PORTABLE BLOOD GLUCOMETERS ARE ROUTINELY USED FOR RAPID ASSESSMENT OF BG CONCENTRATIONS IN COMPANION DOMESTIC RABBITS (ORYCTOLAGUS CUNICULI). PORTABLE INVESTIGATION FOR CORRECTION FORMULAS ON THE BASIS OF PACKED CELL VOLUME FOR BLOOD GLUCOSE CONCENTRATION MEASUREMENTS OBTAINED WITH PORTABLE GLUCOMETERS WHEN USED IN RABBITS

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OBJECTIVE
To determine effects of PCV on blood glucose (BG) concentration measurements obtained with a human portable blood glucometer (HPBG) and a veterinary portable blood glucometer (VPBG) on canine (cVPBG) and feline (fVPBG) settings (test methods) when used in rabbits and to develop correction formulas to mitigate effects of PCV on such measurements.

SAMPLE
48 resuspended blood samples with known PCVs (range, 0% [plasma] to 92% [plasma and packed RBCs]) from 6 healthy research rabbits (experimental sample set) and 252 historic measurements of BG concentration and PCV in 84 client-owned rabbits evaluated at a veterinary hospital (validation data set).

PROCEDURES
Duplicate measurements of BG concentration with each test method and of PCV were obtained for each sample in the experimental sample set, and the mean results for each variable for each test method and sample were compared with results from a clinical laboratory analyzer (reference method) used to determine the true BG concentration for each sample. Mean ± SD differences in measurements between the reference and test methods were calculated. Linear regression and modified Clarke error grid analysis were used to develop correction formulas for the test methods given known PCVs, and these formulas were evaluated on the validation data set with linear regression and a modified Clarke error grid.

RESULTS
Blood glucose concentrations were falsely low for cVPBG and fVPBG used on samples with PCV < 31% and were falsely high for all test methods used on samples with PCV > 43%. Compared with original measurements, formula-corrected measurements overall had better agreement with reference method measurements for the experimental sample set; however, only the formula-corrected HPBG measurements had improved agreement for the validation data set.

CONCLUSIONS AND CLINICAL RELEVANCE
Findings indicated that, in rabbits, HPBG measurements had improved accuracy with the use of the correction formula HPBG measurement of BG concentration + ([0.75 X PCV] – 15); however, the correction formulas did not improve the accuracy of VPBG measurements, and we believe that neither the cVPBG nor fVPBG should be used in rabbits. (Am J Vet Res 2020;81:642–650)
in inaccurate BG measurements with PBGs in that, compared with reference laboratory results, plasma glucose concentrations measured with PBGs are generally falsely lower for hemoconcentrated samples and falsely higher for hemodilute samples. This inaccuracy is thought to be related to the degree of plasma displacement by erythrocytes, and PBGs that use coulometric technology are least affected by Hct interference. Similar effects of hemodilution and hemoconcentration on BG measurements with PBGs have been reported in rabbits. Although that study was performed in a clinical setting, with a mixed population of healthy and diseased animals, the diverse clinical histories and treatments did not allow for the production of a correction formula.

Our primary purpose for the study reported here was to determine effects of PCV on BG concentration measurements obtained with an HPBG, cVPBG, and fVPBG when used in rabbits and to develop correction formulas to mitigate effects of PCV on such measurements. We hypothesized that a correction formula for each PBG would improve agreement between results obtained with the given PBG and the LABgluc.

Materials and Methods

Experimental sample set

Sample collection—Blood samples were obtained from 6 research rabbits that had been randomly assigned numbers as part of an educational teaching lab. The animals were ordered from a commercial laboratory animal vendor. Blood sample collection was approved by the University of Georgia Institutional Animal Care and Use Committee (animal use proposal: A2014-01-013-R3). For each rabbit, a standard aseptic jugular venipuncture technique was performed with a 22-gauge winged infusion catheter and 50 mL of blood was collected into a 60-mL syringe containing 625 U of sodium heparin anticoagulant. Samples were kept at 4°C until processed.

