Accuracy and precision of a point-of-care hemoglobinometer for measuring hemoglobin concentration and estimating packed cell volume in horses

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Objective—To determine accuracy and precision of a point-of-care hemoglobinometer for measuring hemoglobin concentration and estimating PCV in horses.

Design—Prospective trial.

Animals—56 horses.

Procedure—Blood samples were obtained from 43 horses examined at a veterinary teaching hospital. Hemoglobin concentration was measured with the hemoglobinometer and by means of the standard cyanmethemoglobin method; PCV was measured by centrifugation. Blood samples were also obtained from 12 healthy horses, and PCV of aliquots of these samples was altered to approximately 5 to 80% by removing or adding plasma. Hemoglobin concentration and PCV were then measured.

Results—For samples from the clinic patients, hemoglobin concentrations obtained with the hemoglobinometer were less than concentrations obtained with the cyanmethemoglobin method; however, there was a linear relationship between concentrations obtained with the 2 methods. Breed, sex, body weight, and duration of sample storage did not significantly affect the difference between hemoglobin concentrations obtained with the 2 methods. There was a significant linear relationship between PCV and hemoglobinometer hemoglobin concentration (PCV = \[2.83 \times \text{hemoglobin concentration}\] + 0.62). For samples from the healthy horses, a substantial negative bias was evident with the hemoglobinometer when hemoglobin concentration exceeded 16 g/dL.

Conclusions and Clinical Relevance—Results suggest that this hemoglobinometer is reasonably accurate and precise when used to measure hemoglobin concentration in blood samples from horses with a hemoglobin concentration < 16 g/dL. (J Am Vet Med Assoc 2003;223:78-83)

The oxygen-carrying capacity of blood largely depends on its hemoglobin concentration. The standard method for measuring hemoglobin concentration requires conversion of hemoglobin in a sample of blood to cyanmethemoglobin, followed by photometric quantification of the cyanmethemoglobin. This method is technically complex; therefore, samples must typically be submitted to a clinical pathology laboratory for analysis. Similarly, PCV is also often used to estimate the oxygen-carrying capacity of blood, but measuring the PCV requires the use of a centrifuge and takes several minutes. Alternatively, PCV can be estimated by multiplying hemoglobin concentration (g/dL) by 3, but this requires first measuring the hemoglobin concentration.

A portable, rapid, and technically simple method for measuring hemoglobin concentration and estimating PCV at the point of care (ie, in the field, clinic, or operating room) would be useful in the care of critically ill patients and patients under anesthesia. Recently, a point-of-care hemoglobinometer for measuring hemoglobin concentration in people has become available. The device requires 10 µL of blood for each measurement of hemoglobin concentration. The blood is drawn into a disposable microcuvette that is inserted into the measuring device. Reagents in the microcuvette lyse the RBCs and convert free hemoglobin to azidemethemoglobin. The amount of azidemethemoglobin is then measured photometrically at 2 wavelengths: 570 nm for determining hemoglobin concentration and 880 nm to correct for any turbidity in the sample. The conjugation reaction for the azide moiety is 4 times as fast as the conjugation reaction for cyanide, thus substantially reducing the time required for sample analysis. This hemoglobinometer has been evaluated for use with blood samples from horses with hemoglobin concentrations within reference limits for healthy adults (11 to 19 g/dL).

The purpose of the study reported here was to determine the accuracy and precision of a commercial point-of-care hemoglobinometer for measuring hemoglobin concentration and estimating PCV in horse blood. To do this, we compared values obtained with the hemoglobinometer with values obtained by a clinical pathology laboratory that used the standard cyanmethemoglobin method. We hypothesized that hemoglobin concentrations measured with the hemoglobinometer would not be significantly different from concentrations obtained with the standard method and that PCV could be estimated by multiplying the hemoglobin concentration obtained with the hemoglobinometer by 3.

Materials and Methods

Experimental design—Blood samples from 2 groups of horses were used to compare hemoglobin concentration obtained with the hemoglobinometer...
with concentrations obtained by means of the standard cyanmethemoglobin method. The first group consisted of equine patients admitted to the Cornell University Hospital for Animals from which blood had been collected for measurement of hemoglobin concentration as part of their clinical workup. The second group consisted of healthy adult horses from which blood samples were collected, and the hemoglobin concentration of the samples was manipulated by adding or removing plasma.

