

Performance of two portable meters and a benchtop analyzer for blood glucose concentration measurement in rabbits

Paolo Selleri, DMV, PhD; Nicola Di Girolamo, DMV; Gianluca Novari, DMV

Objective—To evaluate performance of a human portable blood glucose meter (PBGM), a veterinary PBGM, and a veterinary benchtop analyzer for measuring blood glucose concentration in rabbits and to evaluate the effect of sample characteristics on their performance.

Design—Observational prospective cross-sectional study.

Sample—Blood samples from 89 pet rabbits.

Procedures—Blood glucose concentration was measured with a human PBGM (n = 89 rabbits), a veterinary PBGM (89), and a benchtop analyzer (32) and compared with results obtained with plasma in a laboratory analyzer (hexokinase method).

Results—The human PBGM underestimated blood glucose concentration, had decreased accuracy at high Hct concentrations, and had the lowest total error observed (11.4%). The veterinary PBGM overestimated blood glucose concentration, had decreased accuracy at low Hct and at high blood glucose concentrations, and had the highest total error (15.5% and 29.8% for canine and feline settings, respectively). The benchtop analyzer had good accuracy and was not influenced by Hct or glucose concentrations. Clinical errors would have occurred in 0% of cases with the human PBGM and with the benchtop analyzer and in 9% (canine setting) to 6.7% (feline setting) cases with the veterinary PBGM.

Conclusions and Clinical Relevance—The use of the human PBGM evaluated in this study is advisable if point-of-care testing of blood glucose in rabbits is needed and benchtop analyzers are not available. The use of the veterinary PBGM evaluated in this study may alter both treatment and diagnostic decisions because of the overestimation of glucose concentrations in some rabbits. (*J Am Vet Med Assoc* 2014;245:xxx-xxx)

Rabbits are among the most common pets in the households of Europe and the United States¹⁻³ and represent a substantial part of most small animal practices' patients in the United Kingdom.⁴ Blood glucose alterations in rabbits are common,⁵ so repeated and accurate glucose measurement may be useful. To obtain more frequent determinations of blood glucose concentration, continuous glucose monitoring and POC glucose testing may be performed. Although continuous glucose monitoring is being increasingly used in human medicine,⁶ its use in veterinary medicine is still limited because of financial and practical concerns.⁷ Besides standard laboratory methods, POC testing permits instantaneous reporting of blood glucose concentrations and has become invaluable in human and veterinary medicine.^{8,9} Point-of-care testing broadly refers to any laboratory testing performed outside the conventional reference laboratory and implies close proximity to patients.¹⁰ A variety of POC testing

ABBREVIATIONS

CI	Confidence interval
CV	Coefficient of variation
MCC	Multiple correlation coefficient
PBGM	Portable blood glucose meter
POC	Point of care

methods for glucose are available, including noninstrumental systems (ie, reagent test strips), portable analyzers (ie, PBGMs), and benchtop analyzers (ie, automated biochemical analyzers).¹⁰⁻¹²

Portable blood glucose meters are handheld instruments that use reagent test strips to provide immediate glucose concentration measurement results. Since the first PBGMs were developed in the 1970s, performance of PBGMs has been a constant concern in human medicine.^{9,13} Most commercially available PBGMs were designed for human use. Recently, PBGMs designed for animal use, which should account for species-specific differences in distribution of glucose between plasma and erythrocytes, have been developed. To assess performance of PBGMs, glucose concentrations measured with PBGMs are typically compared with values obtained by use of a laboratory analyzer that uses the hexokinase reference method.¹⁴ In veterinary medicine, performance of PBGMs has been assessed in dogs,¹⁴ cats,¹⁵ horses,¹⁶ seabirds,¹⁷ Hispaniolan Amazon par-

From the Clinica per Animali Esotici, Centro Veterinario Specialistico, Via Sandro Giovannini 53, 00137 Roma, Italy (Selleri, Di Girolamo), and Ematos Vet Lab, Via Rapagnano, 89, 00138 Roma, Italy (Novari). Presented in abstract form at the 12th Annual Conference of the Association of Exotic Mammal Veterinarians, Indianapolis, September 2013.

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Address correspondence to Dr. Di Girolamo (nicoladiggi@gmail.com).

rots,¹⁸ deer,¹⁹ sheep,²⁰ cattle,²¹ alpacas,²² and ferrets.²³ Results of previous studies indicate that discrepancies between PBGMs and reference methods may be clinically relevant, emphasizing the importance of assessing individual meter performance in the target species. Depending on the species, sample characteristics may differently affect accuracy of PBGMs: for instance, Hct affects accuracy of PBGMs in dogs²⁴ but no effect has been proven in cats.²⁵ Although rapid testing and the low blood volume required make PBGMs particularly attractive in critical care of rabbits, no data evaluating the performance of POC analyzers for blood glucose measurement in rabbits are currently available.

Experience suggests that hypoglycemia and hyperglycemia are common in young, anorexic rabbits and in critically ill rabbits, respectively.²⁶ A recent study⁵ provided the first evidence that clinical monitoring of blood glucose in pet rabbits should be recommended: blood glucose concentration was determined in a large population of pet rabbits ($n = 922$), and results indicated that glucose measurement can be used to distinguish between intestinal stasis and intestinal obstruction.⁵ In that study,⁵ a commercially available PBGM^a developed for humans was used. Unfortunately, accuracy and precision of that instrument have never been evaluated in rabbits.

Therefore, the objective of the study reported here was to compare the performance of a human PBGM, a veterinary PBGM, and a veterinary benchtop analyzer with the performance of a laboratory-based analyzer in the measurement of glucose concentration in rabbits and determine the potential effects on clinical decision making. Furthermore, we wanted to evaluate the relationship between varying Hct and glucose concentrations and performance of the POC analyzers. The specific hypotheses were that the human PBGM would underestimate blood glucose concentration as in other species and that Hct would influence the performance of the instruments.

Materials and Methods

Study design and population—An observational prospective cross-sectional study was planned. Sequentially admitted pet rabbits undergoing blood sampling in the Clinica per Animali Esotici, Rome, for unrelated diagnostic reasons were included in the study. The predefined exclusion criterion was that the rabbit did not need blood collection. All rabbits from which a blood sample was obtained in the clinic in the study period (February to March 2013) were included in the study. Healthy and diseased rabbits were included to ensure that a wide range of analyte concentrations were evaluated. The healthy rabbits were undergoing elective surgery (castration or spaying) and underwent blood analysis for routine preliminary assessment of clinical status (ie, hematologic evaluation and serum biochemical analysis). All other rabbits included in the study were considered diseased; in these rabbits, blood was collected before semielective or urgent surgeries or as necessary to confirm the diagnosis of a pathological status. Diseased rabbits underwent blood sampling for several primary purposes, including hematologic evaluation, serum biochemical analysis, protein electropho-

resis, or serologic testing. The study was performed in compliance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. The owners gave written informed consent to the inclusion of samples in the study.