Sample processing—Within approximately 30 minutes after collection, the BG concentration was measured in duplicate and the PCV was measured in triplicate for each whole blood sample. The BG concentration was measured with an HPBG and a VPBG on canine and feline settings (test methods), yielding 6 measurements of BG concentration/whole blood sample. Measurements of PCV were completed for all whole blood samples by filling 3 separate microhematocrit tubes, then centrifuging to a 150-mL disposable blood transfer bag. After the test method measurements of BG concentration were obtained, the samples were centrifuged at 1,500 X g for 6 minutes. The plasma was decanted and immediately frozen for storage at -20°C. Within 7 days after collection, these plasma samples were batch analyzed on an LABgluc (reference method) that used a hexokinase oxidase reaction method to determine the true BG concentration for each sample. During all phases of processing and handling, all samples, including whole blood, plasma, packed RBCs, and subsequent suspensions, were stored at 4°C.

Validation data set

The validation data set was an external data set of historic BG concentration measurements from a previous study of 84 client-owned healthy and diseased rabbits that had been evaluated at a veterinary hospital and that had BG concentration measurements obtained with the same models of VPBG and HPBG used in the present study and an LABgluc that, similar to the one in the present study, used hexokinase oxidase reaction method to determine BG concentration. For each of the rabbits from the previous study, we used the respective means of historic duplicate measurements of BG concentration performed with each of an HPBG, cVPBG, and fVPBG, compared with the corresponding LABgluc measurement (true BG concentration).

Statistical analysis

Development of correction formulas—Correction formulas for results of BG concentrations measured
with the HPBG, cVPBG, and fVPBG for the experimental sample set were developed on the basis of a simple linear regression model, with the PCV versus the mean ± SD difference in BG concentration (reference method [LABgluc] measurement minus the mean ± SD test method measurement [HPBG, cVPBG, or fVPBG]). Linearity assumption was checked with scatterplots for the results of PCV (independent variable) and BG concentration differences (dependent variables). Normal predicted probability plots were used to check for normal distribution of the residuals of the regression model. Homoscedasticity was checked by plotting the predicted values and residuals on scatterplots and visually inspecting the plots for distribution of the data. Pearson correlation coefficient (r) analysis was performed, and coefficients of determination (R²) were calculated to assess relationships between BG measurements obtained with the reference method (LABgluc) versus the test methods (HPBG, cVPBG, and fVPBG). Available software was used for all analyses, and values of P < 0.05 were considered significant.

In addition, multiple regression models were used to develop alternative correction formulas for results obtained with the HPBG and fVPBG. The multiple regression models included PCV and PBG results as independent variables and LABgluc results as the dependent variables. These formulas were then applied to BG concentration results for the validation data set, and formula-corrected BG concentrations were calculated for the HPBG and the fVPBG. Next, the mean ± SD differences and LoA in formula-corrected BG concentrations (LABgluc results minus formula-corrected PBG results) obtained with formulas derived from the simple linear regression models versus the multiple regression models were compared. The 95% LoAs were determined from Bland-Altman plots, calculating ± 1.96 SD centered on the mean difference. Coefficients of variation were determined from duplicate measurement.

Validation of correction formula—The correction formulas were then evaluated on a validation data set comprising an external data set of historic BG concentration measurements obtained in 84 rabbits from a previous study. Mean BG concentration differences for each test method with and without use of correction formulas were calculated, and results were assessed with linear regression and Clarke error grid analysis, as modified in a previous study. Hypoglycemia was defined as BG concentration < 75 mg/dL, and hyperglycemia was defined as BG concentration > 150 mg/dL. Zone A of the error grids included sample results for which results of both the test and reference method similarly indicated hyper- or hypoglycemia, and thus, use of the test method would have led to appropriate diagnosis of glycemic status. Zone B included sample results for which the reference method indicated euglycemia, but the test method indicated hypo- or hyperglycemia and would have led to underdiagnosis of hypo- or hyperglycemia. Zone D included sample results for which the test method indicated the opposite abnormal glycemic status (hyperglycemia vs hypoglycemia) than did the reference method and would have led to hypoglycemic rabbits being treated for hyperglycemia and to hyperglycemic rabbits being treated for hypoglycemia.