Clinic patients—Forty-three horses admitted to the Cornell University Hospital for Animals during a 2-week period were used in the study. For all horses, blood samples had been submitted to the Cornell University clinical pathology laboratory for determination of the hemoglobin concentration. Median age of the horses was 3 years (range, 2 days to 35 years); median weight was 275 kg (605 lb; range, 50 to 636 kg [110 to 1,390 lb]). There were 16 Thoroughbreds, 8 Quarter Horses, and 5 Standardbreds; breed of the remaining 14 horses was unknown or not recorded. There were 23 mares, 10 stallions, and 10 geldings. Of the 43 horses, 11 were foals < 2 weeks of age. Median age of the foals was 3 days (range, 2 to 9 days), and median weight was 57 kg (125 lb; range, 50 to 100 kg [110 to 220 lb]). There were 5 Thoroughbreds, 1 Standardbred, 4 Quarter Horses, and 1 Clydesdale. Five were female, and 6 were male.

Twenty-five of the 43 horses were > 1 year of age. Median age of these horses was 8 years (range, 2 to 35 years), and median weight was 500 kg (1,100 lb; range, 121 to 636 kg [266 to 1,390 lb]). There were 11 Thoroughbreds, 3 Standardbreds, 2 Quarter Horses, and 9 horses of unknown or mixed breed. Thirteen were mares, 2 were stallions, and 10 were geldings.

For all 43 horses, jugular vein blood samples had been collected into glass tubes containing EDTA. Samples were analyzed immediately or refrigerated at approximately +4°C until analyzed. Hemoglobin concentration was measured by use of the cyanmethemoglobin method.1 The sample was then mixed, and a 0.2-mL aliquot was removed and immediately used to fill a disposable plastic microcuvette and microhematocrit tubes.4 The microcuvette was placed into the hemoglobinometer, and the hemoglobin concentration was recorded. The microhematocrit tubes were centrifuged at 13,700 X g for 5 minutes. The PCV for each sample was calculated as the mean value for the 2 microhematocrit tubes. For each sample, all measurements were completed within 36 hours after blood sample collection. The 2 measurements of hemoglobin concentration and the measurement of PCV were made within 3 hours of each other.

Calibration of the cyanmethemoglobin equipment was verified daily according to the manufacturer’s instructions. Calibration of the hemoglobinometer was verified twice daily with a standard solution containing 12.1 g of hemoglobin/dL. The manufacturer recommends that cuvettes be stored at room temperature (15 to 30°C) in a sealed container and used within 3 months after first opening the container.7 Cuvettes used in the present study were stored in an area where temperature ranged from 19 to 25°C and humidity ranged from 35 to 55% and were used within the prescribed period.

Breed, age, sex, and weight of each animal and storage time of samples before analysis were extracted from the medical records. Color of the plasma in the microhematocrit tubes was classified by the authors (HC) as dark yellow, pale yellow, or pink (hemolyzed).

Healthy horses—Twelve healthy adult horses were also used in the study. Median age was 10 years (range, 3 to 19 years). There were 5 Standardbreds, 4 Thoroughbreds, 2 Quarter Horses, and 1 Warmblood. For each horse, jugular vein blood samples were collected with a 20-gauge needle into 6 evacuated glass tubes containing EDTA. Animal use was approved by the Cornell University Institutional Animal Care and Use Committee.

So that accuracy and precision of the hemoglobinometer could be assessed on blood samples with a wide range of hemoglobin concentrations, hemoglobin concentrations in blood samples from the 12 healthy horses were manipulated. Two tubes from each horse were centrifuged for 4 minutes at 2,772 X g to provide plasma for use as a diluent. From the remaining 4 tubes of blood from each horse, 9 aliquots (< 3 mL each) were obtained. One aliquot was not manipulated and considered the mid-range sample (PCV approx 40%). Four aliquots were centrifuged at 2,772 X g for 4 minutes, and sufficient plasma was removed to result in solutions with PCVs of approximately 80, 70, 60, and 50%. For the remaining 4 aliquots, plasma was added to produce solutions with PCVs of approximately 30, 20, 10, and 5%. Aliquots of blood were mixed by rocking for at least 10 minutes, and PCV was measured as described for samples from the clinical patients. Hemoglobin concentration was then measured with the hemoglobinometer and by use of the cyanmethemoglobin method. To prevent clogging of the sampling port when PCV exceeded 70%, samples used for the cyanmethemoglobin method were diluted 1:1 (vol:vol) with isotonic saline (0.9% NaCl) solution before analysis, and values that were obtained were multiplied by 2.4 Samples that resulted in an error message with the hemoglobinometer, indicating that hemoglobin concentration exceeded the range of the apparatus (> 25.6 g/dL),5 were also diluted 1:1 (vol:vol) with isotonic saline solution and retested. For the mid-range samples, measurements of hemoglobin concentration were repeated to assess repeatability. To measure how long the hemoglobinometer took to analyze a sample, the time from addition of blood to the microcuvette to a value appearing on the screen was recorded for 6 measurements of hemoglobin concentration for the mid-range sample.