Procedures—The rabbits were restrained in lateral recumbency by an experienced operator. A venipuncture skin site over the left saphenous vein was cleaned with a 70% alcohol swab. Blood samples were collected with a 25-gauge needle into 1-mL plastic syringes, and a drop of fresh blood was placed on a glass slide and immediately analyzed by use of the 4 PBGMs (2 identical human PBGMs^a and 2 identical veterinary PBGMs^b). Venipuncture and sample processing were performed by the same operator to minimize procedure time. The order in which PBGMs were used was randomized by use of a random sequence generator to avoid bias caused by the effect of time²⁷ and drop size¹⁴ on glucose concentration. In case of analytic strip failures, another strip from the same vial was immediately used. Blood was not scraped onto the test strips, and it was only applied on one side of the veterinary test strips.^d

The remaining blood was immediately transferred from the syringe into tubes containing lithium-heparin. If the sample needed to be analyzed with the benchtop analyzer, 0.1 mL of blood from the lithium-heparin tube was inserted in a reagent rotor. Hematocrit was determined in duplicate with 32×0.8 -mm heparinized capillary tubes.^e The lithium-heparin tubes were centrifuged at $1,300 \times g$ for 10 minutes, and plasma was harvested within 12 minutes after collection. Plasma glucose concentration was measured in all samples with a laboratory analyzer^f within 4 hours after centrifugation. To minimize delay in the procedures, the PBGMs, benchtop analyzer, and centrifuge were located immediately adjacent to each other and immediately adjacent to the table where blood collection was performed.

Analyzers and quality control—Three commercially available POC instruments (2 PBGMs^{a,b} and 1 benchtop analyzer^c) were evaluated in the present study. Duplicate analysis of the samples²⁸ via PBGMs was performed with 2 identical human PBGMs^a and 2 identical veterinary PBGMs^b as in a previous study.²⁹ The meters and test strips were maintained and operated within a temperature range of 20° to 24°C. Test strips were stored in their original vial in a cool, dry place between 20° and 25°C, away from sunlight and heat. The vial was immediately closed after removing 2 test strips. Strips belonging to 2 lots were used in the veterinary PBGM, and strips belonging to 4 lots were used in the human PBGM to account for lot-to-lot variability.³⁰ Control solutions^{g,h} were used once per week to check the performance of the PBGMs. Control solutions were also used before a new box of test strips was used. The system was considered to perform correctly if the control solution test result was within the specific control solution ranges listed on the strip vial.

Human amperometric PBGMs—Single-use test stripsⁱ were used in the human PBGMs. The human PBGMs determined blood glucose concentration amperometrically by means of a reaction catalyzed by pyr-

roloquinoline quinone–glucose dehydrogenase. Results were provided in approximately 5 seconds, and the manufacturer's reported range for blood glucose concentration measurements was 10 to 600 mg/dL.¹

Veterinary colorimetric PBGMs—Single-use test strips^k were used in the veterinary PBGMs. Blood glucose concentration was determined colorimetrically by means of a flavin-adenine dinucleotide–glucose dehydrogenase–catalyzed reaction.^h The veterinary PBGMs provided results in approximately 8 seconds, and the manufacturer's reported range for blood glucose concentration measurements was 20 to 750 mg/dL.⁴ The 2 veterinary PBGMs were set on the canine setting for the first 40 samples and on the feline setting for the remaining samples. Glucose concentrations obtained with the canine code were converted to the feline code and vice versa by use of a proprietary algorithm of the manufacturer.¹

Benchtop analyzer—The benchtop POC analyzer^l with reagent rotors specific for mammalian biochemical analyses^m was used in the cases in which a general biochemical profile was needed. The benchtop analyzer used a modified version of the hexokinase method to measure glucose concentration.³¹ The manufacturer's reported range for glucose concentration measurements was 10 to 700 mg/dL.³¹ Rotors of the benchtop analyzer were stored at 4°C. The device was serviced by the local provider a few days before the beginning of the study.

Laboratory analyzer—A laboratory-based automated biochemical analyzer^f that measures glucose concentration via an enzymatic hexokinase oxidase reaction was used as the reference analyzer. Linear calibrationⁿ of the laboratory analyzer was performed weekly during the study period.

Statistical analysis—Accuracy of the POC analyzers was assessed with Bland-Altman bias plots and regression techniques. Deming regression analysis was used for the PBGMs because duplicate measurements were obtained, and Passing-Bablok regression analysis was used for the benchtop analyzer because only single measurements were obtained.

The limits of agreement were determined from Bland-Altman plots by ± 1.96 SD centered on the mean difference. The association between the difference and the analyte concentration was examined by standard re-

gression analysis of the difference between the 2 methods. If a significant ($P \leq 0.05$) slope of the regression line was detected, logarithmic transformation of both measurements before analysis was performed.³² The limits of agreement derived from log-transformed data were back transformed to give limits for the ratio of the actual measurements.³³

By use of Deming regression, the slope and intercept were calculated with SE and 95% CI values.³⁴ Passing-Bablok regression was performed as described elsewhere.^{35–37} Constant bias was present if the 95% CI for the y-intercept did not include 0. Proportional bias was present if the 95% CI for the slope did not include 1.³⁶

To assess relationship between accuracy, blood glucose concentration, and Hct, the differences among laboratory results and POC analyzer results (ie, benchtop analyzer results or PBGM results) were included as dependent variables in a multiple regression stepwise model, with Hct and blood glucose concentration as independent variables. If the 2 independent variables concurred in the prediction of the dependent variable, the MCC was provided. If only 1 variable fit the model, the Pearson correlation coefficient was provided for the significant variable.

As a measure of precision, repeatability of the PBGMs was measured, calculating the CV from duplicate measurements.³⁸ Because no data were found in the current literature describing repeatability of the reference method (hexokinase) in pet rabbits, the CV for the hexokinase method was calculated. Four plasma samples were aliquoted in 4 vials each and were sent to the laboratory as different samples. This way, the technician was not aware that multiple aliquots of the same samples were measured.

To assess relationships among repeatability, blood glucose concentration, and Hct in the PBGMs, the difference (absolute value) between duplicate measurements was included as a dependent variable in a multiple regression stepwise model, with Hct and blood glucose concentration as independent variables. If the 2 independent variables concurred in the prediction of the dependent variable, the MCC was provided. If only 1 variable fit the model, the Pearson correlation coefficient was provided for the significant variable.

Total analytic error reflects the sum of random error (imprecision) and systematic error (bias). No clear

Table 1—Blood glucose concentrations obtained with a reference laboratory analyzer, a human PBGM, a veterinary PBGM on the canine setting, a veterinary PBGM on the feline setting, and a benchtop analyzer in samples from healthy and diseased pet rabbits.