Results

Experimental sample set

For the samples of whole blood from the 6 healthy research rabbits used in the experimental sample set, the mean baseline PCV was 36% (range, 32% to 42%; reference range, 31% to 43%2), and the mean baseline BG concentrations with the test methods were 257.3 mg/dL (range, 194 to 362.5 mg/dL) for the cVPBG, 234.3 mg/dL (range, 187.5 to 334 mg/dL) for the fVPBG, and 209.6 mg/dL (range, 165 to 288.5 mg/dL) for the HPBG. Following processing of the whole blood samples to obtain packed RBCs and plasma, 8 resuspended samples were generated for each rabbit, yielding 48 suspension samples with PCVs that ranged from 0% (plasma) to 92% (packed RBCs). Of the 48 suspension samples, 16 had PCVs within reference limits (PCV, 31% to 43%), 24 had PCV < 31%, and 8 had PCV > 43%. All samples yielded readable BG concentration measurements for the devices used. At PCVs below the lower reference limit (< 31%), results for BG concentration measured with the cVPBG and fVPBG were higher than those obtained with the LABgluc, and at PCVs above the upper reference limit (> 43%), BG concentration results measured with the cVPBG, fVPBG, and HPBG were lower than those obtained with the LABgluc (Figure 1). Overall, the mean ± SD difference-
es in results for BG concentration measured with the LABgluc versus the HPBG, cVPBG, and fVPBG were 8.1 ± 12.7 mg/dL, −27.9 ± 48.7 mg/dL, and −54.2 ± 55.5 mg/dL, respectively (Table 1).

On the basis of the slopes and intercepts of the linear regression lines, with the PCV versus the mean ± SD difference in BG concentration (reference method [LABgluc] measurement minus the mean ± SD test method measurement [HPBG, cVPBG, or fVPBG]), predictive formulas were developed to correct results obtained with the PBGs to more accurately predict the true BG concentrations. The mean differences in formula-corrected BG concentrations were similar for the correction formulas developed on the basis of simple linear versus multiple regression; therefore, these simpler linear regression correction formula for each PBG were used for further assessment:

HPBG formula-corrected measurement = HPBG measurement of BG concentration + ([0.75 X PCV] − 15)

fVPBG formula-corrected measurement = fVPBG measurement of BG concentration + ([3.3 X PCV] − 130)

cVPBG formula-corrected measurement = cVPBG measurement of BG concentration + ([3.6 X PCV] − 167)

After the developed formulas were applied, the mean ± SD differences in BG concentration between LABgluc measurements and formula-corrected HPBG, fVPBG, and cVPBG measurements decreased to −0.3 ± 7.1 mg/dL, −0.7 ± 15.2 mg/dL, and 0.7 ± 21.7 mg/dL, respectively, compared with original measurements (Table 1). Mean slopes of the regression lines were 0.8, with an intercept of −15.3 (R² = 0.97; P < 0.001), for the formula-corrected HPBG model; 3.3, with an intercept of −130.5 (R² = 0.97; P < 0.001), for the formula-corrected fVPBG model; and 3.6, with an intercept of −167.5 (R² = 0.85; P < 0.001), for the formula-corrected cVPBG model (Figure 2).

Validation data set

Two hundred fifty-two historic BG concentration measurements from 84 rabbits were used for the validation data set. The reported PCVs ranged from 14% to 53%, and BG concentration measurements ranged from 29 to 417.5 mg/dL for the HPBG, 28 to 494.5 mg/dL for the fVPBG, and 31 to 405 mg/dL for the cVPBG. Because of the variability we detected in cVPBG results and on the basis of findings from a previous study that used the same glucometer, we considered cVPBG measurements inferior to fVPBG measurements and therefore did not include cVPBG measurements in subsequent linear regression or modified Clarke error grid analyses.

HPBG—Mean ± SD difference in original BG concentration measurements between the LABgluc and HPBG was 9.6 ± 12.2 mg/dL, with a maximum difference of 44 mg/dL and 95% LoA of 14.7 to 33.9 mg/dL for the validation data set. However, when formula-corrected HPBG measurements were considered, the mean ± SD difference in BG concentration was −3.86 ± 10.8, with a maximum difference of 38 mg/dL and 95% LoA of −25.5 to 17.7 mg/dL.

