer) and reticulocyte count and the severity of hemolysis, graded from 1 to 4 (1 = slight, 2 = mild, 3 = moderate, and 4 = severe) by an automated system within a LAB hematology analyzer. Lipemia and autoagglutination were not graded in severity and were recorded as visible or not visible. All blood samples were gently inverted 8 times to resuspend cells and then were tested in duplicate on the POC meter according to the manufacturer’s directions. A syringe and needle were used to apply a hanging drop to the test strip. The mean values of the duplicate measurements were calculated and recorded as POC Hgb concentrations and POC Hct values. These measurements were compared with results from a LAB analyzer (ie, LAB Hgb concentrations and LAB Hct values), which were obtained through review of patient medical records.

Statistical analysis

Agreement between measurements from the POC meter and LAB analyzer was determined by use of Bland-Altman analysis for all blood samples and separately for blood samples with icterus, autoagglutination, lipemia, hemolysis, and high concentrations of reticulocytes. Blood samples from cats and dogs were analyzed separately and together. Mean differences (bias) and 95% LOAs were calculated. Mean bias was calculated as the mean of the difference between the mean value of the duplicate measurements from the POC meter and the value obtained with the LAB analyzer. A Welch t test was used to evaluate the effects of bilirubin, autoagglutination, lipemia, and hemolysis on the differences between measurements from the POC meter and LAB analyzer.

For the Bland-Altman analysis, confounding variables were designated as present or absent. A serum total bilirubin concentration > 1.0 mg/dL was considered indicative of icterus. A hemolysis grade ≥ 1 was considered present for statistical purposes. Reticulocytosis was defined as an absolute reticulocyte count of > 180,000 cells/µL (the upper reference limit at our laboratory). The mean value of the duplicate measurements from the POC meter was used. Blood samples with values below the lower limit of detection of the POC meter were assigned the following value:

\[
\text{Lowest limit of detection} \div (2)^{1/2}
\]

with the lowest limit of detection being 12% and 4.1 g/dL for POC Hct value and POC Hgb concentration, respectively (these were the lowest measurable values obtainable in titration studies [data not shown]).
Differences between duplicate measurements from the POC meter were determined for POC Hct values and POC Hgb concentrations. Measurements below the limit of detection for the POC meter were excluded from this analysis.

Effects of serum total bilirubin concentration on the agreement between measurements from the POC meter and LAB analyzer were assessed with a Pearson correlation coefficient ($r$). Additionally, a linear regression with 95% CI was used to quantify the association of serum total bilirubin concentration and the difference between measurements from the POC meter and LAB analyzer. The Spearman correlation coefficient ($\rho$) was calculated to quantify the association of hemolysis grade and the agreement between measurements from the POC meter and LAB analyzer.

Descriptive statistics$^4$ were calculated for measurements from the POC meter that were below the limit of detection. $\kappa$ Statistics with 95% CI for agreement between the LO reading on the POC meter and Hct $\leq$ 12% on the LAB analyzer were calculated. Values of $\kappa < 0.4$ represent poor agreement, values between 0.4 and 0.75 indicate fair to good agreement, and values $\geq 0.75$ represent excellent agreement.$^{19}$

**Results**

Blood samples from 104 animals (86 dogs and 18 cats) were tested (Figure 1). Of these, 14 samples (from 13 dogs and 1 cat) were lipemic, 23 samples (from 18 dogs and 5 cats) were icteric, 12 samples (from 12 dogs) were hemolyzed (Figures 2–4), and 14 samples (from 12 dogs and 2 cats) were autoagglutinating. For the icteric blood samples, serum total bilirubin concentration ranged from 1.0 to 21.7 mg/dL (median, 7.2 mg/dL; mean, 8.4 mg/dL). Of the 12 samples with hemolysis, 1 was graded as grade 1, 4 as grade 2, 2 as grade 3, and 2 as grade 4, and a grade was not available for 3 samples. Thirty-five blood samples had a reticulocyte count available, 15 of which had a high reticulocyte count that ranged from 82,700 to 490,300 cells/
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µL (median, 173,400 cells/µL; mean, 211,367 cells/µL), compared with 700 to 72,100 cells/µL (median, 18,500 cells/µL; mean, 27,060 cells/µL) for the blood samples with reticulocyte counts within reference range.

The mean ± SD LAB Hct value was 33.9 ± 15.73% (range, 3.9% to 75.8%). The mean LAB Hgb concentration was 11.2 ± 5.4 g/dL (range, 1 to 24.6 g/dL). The POC meter provided a value of LO for 13 blood samples, 9 of which had a LAB Hct value of ≤12%. Of these 9 samples, 6 were autoagglutinating. The 4 other blood samples with measurements identified as LO had a LAB Hct value between 13% and 17%; 3 of these 4 blood samples were also autoagglutinating. All the LAB Hct values ≤12% were identified as LO by the POC meter. Agreement between the reading of LO on the POC meter and Hct ≤12% on the LAB analyzer was very good (κ = 0.91; 95% CI, 0.78 to 1.0), indicating the POC meter accurately identified all blood samples with a severely low Hct with a sensitivity of 100% and specificity of 97.8%.