Variable	Reference method (n = 89)	Human PBGM (n = 89)	Canine PBGM (n = 89)	Feline PBGM (n = 89)	Benchtop analyzer (n = 32)
Concentration (mg/dL)					
Median (range)	161 (31–405)	152 (29–417.5)	206.5 (30–550.5)	185.5 (28–494.5)	158.5 (80–360)
Mean \pm SD	175.2 \pm 70.1	165.6 \pm 70.2	223.3 \pm 96.6	200.6 \pm 86.9	172.9 \pm 53.3
Euglycemic values	25	37	10	19	12
Hypoglycemic values	5	6	5	5	0
Hyperglycemic values	59	46	74	65	20

The distribution of values corresponding to euglycemia, hypoglycemia, and hyperglycemia is indicated.

consensus exists as to the best method to calculate total analytic error for instrument validation.^{28,39,40} In the present study, total error observed was calculated as described elsewhere⁴¹:

$$\text{Total error observed} = 2 \text{ CV} + \text{Bias} (\%)$$

Bias (%) was calculated as follows:

$$\text{Bias} (\%) = \frac{\text{Mean determined by hexokinase method} - \text{Mean determined by PBGM}}{\text{Mean determined by hexokinase method}} \times 100$$

Table 2—Results of Bland-Altman and regression analyses of accuracy of a human PBGM, a veterinary PBGM on the canine setting, a veterinary PBGM on the feline setting, and a benchtop analyzer to measure blood glucose concentration in blood samples from healthy and diseased pet rabbits.

Instrument	Bland-Altman analysis			Regression analyses					
	Mean difference (mg/dL)	Limits of agreement (mg/dL)	Log transformation	Regression equation	95% CI		CV (%)	r*	95% CI
					Intercept	Slope			
Human PBGM (n = 89)	-9.56	-33.80 to 14.68	—	$y = -7.51 + 0.98x$	-16.05 to 1.02	0.93 to 1.04	2.99	0.98	0.97 to 0.98
Canine PBGM (n = 89)	48.17	-20.04 to 116.39†	-1.2 to 59.8	$y = -9.75 + 1.33x$	-25.41 to 5.89	1.23 to 1.42	4.16	0.96	0.94 to 0.97
Feline PBGM (n = 89)	25.43	-27.78 to 78.66†	-11.3 to 43.4	$y = -9.04 + 1.19x$	-23.10 to 5.02	1.10 to 1.28	4.13	0.96	0.94 to 0.97
Benchtop analyzer (n = 32)	1.25	-7.03 to 9.53	—	$y = 2 + 1x‡$	-2.64 to 9.42	0.95 to 1.02	—	0.99	0.99 to 0.99

Deming regression was used for PBGMs because duplicate measurements were obtained. Passing-Bablok regression was used for the benchtop analyzer because only single measurements were obtained.
 *For all r values, $P < 0.001$. †Variables with differences proportional to the mean underwent logarithmic transformation. Antilogs of the limits of agreement calculated on the log-transformed data are presented as a percentage.^{32,33} ‡Relative SD was 5.2, and there was no significant (Cusum test, $P = 0.49$) deviation from linearity.
 — = Not applicable.

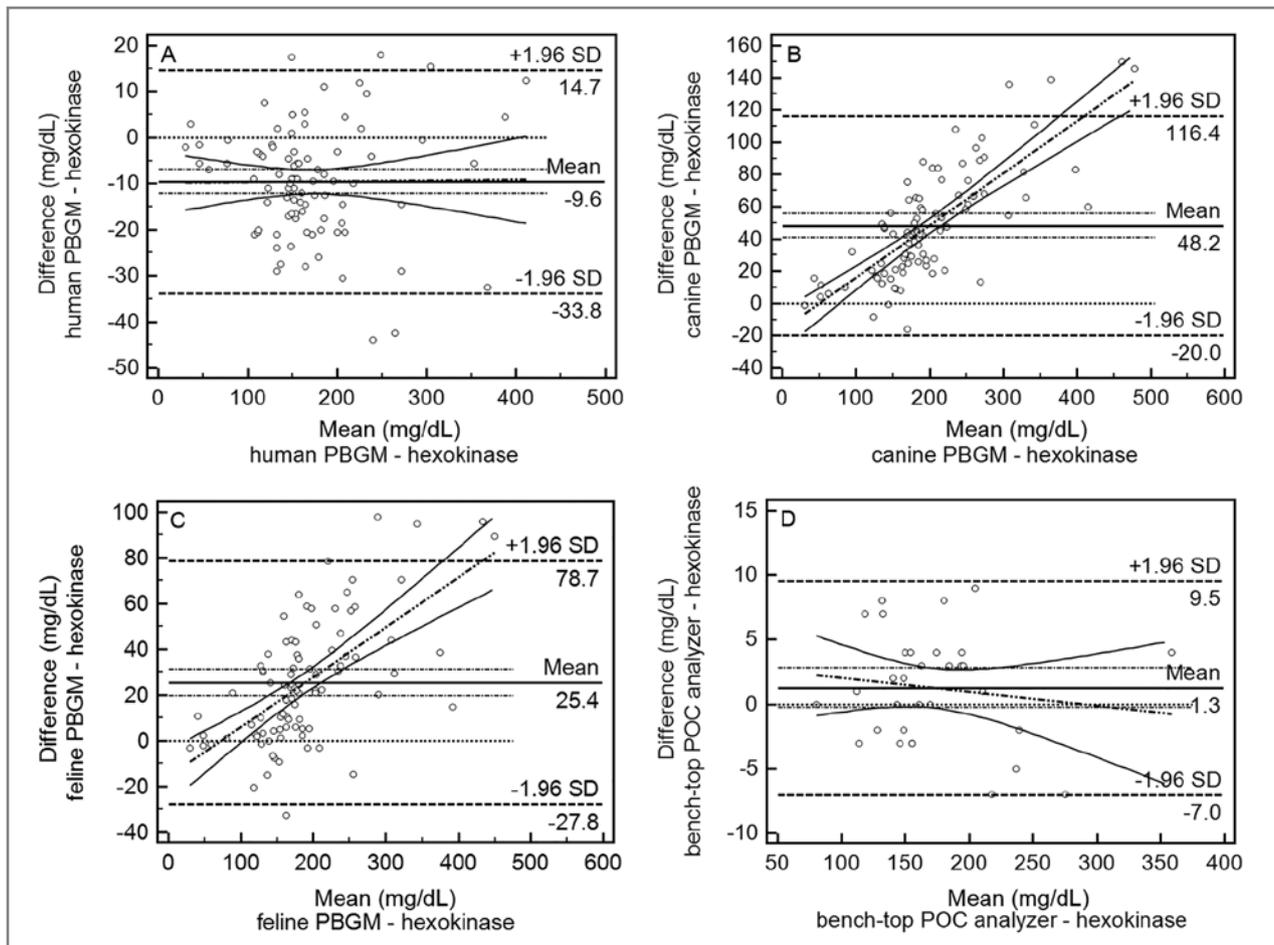


Figure 1—Bland-Altman agreement plots for results of analysis of venous blood samples collected from pet rabbits for glucose concentration and analyzed with 4 POC analyzers, including a human PBGM (A), a veterinary PBGM on the canine setting (B), a veterinary PBGM on the feline setting (C), and a benchtop analyzer (D), versus a laboratory analyzer (hexokinase reference method). Circles represent individual measurements. The middle solid horizontal line represents the mean difference between the pairs of measurements. The upper and lower horizontal dashed lines represent the 95% limits of agreement. The dashed and dotted lines represent 95% CIs of the mean of differences. If the 95% CIs of the mean of differences do not include 0 (dotted line), there is a constant bias. The regression line with its 95% CIs is depicted to assist the detection of a proportional bias.