Table 1—Summary of differences and agreement in results for BG concentration measurements between those obtained with a reference method (LABgluc) and test methods (original and PCV formula-corrected measurements obtained with an HPBG, cVPBG, and fVPBG) for 48 blood samples with known various PCVs obtained by processing and resuspending (plasma and packed RBCs) samples from 6 healthy research rabbits.

<table>
<thead>
<tr>
<th>PBG measurements</th>
<th>Mean ± SD difference in BG (mg/dL)</th>
<th>95% LoA (mg/dL)</th>
<th>Maximum absolute difference (mg/dL)</th>
<th>Median (range)</th>
<th>IQR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPBG Original</td>
<td>8.1 ± 12.7</td>
<td>−16.9 to 33.0</td>
<td>58</td>
<td>1.45 (0–10.7)</td>
<td>0.7–3.2</td>
</tr>
<tr>
<td>Formula corrected</td>
<td>−0.3 ± 7.1</td>
<td>−14.1 to 13.6</td>
<td>29.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>fVPBG Original</td>
<td>−27.9 ± 48.7</td>
<td>−123.5 to 67.5</td>
<td>134</td>
<td>1.2 (0–5.3)</td>
<td>0.4–2.4</td>
</tr>
<tr>
<td>Formula corrected</td>
<td>−0.7 ± 15.2</td>
<td>−30.5 to 29.2</td>
<td>46.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>cVPBG Original</td>
<td>−54.2 ± 55.5</td>
<td>−163.1 to 54.6</td>
<td>185</td>
<td>1.45 (0–34.0)</td>
<td>0.6–2.5</td>
</tr>
<tr>
<td>Formula corrected</td>
<td>0.7 ± 21.7</td>
<td>−30.5 to 29.2</td>
<td>64.8</td>
<td>—</td>
<td>—</td>
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</table>

—— = Not calculated because the coefficient of variation should be calculated only on native data rather than on data after correction. CV = Coefficient of variation. IQR = Interquartile (25th to 75th percentiles) range.

Table 2—Results of regression analysis of the differences in BG concentration measurements between those obtained with a reference method (LABgluc) and test methods (HPBG, cVPBG, and fVPBG) for the 48 rabbit blood samples described in Table 1.

<table>
<thead>
<tr>
<th>PBGs</th>
<th>Regression equation</th>
<th>Intercept</th>
<th>Slope</th>
<th>r</th>
<th>R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPBG</td>
<td>y = −15.25 + 0.75x</td>
<td>−20.32</td>
<td>0.70</td>
<td>0.83</td>
<td>0.69</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>fVPBG</td>
<td>y = −130.08 + 3.28x</td>
<td>−141.00</td>
<td>3.60</td>
<td>0.95</td>
<td>0.90</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>cVPBG</td>
<td>y = −166.95 + 3.62x</td>
<td>−182.55</td>
<td>3.17</td>
<td>0.92</td>
<td>0.85</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
fVPBG—Mean ± SD difference in BG concentration measurements between the LABgluc and fVPBG was –24.7 ± 27.5 mg/dL, with a maximum difference of 32.5 mg/dL and 95% LoA of –79.7 to 30.3 mg/dL. However, when formula-corrected fVPBG measurements were considered, the mean ± SD difference in BG concentration was –19.8 ± 27.1 mg/dL, with a maximum difference of 52.3 mg/dL and 95% LoA of –74.1 to 34.5 mg/dL.

Linear regression and modified Clarke error grid analyses—Results of linear regression indicated that correlation with results obtained with the reference method was higher ($r = 0.99$) for formula-corrected HPBG measurements, compared with original HPBG measurements ($r = 0.98$); however, the correlation was the same for original and formula-corrected fVPBG measurements ($r = 0.96$; Table 3). Results of modified Clarke error grid analysis indicated fewer...
zone C errors (those that would have led to underdiagnosis of hypo- or hyperglycemia) for the formula-corrected (1/84 [1%]) versus original (14 [17%]) HPBG measurements, but more zone C errors for the formula-corrected (4 [5%]) versus original (2 [2%]) fVPBG measurements (Figure 3). Because no results of the modified Clarke error grid analysis were in zone D, which represented results that would have led to opposite diagnoses (hyper- vs hypoglycemia), zone D was not depicted in the error grids.