The mean differences between duplicate measurements with the POC meter were 2.4 ± 4.22% and 0.78 ± 1.39 g/dL for POC Hct values and POC Hgb concentrations, respectively. For this analysis, blood samples for which the meter read LO were excluded.

For all blood samples, mean bias between POC Hct and LAB Hct values was -1.8% with 95% LOAs of -11.1% to 7.5% (Figure 5), and mean bias between POC Hgb and LAB Hgb concentrations was -0.5 g/dL with 95% LOAs of -3.8 to 2.8 g/dL. Presence of icterus, lipemia, autoagglutination, and hemolysis did not result in larger mean bias between measurements from the POC meter and LAB analyzer, compared with the bias between measurements in the absence of these conditions. Blood samples with high reticulocyte counts had a bias for Hct values of -2.5% (95% LOA, -6.8% to 1.7%) between POC meter and LAB analyzer measurements, compared with that of blood samples without a high reticulocyte count (bias for Hct values, -0.4%; 95% LOAs, -2.0% to 1.1%; Figure 6). The Welch t test revealed no significant (all values of P > 0.19) differences in bias between LAB Hct values and LAB Hgb concentrations versus POC Hct values and POC Hgb concentrations in blood samples with and without lipemia, hemolysis, or autoagglutination.

**Icteric and hemolyzed blood samples**

With increasing serum total bilirubin concentrations, there was a low positive correlation in the difference between POC Hct and LAB Hct values ($r =$

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**Figure 4**—Comparison of LAB Hct and POC Hct values for 12 samples with hemolysis from 12 dogs. The line of perfect agreement is shown.

**Figure 5**—Bland-Altman plot of the differences in Hct values between the LAB analyzer and POC meter for all samples. Mean bias between Hct values for the POC meter and LAB analyzer was -1.8% (solid line) with 95% LOAs of -11.1% to 7.5% (upper and lower dashed lines).

**Figure 6**—Comparison of Hct values of the LAB analyzer and POC meter for patients with reticulocytosis (white circles) and without reticulocytosis (black circles). The dotted and solid lines represent the correlation between LAB Hct and POC Hct values for patients with and without reticulocytosis, respectively.
The results of the present study demonstrated good agreement between measurements obtained from the tested POC meter and the LAB analyzer, although the meter did not read any values of Hct ≤ 12%. The small negative bias for each confounding variable indicated that the POC meter generally underestimated the Hct and Hgb concentration. However, the LOAs were fairly wide. Ascertainment of the acceptability of the LOAs must be done in light of the potential for the meter to provide a clinically relevant error. The American Society for Veterinary Clinical Pathology recommends that the amount of error (combined imprecision and bias) that is acceptable for an analyzer is no more than 10% for both Hct and Hgb concentration.20 This reflects the degree of measurement error that would likely alter interpretation of patient data for medical decision-making. This meter had wide LOAs associated with constant bias, meaning the risk of a potentially substantial clinical error (> 10% difference in measurements from that of the LAB analyzer) would be greater for blood samples with lower Hct values. Although the POC meter did not read any values of Hct ≤ 12%, it did accurately identify the blood samples as LO (ie, Hct ≤ 12%) with an excellent sensitivity and specificity. Although having an actual measurement from the POC meter would be optimal, acquiring a reading of LO would likely convey enough information to ultimately not affect clinical decision-making.

There were several blood samples for which the difference between duplicate measurements and the difference between measurements from the POC meter and LAB analyzer were larger than expected. This may have contributed to the wide LOA observed. Although we could not identify a definitive cause for this, 1 possible explanation is a difference in amount of sample added to the test spot or other user error.21 The manual for the POC meter reports that the test spot must be covered with blood but does not specify how thick the layer should be. Different sample sizes could cause differences in light dispersion and cause aberrant values. Agglutination could also contribute to these discrepancies if the RBC clumps settle rapidly in the syringe prior to placing the blood drop on the test spot. Some of the large differences between measurements from the POC meter and LAB analyzer could be clinically important. Therefore, results should always be interpreted in light of the patient’s clinical signs and agglutination status.

On the basis of the findings in the present study, the POC meter was negligibly impacted by common endogenous compounds; the presence of lipemia and hemolysis did not affect meter function. The meter was unable to read several blood samples with severe autoagglutination. However, most of these samples concurrently had an Hct ≤ 12%, which made it more difficult to evaluate the exact cause of the LO reading. The meter accurately read 6 of 8 blood samples with autoagglutination with an Hct > 12%, suggesting that the Hgb concentration was more important than autoagglutination in creating this error. Agglutination of RBCs may falsely lower their concentration and falsely increase mean corpuscular volume on hematology analyzers.22-24 This is because RBC clumps are larger than a single RBC and therefore are not recognized as an RBC by the analyzer.23 Because the POC meter uses optical reflectance for its measurements, RBC clumps may reflect light differently than a single RBC. Additional agglutinated samples from less severely anemic animals must be tested to clarify this limit. The impact of cell or clump size may also be reflected in the larger bias associated with samples with a high reticulocyte count, compared with those without.