On the basis of the guidelines of the American Society for Veterinary Clinical Pathology,⁴¹ allowable total error for glucose measurement was 10% in hypoglycemic samples, 20% in normoglycemic samples, and 20% in hyperglycemic samples.

Alteration in diagnostic and treatment decisions—Error grid plots⁴² were developed to evaluate clinical effect if the POC analyzers were used rather than the laboratory analyzer. The Clarke error grid analysis method and acceptance criteria was modified because veterinary critical limits are different than those used in humans.^{16,43,44} The grid system assigns predicted glucose concentrations (POC analyzers) versus actual glucose concentrations (laboratory hexokinase method) to 4 zones (A through D). Zone A included POC readings that deviated from the laboratory result by no more than 20% or POC readings that were in the hyper- or hypoglycemic range when the laboratory result was also in the hyper- or hypoglycemic range, respectively. Twenty percent limits were plotted around the line of equality, and the hypoglycemic limit was considered < 75 mg/dL

and the hyperglycemic limit > 150 mg/dL, according to a recent survey⁵ and to reference ranges.^{45,46} Zone B was defined as values outside the reference range, based on the POC analyzers, but in the reference range, based on the reference analyzer (ie, including POC readings that would lead to overdiagnosis and treatment of either hypo- or hyperglycemia). Zone C was defined as values in the reference range, based on the POC analyzers, but outside the reference range, based on the reference analyzer (ie, including POC readings that would lead to underdiagnosis and treatment of either hypo- or hyperglycemia). Zone D was defined as POC readings that were opposite of the hexokinase glucose readings, leading to treatment of hypoglycemia rather than hyperglycemia or vice versa. The POC analyzer would be considered clinically acceptable if at least 95% of its readings were within zone A.^{16,43,47}

All data obtained, including those gathered after a previous strip failed, were included in the statistical analysis. Data were analyzed with commercial software.⁶ Two-tailed values of $P < 0.05$ were considered significant.

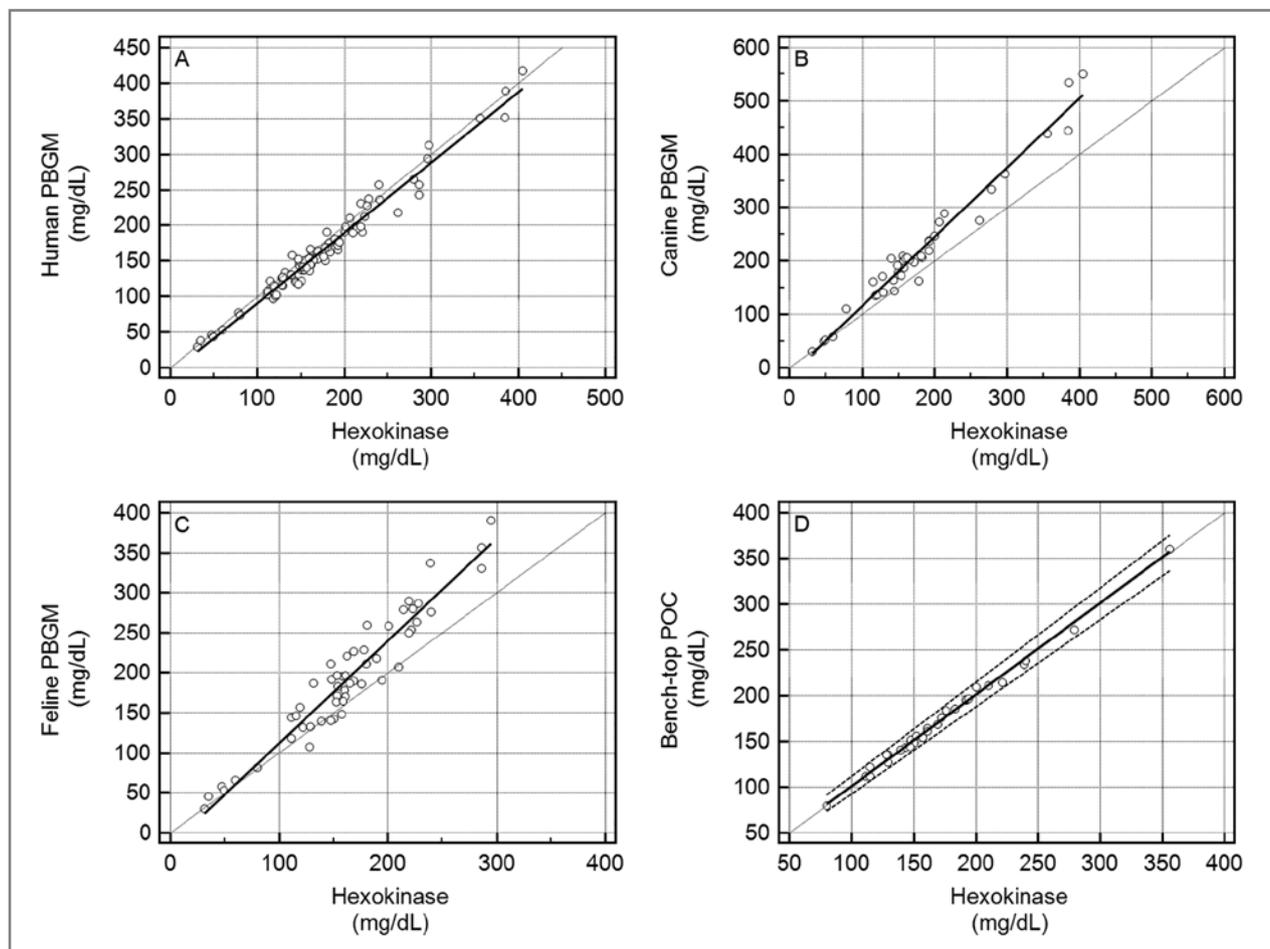


Figure 2—Regression analyses between results of measurement of blood glucose concentration in pet rabbits by use of 4 POC analyzers and a laboratory analyzer (hexokinase method). Circles represent individual measurements. A—Deming regression for results obtained by use of a human PBGM versus a hexokinase method. B—Deming regression for results obtained by use of a veterinary PBGM on the canine setting versus a hexokinase method. C—Deming regression for results obtained by use of a veterinary PBGM on the feline setting versus a hexokinase method. D—Passing-Bablok regression for results obtained by use of a benchtop analyzer versus a hexokinase method. The identity line ($x = y$) is indicated by the dotted line. The regression line is indicated by the solid line. In the Passing-Bablok regression, the 95% CIs of the regression line are marked as dashed lines.