**Discussion**

Portable blood glucometers are frequently used in the initial triage of critical animals and in serial monitoring of intensively managed patients. The inaccuracy of these point-of-care devices is recognized;
however, their rapid results on small sample sizes contributes to their frequent use, particularly with critical patients. An ideal solution would be the development of an accurate point-of-care glucometer that uses small sample sizes and that can be implemented in clinical settings. In lieu of such a device, understanding factors that affect the accuracy of point-of-care glucometers and developing methods to mitigate these factors should be pursued. Improved BG monitoring would enhance patient care in clinical settings and improve clinical research in which point-of-care glucometers are used.

A study of 907 rabbits shows that BG concentration is associated with prognosis and subsequent treatment in clinically ill rabbits in that rabbits with BG concentration > 360 mg/dL had worse prognoses than did those with a BG concentration < 360 mg/dL and that the mean BG concentration was 445 mg/dL for rabbits with gastrointestinal obstruction, compared with 153 mg/dL for rabbits with gastrointestinal stasis. In that study, the same model of PBG was used to generate all BG measurements; however, measurements of PCV were not reported. On the basis of findings in the present study, we suspect that the BG measurements in the previous study may have been affected by low PCV, resulting in altered results for BG concentration. Moreover, anemia has been associated with disease in rabbits, which reinforces the importance of analyzing concurrent factors when assessing BG concentration in sick rabbits.

In addition, a study shows that rabbits with a hyponatremia of < 129 mEq/L (reference range, 136 to 147 mEq/L) had a 2.3-fold increased risk of death within 7 days after clinical presentation. As in humans, plasma sodium concentration is often low in rabbits with hyperglycemia, and the investigators of that previous study recommended that BG and serum sodium concentrations be measured concurrently. On the basis of findings in the present study, we suggest that PCV should also be measured and considered along with BG and serum sodium concentrations. In the present study, our use of healthy research rabbits for the experimental sample set helped mitigate potential health factors that could have otherwise contributed to altered measurements with PBGs.

Results of the present study were consistent with previous studies that show PCV affects BG concentration measurements in dogs and humans. In addition, our findings that the HPBG yielded results more consistent with the LABgluc results than did the VPBG when used to measure BG concentration in rabbits was consistent with a previous study. Although the primary objective of the present study was to derive correction formulas for BG concentration measurements obtained with the particular PBGs when used in rabbits, our findings also provided the opportunity to confirm previous recommendations for the use of the particular model of HPBG, versus the particular model of VPBG, when measuring BG concentration in rabbits. Similarly, a study of 142 nonhuman primates shows that the evaluated HPBGs were more accurate than the evaluated VPBGs. In addition, findings from that study indicate that BG concentration measurements were lower when obtained from venous blood samples, compared with capillary blood samples. The difference in measurements may be affected by whether food has been withheld and by differences in PBG. Thus, the effect of sample site collection should be taken into account when PBGs are used. In the present study, measurements were taken from venous blood (jugular veins for the experimental sample set; saphenous veins for the validation data set).

Humans, along with nonhuman primates and odontocete whales, have a higher concentration of glucose in the RBCs, compared with other mammals, such as dogs and cats. For instance, glucose within RBCs is approximately 60%, 12%, and 7% of the total BG concentration in humans, dogs, and cats, respectively, and is approximately 15% in rabbits. Whether such differences in glucose distribution affected BG measurements in the present study was unclear but should be considered in future research.

It should be noted that the preference for the HPBG over the VPBG is specific to the models used in the present study, and extrapolation to other HPBGs cannot be guaranteed. Human glucometers have been shown to have varying accuracy, and human correction formulas are specific to each PBG model. Correction formulas for specific PBGs have been developed for dogs, cats, and humans and should be applied only to measurements obtained with those PBGs when used in those species.