Statistical analyses—For each set of data, linear regression analysis was used to describe the relationships between hemoglobin concentrations obtained with the 2 methods and between measured PCV and hemoglobin concentration obtained with the hemoglobinometer. For each set of data, the difference between
hemoglobin concentrations obtained with the 2 methods was plotted in modified Bland-Altman format. Two subsets of the clinical patients were identified (foals < 2 weeks old and adults > 1 year old), and differences between regression analyses of these subsets were calculated. For data from the clinical patients, a 1-way ANOVA was used to evaluate whether breed, plasma color, or sex had an effect on the difference between hemoglobin concentrations obtained with the 2 methods. For data from the clinical patients, frequency distributions of weight and duration of sample storage were non-Gaussian; hence, whether these factors affected the difference between hemoglobin concentrations obtained with the 2 methods was evaluated with the Spearman rank correlation method.

Simple linear regression analysis was used to summarize hemoglobin concentrations obtained for the 9 replicate samples for each of the healthy horses. A t test was used to determine whether slopes and intercepts of the regression equations were significantly different from 0. A paired t test was used to determine if the replicate mid-range samples were significantly different from one another. For all analyses, values of $P \leq 0.05$ were considered significant.

**Results**

Clinic patients—Hemoglobin concentrations obtained with the hemoglobinometer were less than concentrations obtained with the standard cyanmethemoglobin method; however, there was a linear relationship between concentrations obtained with the 2 methods (Fig 1). The difference in hemoglobin concentrations obtained with the 2 methods exceeded 1 g/dL for only 2 of the 43 samples, and the difference appeared to be unrelated to the concentration obtained with the cyanmethemoglobin method (Fig 2). Breed, sex, body weight, and duration of sample storage prior to analysis did not have any significant effects on the difference between hemoglobin concentrations obtained with the 2 methods ($P \geq 0.14$). Only 3 samples had pink plasma, suggesting hemolysis; the difference between hemoglobin concentrations obtained with the 2 methods was significantly ($P = 0.03$) greater for hemolyzed samples than for nonhemolyzed samples.

Regression lines for hemoglobin concentration obtained with the cyanomethemoglobin method versus concentration obtained with the hemoglobinometer were generated for foals and adult horses. Slopes of the 2 regression lines were not significantly different, but the intercepts were (0.64 g/dL in foals and 1.40 g/dL in adult horses, $P = 0.01$).

The relationship between measured PCV and hemoglobin concentration obtained with the hemoglobinometer was linear (Fig 3). For the whole population of clinic patients, the slope of the regression equation was 2.83. For the foals and adult horses, the slopes of the regression equations were significantly ($P < 0.001$) different (3.08 and 2.65, respectively); the intercepts were not significantly different.

Healthy horses—Hemoglobin concentrations obtained with the cyanmethemoglobin method ranged from 1.6 to 33.4 g/dL for samples from the healthy horses. For each horse, a regression equation for hemoglobin concentration obtained with the cyanmethemoglobin method as a function of hemoglobin concentra-
tion obtained with the hemoglobinometer was calculated. Slopes of the regression equations for these 12 horses ranged from 1.01 to 1.08, and intercepts ranged from –0.40 to 0.24. By inspection, these regression equations were similar; therefore, a modified Bland-Altman plot of the pooled data was used to illustrate the difference between hemoglobin concentrations obtained with the 2 methods (Fig 4). Inspection of the plot suggested that there was a substantial negative bias when hemoglobin concentration obtained with the cyanmethemoglobin method exceeded 16 g/dL. The highest hemoglobin concentration obtained with the hemoglobinometer was 25.4 g/dL. For 12 samples, the PCV exceeded 72%, and the hemoglobinometer gave an error message. These samples were diluted and retested as described.

Mean ± SD difference in hemoglobin concentration when mid-range samples (n = 10) were tested multiple times was 0.02 ± 0.16 g/dL; the mean value was not significantly different from 0. Median time to complete a measurement with the hemoglobinometer was 46 seconds (range, 44 to 47 seconds; n = 6).