Results

Population summary—Overall, 356 test strips were used on samples from 89 rabbits that underwent measurement of blood glucose by use of 2 identical human PBGMs, 2 identical veterinary PBGMs, and the laboratory analyzer (Table 1). The benchtop analyzer was used to measure blood glucose concentration in 32 samples. Hematocrit was measured in 84 rabbits. Rabbits ranged from 1 to 132 months of age (median, 24 months); 46 were female (of which 23 were neutered), and 43 were males (of which 11 were neutered). Thirty-five rabbits were healthy, of which 11 were undergoing neutering. Veterinary PBGMs did not yield results on 7 occasions. On 2 occasions, one of the veterinary PBGMs did not read the samples twice (for a total of 4 errors). On 3 occasions, the other veterinary PBGM did not read the sample once (for a total of 3 errors).

Accuracy—The degree of agreement between the POC analyzers and the laboratory method varied among instruments (Table 2). Bland-Altman plots revealed proportional and constant bias for the veterinary PBGM (on the canine and feline settings) and at least

constant bias for the human PBGM. No significant bias was detected for the benchtop analyzer (Figure 1). Results of regression analyses (Figure 2) confirmed at least proportional bias for the veterinary PBGM (on either setting). On the basis of these findings, the veterinary PBGM significantly overestimated blood glucose concentrations and the human PBGM significantly underestimated blood glucose concentrations.

An increase in Hct was associated with a decrease in accuracy ($r = 0.46$; $P < 0.001$) in the human PBGM (Figure 3), whereas blood glucose concentration had no effect on accuracy. In the veterinary PBGM, accuracy decreased with increasing blood glucose concentration and decreasing Hct (canine [MCC = 0.77; $P < 0.001$] and feline [MCC = 0.68; $P < 0.001$] settings). Accuracy of the benchtop analyzer was not associated with blood glucose concentration or Hct.

Precision—The human PBGM had better overall repeatability (CV, 2.99%) than the veterinary PBGMs, with either canine (4.13%) or feline (4.16%) settings. Coefficient of variation of the hexokinase-based laboratory analyzer was 0.8%.

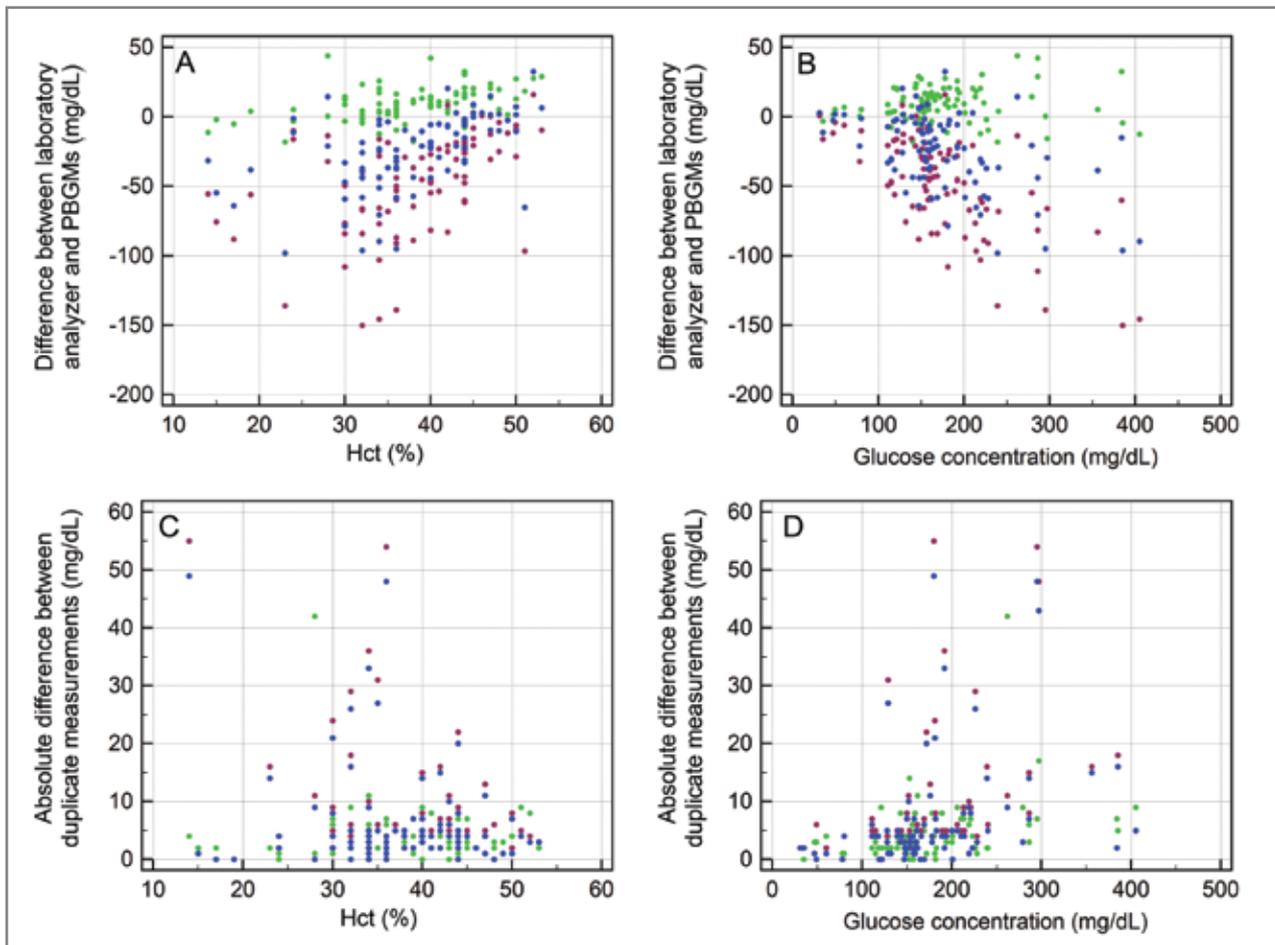


Figure 3—Scatterplots of differences between results of analyses of blood glucose concentration and Hct with a laboratory analyzer versus a human PBGM (green dots) and a veterinary PBGM on the canine setting (purple dots) and feline setting (blue dots) used in pet rabbits, and absolute differences between paired measurements. A—Notice that accuracy decreases with increasing Hct for the human PBGM, and accuracy decreases with decreasing Hct for the veterinary PBGM (either canine or feline setting). B—Notice that blood glucose concentration does not affect accuracy of the human PBGM. Increasing blood glucose concentrations decrease accuracy of the veterinary PBGM. C—Notice that precision decreases with decreasing Hct in the veterinary PBGM on the feline setting. D—Notice that precision decreases with increasing blood glucose concentration in all 3 PBGMs.