Although use of the correction formulas that we developed to mitigate the effect of PCV on BG concentration measurements decreased the mean and absolute differences in BG concentration measurements between the LABgluc and PBGs, the formula-corrected HPBG results were still more accurate than the formula-corrected VPBG results. This may have been because of the higher actual random error present in results for the VPBG. In addition, because PBGs were tested side by side and the same VPBG was used sequentially on the canine setting and then on the feline setting, there was potential for mis-selection (ie canine setting vs feline setting); however, if such would have occurred, it was likely that the results would have been essentially identical. Randomized testing order could have allowed us to confirm this; however, considering the timing between analyses, testing order and carryover potential likely had minimal to no impact on the final results. Furthermore, the noticed inherent random error of the VPBG may have affected the results. Owing to lack of improvement with the correction formulas and inherent random error, the model of VPBG used in the present study did not appear to be as accurate in assessing rabbit BG concentration, compared with the HPBG. Another limitation of the present study was the lack of manufacturer-established accuracy of
the PBGs, compared with a gold standard, for use in rabbits; however, not having such was understandable because measuring BG concentration in rabbits is not the intended use of the devices. Nonetheless, a known accuracy for each would have allowed a robust demonstration of bias reduction but also would have required a much larger sample size and was outside the scope of the present study. Further research with a larger sample size is recommended for future research regarding use of PBGs in rabbits. Another limitation of the present study was that although the LABgluc for the experimental sample set and the LABgluc for the validation data set used the same analysis method (hexokinase oxidase), 2 different types of analyzers were used, which likely introduced variability. This limitation could have been reduced by use of the same LABgluc, which may have resulted in improved correction in the validation data set.

The validation data set comprised historic BG measurements of healthy and ill rabbits, and various factors (eg, drugs, ascorbate, and maltose31,32) affect PBG results. Further, maltose and ascorbate were not measured in the rabbits of the validation data set. However, with use of healthy rabbits for the experimental sample set and because 6 rabbits were the source of the full range of sample PCVs tested, various physiologic and pharmacological factors that could impact BG concentration results were minimized, compared with samples for the validation data set. Glucometers4 for use in humans have been developed to measure potentially interfering factors (eg, Hct and concentrations of ascorbic acid, uric acid, acetaminophen, bilirubin, maltose, and galactose) and correct the BG concentration measurement before displaying it.

The present study was successful in the creation and validation of correction formula to help mitigate the effect of PCV on BG concentration measurement to improve the accuracy of HPBGs when used in rabbits. In addition, findings partially supported our hypothesis that correction formulas would improve agreement in results between the PBGs and an LABgluc in that agreement was improved for the HPBG but not the VPBG on either setting (canine or feline). On the basis of results for the PBGs tested, we would prefer use of the HPBG, instead of the VPBG, when use of a PBG in rabbits is needed, and we do not recommend use of the tested model of VPBG (on either setting) when measuring BG concentration in rabbit plasma. Our findings underscored the importance of considering PCV regarding BG concentration of animals in research and clinical settings. Future research on BG concentration measurement should include development of point-of-care devices with improved accuracy, creation and validation of correction formulas for use in PBGs in other animal species and with other PBG models, and analysis of contributing factors in conjunction with PCV that may affect BG results. Additionally, development of a VPBG that can simultaneously measure Hct and BG concentration and make appropriate corrections would be ideal.

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Footnotes


b. Heparin sodium injection USP, 1,000 U/mL, Hospira, Lake Forest, Ill.

c. Accu-Chek Aviva, Roche Diagnostics, Indianapolis, Ind.

d. AlphaTRAK 2, Abbott Laboratories, Abbott Park, Ill.

e. Triac Centrifuge, BD, Franklin Lakes, NJ.


g. Teruflex transfer bag, Terumo Medical Corp, Somerset, NJ.


i. Hitachi P-Modular 800, Roche Diagnostics, Indianapolis, Ind.

j. Dimension EXL, Siemens Healthcare Diagnostics Inc, Tarrytown, NY.

k. SPSS Statistics, version 22.0, IBM Corp, Armonk, NY.


References