For each of the 12 horses, a regression equation for the relationship between measured PCV and hemoglobin concentration obtained with the hemoglobinometer was calculated. Slopes of the regression equations ranged from 2.68 to 2.99, and intercepts ranged from –2.5 to –0.04.

Discussion

Results of the present study suggest that this point-of-care hemoglobinometer may be reasonably accurate when used to measure hemoglobin concentration in blood samples from adult and juvenile horses. For samples with hemoglobin concentrations obtained with the standard cyanmethemoglobin method that ranged from 10 to 16 g/dL, the hemoglobinometer typically underestimated the hemoglobin concentration. However, the difference was generally small and exceeded 1 g/dL for only 2 of 43 samples. Accuracy was not affected by breed, sex, body weight, or storage of the samples for up to 36 hours at 4°C. Any difference in accuracy of the hemoglobinometer between foals < 2 weeks old and adults > 1 year old was not likely to be clinically important. When the range of hemoglobin concentration was expanded artificially to include values from 1.6 to 33.4 g/dL, accuracy was not affected with low hemoglobin concentrations. However, when the hemoglobin concentration...
Aliquots of blood samples from each horse were manipulated by removing or adding plasma to result in samples with hemoglobin concentrations ranging from 1.6 to 33.4 g/dL.

When hemoglobin concentration measured with the hemoglobinometer exceeded 25.4 g/dL or the PCV of samples tested with the cyanmethemoglobin method exceeded 70%, samples were diluted with saline solution and reanalyzed. This may have contributed somewhat to the increased variability and bias seen with higher hemoglobin concentration. However, visual inspection of the data suggested that variability and negative bias were present even with hemoglobin concentrations less than those necessitating sample dilution. The manufacturer of the hemoglobinometer states that output of the device may no longer be linear and that it will give an error message when hemoglobin concentration exceeds 23.5 g/dL; however, when hemoglobin concentration exceeds 16 g/dL, the amount of underestimation is small enough to be clinically unimportant when hemoglobin concentration by as much as 3.2 g/dL.

The small difference between repeated measurements (0.02 ± 0.16 g/dL) suggested that the hemoglobinometer provides precise measurements of hemoglobin concentration. However, samples for which repeated measurements were obtained had PCVs of approximately 40%, and it is possible that precision is less at extreme hemoglobin concentrations.

In summary, we conclude that the hemoglobinometer can be used to measure hemoglobin concentration in blood samples from adult and neonatal horses, but that values tend to be less than those obtained with the standard cyanmethemoglobin method. The difference is small enough to be clinically unimportant when hemoglobin concentration <16 g/dL; however, when hemoglobin concentration exceeds 16 g/dL, the amount of underestimation may be clinically relevant. We further conclude that PCVs in horses can be estimated by multiplying hemoglobin concentrations obtained with the hemoglobinometer by 2.8 and subtracting 0.6. In foals <2 weeks old, PCV can be estimated by multiplying hemoglobin concentration obtained with the hemoglobinometer by 3.1 and subtracting 3.2.

Storage of horse blood in EDTA at 4°C for up to 48 hours does not cause hemolysis. All blood samples used in the present study were analyzed within 36 hours after collection. Therefore, the hemolysis that we observed in 3 of the 43 samples was unlikely to be a result of storage. All 3 hemolyzed samples yielded lower hemoglobin concentrations with the hemoglobinometer than with the cyanmethemoglobin method. The hemoglobinometer corrects for turbidity associated with hemolysis by measuring absorption at 880 nm in addition to absorption at 570 nm. Such a correction is not automatic with the cyanmethemoglobin method; therefore, the cyanmethemoglobin method may systematically overestimate the hemoglobin concentration in hemolyzed samples.

In 1 study, regression analysis yielded a regression equation of PCV = (2.8 × hemoglobin concentration) + 0.8. This is similar to results for the present study in which the regression equation was PCV = (2.8 × hemoglobinometer hemoglobin concentration) − 0.6. In foals <2 weeks old, PCV could be estimated by multiplying hemoglobin concentration measured with the hemoglobinometer by 3.1 and subtracting 3.2. The significantly higher slope of the regression equation for foals, compared with adult horses, may be explained by the lower concentration of hemoglobin in foal RBCs.

With the hemoglobinometer used in the present study, results were available in <1 minute, making it convenient for use at the point of care.
References