Table 3—Accuracy, precision and total error of a human PBGM, a veterinary PBGM on the canine setting and a veterinary PBGM on the feline setting for determination of glucose concentration in blood samples from pet rabbits at different ranges of glucose concentrations tested.

Glucose range	Human PBGM				Canine PBGM				Feline PBGM			
	Mean difference (mg/dL)	Limits of agreement (mg/dL)	CV (%)	Total error (%)	Mean difference (mg/dL)	Limits of agreement (mg/dL)	CV (%)	Total error (%)	Mean difference (mg/dL)	Limits of agreement (mg/dL)	CV (%)	Total error (%)
Euglycemic (n = 25)*	-8.3	-31.1 to 14.3	2.1	10.7	32.2	-16.2 to 80.7	3.2	24.8	15.9	-27.1 to 59.1	3.2	7.3
Hypoglycemic (n = 5)	-2.6	-10.2 to 5.0	4.6	15.0	7.3	-5.6 to 20.2	4.2	31.6	1.4	-9.8 to 12.6	2.1	18.9
Hyperglycemic (n = 59)	-10.6	-36.1 to 14.8	2.9	10.9	58.3	-10.1 to 126.9	4.1	36.4	31.4	-23.6 to 86.5	4.0	23.2

*Values between 75 and 150 mg/dL as determined by use of the laboratory analyzer.

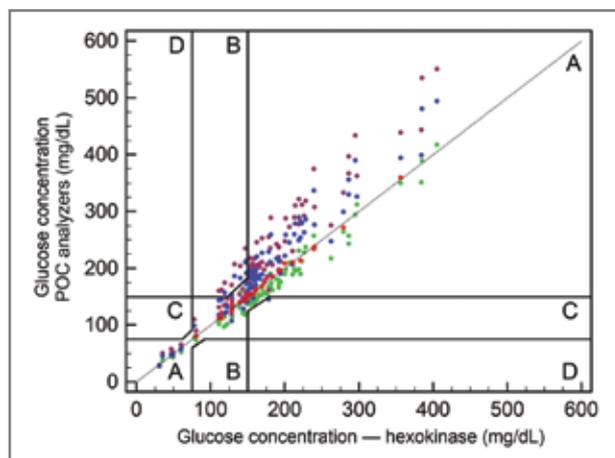


Figure 4—Modified error grid analysis for rabbit blood glucose concentrations obtained by use of POC glucometers, including a human PBGM (green dots), a veterinary PBGM with canine (purple dots) and feline (blue dots) settings, and a benchtop analyzer (red dots), versus a laboratory analyzer (hexokinase method). The dotted diagonal line indicates $x = y$, and lines representing the 10% limits of the $x = y$ line are present (zone A). The hypoglycemic limit was considered < 75 mg/dL and the hyperglycemic limit > 150 mg/dL. Zone B was defined as POC readings that would lead to overdiagnosis and overtreatment of either hypo- or hyperglycemia. Zone C was defined as POC readings that would lead to inadequate diagnosis and treatment for either hypo- or hyperglycemia. Zone D was defined as POC readings that were opposite of the hexokinase glucose readings, leading to treatment of hypoglycemia rather than hyperglycemia or vice versa. A POC analyzer would be considered clinically acceptable if at least 95% of its readings were within zone A.

In all the PBGMs, repeatability decreased with increasing glucose concentration (human PBGM [$r = 0.38$; $P = 0.0002$] and veterinary PBGM on the canine setting [$r = 0.34$; $P = 0.001$]) and decreased with decreasing Hct in the veterinary PBGM on the feline setting (MCC = 0.38; $P = 0.002$; Figure 3).

Total error—The total error for the human PBGM was 11.4%. With the veterinary PBGM, the canine setting resulted in a total error of 29.8%, and the feline setting resulted in a total error of 15.5%. The total errors at different glucose concentration were summarized (Table 3).

Alteration in diagnostic and treatment decisions—On the basis of results of the error grid analysis (Figure 4), all the glucose concentrations obtained with the human PBGM and the benchtop analyzer deviated from the reference by $\leq 20\%$ or were correctly identi-

Table 4—Results (%) of modified Clarke error grid analysis used to determine the percentage of results that would have potentially led to inappropriate diagnostic or therapeutic decisions with POC analyzers used for determination of blood glucose concentrations in rabbits.

Error grid zone	Human PBGM (n = 89)	Canine PBGM (n = 89)	Feline PBGM (n = 89)	Benchtop analyzer (n = 32)
A	100	91	93.3	100
B	0	9	5.6	0
C	0	0	1.1	0
D	0	0	0	0

Zone A included POC values that deviated from the laboratory result by $\leq 20\%$, or POC values that were in the hyper- or hypoglycemic range when the laboratory result was also in the hyper- or hypoglycemic range, respectively. Zone B included values that would lead to overdiagnosis of either hypo- or hyperglycemia. Zone C included values that would lead to underdiagnosis of either hypo- or hyperglycemia. Zone D included readings that were the opposite of the hexokinase glucose readings, leading to treatment of hypoglycemia rather than hyperglycemia or vice versa. A POC analyzer would be considered clinically acceptable if at least 95% of its readings were within zone A.

fied as hypoglycemic, normoglycemic, or hyperglycemic. Use of the canine setting of the veterinary PBGM would have resulted in misdiagnosis of hyperglycemia in normoglycemic pet rabbits in 9% of the cases. Use of the feline setting would have resulted in misdiagnosis of hyperglycemia in normoglycemic pet rabbits in 5.6% of the cases and misdiagnosis of normoglycemia in hyperglycemic pet rabbits in 1.1% of the cases (Table 4).

Discussion

Tight glucose control is increasingly being recognized as a priority in the management of critically ill human and animal patients.^{48–50} For instance, hypoglycemia and hyperglycemia are both associated with increased morbidity and mortality rates in pediatric intensive care units.⁴⁸ Furthermore, blood glucose concentrations may assist in the diagnosis of disease in most animals,^{51–55} including rabbits.⁵ Nevertheless, PBGMs should be always evaluated in the target species, where their results may diverge substantially from true glucose concentration.^{17,18,20,43} In the present study, the benchtop analyzer had better agreement with the laboratory analyzer for measurement of glucose concentration in blood of rabbits, compared with the other analyzers. This was not unexpected, considering that the benchtop analyzer used a hexokinase-based methodology for glucose determination. Among PBGMs, the

one intended for human use was more accurate and precise in measurement of rabbit blood glucose concentrations. Both canine and feline settings of the PBGM designed for veterinary use typically proportionally overestimated blood glucose concentrations. The overestimation was substantial for canine (mean difference, 48 mg/dL; limits of agreement, -20 to 116 mg/dL) and feline (mean difference, 25 mg/dL; limits of agreement, -27 to 78 mg/dL) settings and clinically important, as indicated by the modified error grid analysis.