The bias between measurements from the LAB analyzer and the POC meter became more positive with increasing total serum bilirubin concentration. Bilirubin is known to produce interference with clinical laboratory analysis as result of either its spectral properties (strong absorbance around 450 nm) or its ability to react chemically with certain assays.14,25 The
POC meter uses optical reflectance to measure Hgb, and therefore, bilirubin could interfere with accuracy of the measurements from the POC meter. Despite the increased bias in meter readings in samples with high serum total bilirubin concentrations, the differences were small, even when the serum total bilirubin concentration was markedly elevated. Additionally, there were only a few samples with a serum total bilirubin concentration > 10 mg/dL, and the change in bias may not have been evident if more samples with higher bilirubin concentrations were tested. Although accuracy of the POC meter does not appear to be affected by mild increases in serum total bilirubin concentration, until additional samples with markedly high bilirubin concentrations are studied, the POC meter should be used with caution in patients with severe icterus.

Although the total number of evaluated samples exceeded our initial goal, one of the limitations of the present study was the low number of blood samples from cats and low number of samples representing each of the potentially confounding variables. Given the lack of previously described data for testing of confounding blood variables on the POC meter, an a priori power analysis was not performed, and we used recommendations for statistical analysis for pathology samples.\(^\text{15,16}\) Additionally, use of convenience samples also influenced sample numbers. Thus, it is possible that the number of samples representing each confounding variable was too small to detect a clinically important difference between the measurements from the POC meter and LAB analyzer.

Another study limitation was the use of stored, refrigerated samples. According to the manufacturer’s instruction manual, stored samples should be tested within 24 hours after collection. Storage affects RBC shape and can cause hemolysis that could affect the accuracy of the POC meter.\(^\text{20}\) However, it is considered unlikely that storage for 24 hours would cause clinically important changes.\(^\text{27}\) A study\(^\text{28}\) in horses showed storage for up to 36 hours did not make a difference in the accuracy of an Hgb meter. Furthermore, the stored samples were refrigerated and allowed to equilibrate to room temperature for 10 minutes before testing, although the temperature of the sample was not measured, so it was not possible to verify that the samples were at room temperature. Subjectivity of hemolysis grade and the unavailability of serum total bilirubin concentrations for some samples were also limitations.

In conclusion, the POC meter showed good agreement with a reference LAB analyzer for the measurement of Hct values and Hgb concentrations in blood samples from cats and dogs with a wide spectrum of conditions. However, the LOAs were fairly wide, indicating that there may be clinically important differences between the measurements from the POC meter and LAB analyzer. Although the meter provides Hgb and Hct readings, Hct must always be interpreted in light of total protein concentration, which was not provided.\(^\text{29}\) The POC meter should be used with caution in patients with autoagglutination, severe icterus, and an Hct ≤ 12%.

With that in mind, the authors consider the POC meter a potentially useful tool for serial evaluation of Hct in cats and dogs given its low cost, portability, use of a small sample volume, and speed of analysis.

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**Footnotes**

c. Roche Cobas c501, Roche Diagnostics Inc, Indianapolis, Ind.
d. Advia120, Siemens Medical Solutions USA, Inc, Malvern, Pa.
f. SAS, version 9.4, SAS Institute Inc, Cary, NC.

**References**

Use of serum hyaluronic acid as a biomarker of endothelial glycocalyx degradation in dogs with septic peritonitis
Kaela E. Shaw et al

**OBJECTIVE**
To describe daily changes in serum concentrations of hyaluronic acid (HA), a biomarker of endothelial glycocalyx degradation, in dogs with septic peritonitis and to determine whether relationships exist among serum concentrations of HA and biomarkers of inflammation and patient fluid status.

**ANIMALS**
8 client-owned dogs.

**PROCEDURES**
Serum samples that had been collected for a previous study and stored at –80°C were used. Blood samples were collected at admission and daily thereafter during hospitalization and were analyzed for concentrations of HA and interleukins 6, 8, and 10. Patient data including acute patient physiologic and laboratory evaluation score, type and amount of fluids administered daily, and daily CBC and lactate concentration results were recorded. To determine the significant predictors of HA concentration, a general linear mixed model for repeated measures was developed.

**RESULTS**
All dogs survived to discharge. Concentrations of HA ranged from 18 to 1,050 ng/mL (interquartile [25th to 75th percentile] range, 49 to 119 ng/mL) throughout hospitalization. Interleukin-6 concentration was a significant predictor of HA concentration as was total administered daily fluid volume when accounting for interleukin-6 concentration. When fluid volume was analyzed independent of inflammatory status, fluid volume was not a significant predictor. Concentrations of HA did not significantly change over time but tended to increase on day 2 or 3 of hospitalization.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Results supported the theory that inflammation is associated with endothelial glycocalyx degradation. Dogs recovering from septic peritonitis may become more susceptible to further endothelial glycocalyx damage as increasing fluid volumes are administered. (Am J Vet Res 2021;82:566–573)