The veterinary PBGM used in the present study has been already evaluated in dogs,⁵⁶ cats,⁵⁷ horses,¹⁶ alpacas,²² and ferrets.²³ The accuracy of the veterinary PBGM in rabbits was similar to the accuracy reported in alpacas.²² In blood from alpacas, the veterinary PBGM typically overestimated glucose concentration (mean difference, 5.2 mg/dL) and had wide limits of agreement (-46.6 to 57.1 mg/dL), compared with a laboratory analyzer.²² In contrast, accuracy of the veterinary PBGM in dogs, cats, horses, and ferrets was different than that in rabbits. In dogs, the veterinary PBGM did not provide results that were consistently lower or higher than the reference glucose concentration, although 43% of the results yielded blood glucose concentrations higher than those of the reference analyzer.⁵⁶ As in the present study, the veterinary PBGM in dogs had decreased accuracy with increasing blood glucose concentration (ie, proportional bias).⁵⁶ Reporting of the mean difference with the 95% CI would have assisted in the evaluation of a significant constant overestimation of blood glucose concentration.^{32,58} In cats, the results obtained with the veterinary PBGM did not differ significantly from results obtained with the laboratory analyzer.⁵⁷ Unfortunately, no data from cats are available regarding mean difference between the veterinary PBGM and the reference analyzer. In horses, constant over- or underestimation of blood glucose by use of the veterinary PBGM was not reported.¹⁶ The veterinary PBGM was considered clinically acceptable in horses, and nearly 97% of the readings would have resulted in appropriate clinical decisions.¹⁶ In ferrets, the canine setting of the PBGM had a negligible mean difference (1.9 mg/dL), compared with results of the laboratory analyzer, but wide limits of agreement (-29 to 34 mg/dL; values extrapolated from the difference plot) that may have an effect on clinical decision making, as also stated by the authors.²³ Dissimilarly, use of the feline setting resulted in significant underestimation of the blood glucose concentration.²³ These interspecific differences emphasize the importance of assessing the performance of veterinary meters in their target species. Furthermore, the disparate statistical analyses performed in each study^{32,41} make comparison of results difficult. This emphasizes the need for a common design and statistical protocol in method comparison studies.

The human PBGM constantly underestimated blood glucose concentration in rabbits (mean difference, 9.5 mg/dL). Identical human PBGMs have been evaluated in horses,⁵⁹ parrots,¹⁸ alpacas,^{22,43} and ferrets.²³ In all those species, the same human PBGM consistently underestimated blood glucose concentrations. A possible explanation for this finding is that such instruments are designed for self-monitoring of blood

glucose concentration by humans with diabetes, who adjust their dosage of insulin according to the PBGM reading. With PBGM readings that are slightly less than actual blood glucose concentrations, the diabetic would avoid hypoglycemia by injecting less insulin or by treating potential hypoglycemia earlier.¹⁵ This explanation seems unlikely, considering that in humans, the identical PBGM has some proportional bias (mean difference among the PBGM and the reference analyzer, -2.7 mg/dL [-0.15 mmol/L]; limits of agreement, 26.1 to -31.3 mg/dL [1.45 to -1.74 mmol/L])⁶⁰ but not a constant underestimation of blood glucose.^{9,60}

Furthermore, it has been proposed that filters used in test strips to separate erythrocytes from plasma may cause inaccuracy in PBGMs,⁵⁹ accounting for the differences between results for the PBGM and the reference hexokinase method. That hypothesis is challenged by the finding that dogs, humans, and rabbits have erythrocytes of similar size (approx 7, 6 to 8, and 6.5 μm , respectively).⁶¹⁻⁶³

Another proposed explanation is that values obtained with the reference method, for which plasma or serum was used, can be expected to be higher than values obtained with the PBGM, for which blood was used, because of the quantity of water in plasma and blood. In fact, glucose equilibrates into the aqueous portion of a blood sample. The concentration of water in serum and plasma is higher than the concentration of water in the cellular portion of blood. Therefore, serum and plasma have higher water content and higher glucose concentration than does blood.⁶⁴ In humans, by volume, plasma is 93% water, and the packed cell component is 71% water.⁶⁵ Considering a mean Hct of 43%, the constant factor to convert blood molality to the equivalent plasma glucose molarity in humans is 1.11.⁶⁶ For instance, a fixed volume of plasma has higher water content and therefore has higher glucose concentrations of approximately 11% to 12%, compared with blood, with an Hct of approximately 45%.²⁷ Some PBGMs have calibrations to correct this incongruity, assuming the patient has Hct within a given reference interval.⁶⁴ Difference among blood glucose molality and equivalent plasma glucose molarity is clearly more pronounced in the case of high Hct.

A further explanation could be the different distribution of glucose in plasma and erythrocytes among species.¹⁶ In dogs and cats, approximately 12% and 7% of glucose is within erythrocytes, respectively.⁶⁷ In human blood, glucose is distributed approximately 60% within erythrocytes and 40% within plasma.⁶⁸ Interestingly, in rabbits, approximately 15% of the glucose is distributed in the erythrocytes,⁶⁸ with some differences among studies.⁶⁷⁻⁶⁹ The better performance of the human PBGM in rabbits than in dogs and cats may be justified by the difference in distribution of glucose among species. However, it should be considered that results obtained with the PBGM used in this study may not apply to different PBGMs intended for human use.

In human medicine, although modern glucose meters still have variable results with respect to analytic measures of accuracy, error grid analysis often indicates that measurements are clinically acceptable.⁷⁰ Poor clinical agreement of the veterinary PBGM with the

reference analyzer was unexpected, considering that in other studies,^{23,56} use of the veterinary PBGM proved to be more or equally accurate, compared with use of human PBGMs in animal patients. The canine and feline settings of the veterinary PBGM did not provide clinically acceptable results in rabbits because it inaccurately indicated normoglycemic blood samples as hyperglycemic; this occurred in 9% of samples tested with the canine setting and 5.6% of samples with the feline setting. Although the human PBGM used in this study constantly underestimated blood glucose in rabbits, on the basis of the modified error grid analysis, it was acceptable for clinical use in rabbits. Our findings differed from what has been previously found in ferrets, in which 31% of the samples were incorrectly labeled as hypoglycemic with the same human PBGM.²³ Rabbits and ferrets together represent a large proportion of the small exotic mammals kept as pets.¹ The ability to use a single PBGM to measure blood glucose in both species would be beneficial. Unfortunately, on the basis of our results and those of the previous study²³ conducted on ferrets, veterinarians caring for exotic mammals should have available both the veterinary PBGM (for ferrets) and the human PBGM used in this study (for rabbits).

To use the veterinary PBGM in rabbits, it may be possible to mathematically correct for the constant and proportional bias detected in this study. Another option would be to validate independent reference intervals and critical decision limits for that particular PBGM, but the lower repeatability would still be a problem. In this study, we observed that repeatability of the veterinary PBGM was similar to that reported in dogs (approx 4%)⁵⁶ and that repeatability was influenced by blood glucose concentration and Hct.

Total analytic error can be used as an assessment of an individual instrument's analytic performance for instrument selection or for assessment of in-clinic instrument performance.⁴⁰ Analytic imprecision (repeatability of the result) and bias (constant error) were combined into a single measure of the uncertainty of a test result. The ideal situation is to have highly accurate and precise measurement (ie, low bias and low CV, respectively). Although total error when first introduced was proposed with a CV multiplication factor of 1,^{66,71} several multiplication factors for the CV have been used by different authors for method validation.⁴⁰ In the present study, a factor of 2 CV was used, as suggested by the guidelines for total allowable error of the American Society for Veterinary Clinical Pathology,⁴¹ which is consistent with the Clinical Laboratory Improvement Amendments of 1988.⁷² Allowable total error for glucose measurement should be 10% in hypoglycemic samples, 20% in normoglycemic samples, and 20% in hyperglycemic samples.⁴¹ Both the human and the veterinary PBGM evaluated in this study exceeded the allowable total error. Nevertheless, no single standard exists to assess acceptable accuracy of a PBGM, so the determination of accuracy and the definition of acceptable accuracy will vary by country.⁶⁴ Therefore, in the present study, we calculated the total error observed to compare the PBGMs, more than for describing absolute acceptability.

Several factors can adversely affect blood glucose concentrations reported by PBGMs.⁶⁶ Hematocrit has

long been known to affect the accuracy of PBGMs.^{73,74} Several hypotheses have been proposed to explain the effect of abnormal Hct on blood glucose testing, such as altered viscosity of blood, prevention of plasma from reaching the reaction surface of the test strip, change in diffusion kinetics, or increased PCV and displacement of plasma volume leading to insufficient plasma volume for accurate testing.⁷⁵ In rabbits, we observed that Hct influenced accuracy of all the PBGMs, but on an instrument-dependent basis: the human PBGM had increased underestimation of blood glucose with increased Hct, whereas the veterinary PBGM had increased overestimation of blood glucose with decreased Hct. Therefore, the PBGM for use in humans is likely to be less accurate in polycythemic rabbits and more accurate in anemic ones, whereas the PBGM for veterinary use is more likely to be more accurate when used in polycythemic rabbits but less accurate in anemic ones. Interestingly, similar results have been observed when human and veterinary PBGMs were used in dogs²⁴; the effect of Hct on accuracy of PBGMs has been assessed in Greyhounds (ie, with naturally high Hct) and in anemic dogs. As observed in rabbits, in dogs, the veterinary PBGM had decreased accuracy at low Hct, whereas the human PBGM had decreased accuracy at high Hct.²⁴ Considering that the effect of Hct in dogs and rabbits was similar, this difference was probably secondary to the different technology used by the veterinary and the human PBGMs. To diminish Hct interference, mathematical algorithms based on the differences in the kinetics of the electrochemical reactions of glucose and confounding substances have been developed. This method is referred to as dynamic electrochemistry and has been incorporated into commercially available PBGMs⁷⁶ with promising results.⁷⁷

Apart from Hct, other analytes in the blood may alter the accuracy of PBGMs. One study⁶⁶ on PBGMs for use in humans investigated the quantity of total error contributed by variations in Hct, maltose, and ascorbate concentration. Interestingly, all the PBGMs performed well so long as Hct was not abnormal and no maltose or ascorbate was present. However, with Hct of 20% and ascorbate concentration of 0.29 mmol/L, the estimated total analytic error approached 30% for the meters evaluated.⁶⁶ Future studies should take in account also these sources of analytic error when PBGMs are evaluated in animal patients.

In the present study, 1 drop of blood was immediately analyzed with the 4 glucose meters and the rest of the sample was placed in lithium-heparin tubes. Although the anticoagulant could be a source of analytic variation, in cats and dogs, significant differences were not detected among glucose concentrations of fresh blood without anticoagulant, EDTA-anticoagulated blood, and lithium-heparinized blood.^{14,15} It should be also considered that glucose is unstable in blood; erythrocytes metabolize glucose at a rate of 6 to 10 mg/dL/h at 25°C in human blood.²⁷ Therefore, to prevent artifactual hypoglycemia caused by glycolysis, separation of the erythrocytes should be performed soon after sample collection. All samples in the study reported here were evaluated on each PBGM immediately after collection, and the remaining sample was centrifuged to separate

the plasma 2 minutes after collection. Considering the strict procedure timing, glycolysis in the present study should have been minimal. This assumption was supported by the good agreement observed between the benchtop analyzer (which used nonanticoagulated blood) and the laboratory analyzer. Therefore, the overestimation observed in the readings of the veterinary PBGM was not a consequence of falsely low concentrations detected by the laboratory analyzer.

Portable blood glucose meters are designed to measure glucose concentration in capillary blood. The present study was performed on pet rabbits that needed to have venous blood collection performed for other purposes. Although we cannot be certain that repeating the study with capillary blood samples would yield identical results, in cats, no clinically important differences were found among capillary samples (obtained from the marginal ear vein) and venous samples.⁷⁸ Considering that all samples evaluated in this study were of venous origin, all the instruments should have been affected similarly.

Future studies evaluating PBGMs in rabbits should take in account other relevant factors in POC measurement of blood glucose, such as differences between plasma and blood measurements, difference between capillary and venous blood samples, and interference of other analytes (ie, maltose and ascorbate) with glucose determination.

Collectively, the data indicated that the human PBGM and benchtop analyzer in the present study can be safely used for glucose measurement of pet rabbits among a wide range of Hct values and blood glucose concentrations. The feline setting of the veterinary PBGM provided more accurate results, compared with the canine setting. Nevertheless, on the basis of wide limits of agreement, presence of constant and proportional bias, high total error observed, and < 95% results in zone A of the error grid analysis, results obtained with both settings of the veterinary PBGM were not deemed acceptable in pet rabbits. Further work is required for calibrating and validating the veterinary PBGM for use in pet rabbits.

- a. Accu-Chek Aviva, Roche Diagnostics, Indianapolis, Ind.
- b. AlphaTrak 2 Blood Glucose Monitoring System, Abbott Laboratories, Abbott Park, Ill.
- c. VetScan VS2, Abaxis Inc, Union City, Calif.
- d. AlphaTrak, User Guide, Abbott Laboratories, Abbott Park, Ill.
- e. Capillary Hct tube, Drummond Scientific Co, Broomall, Pa.
- f. Dimension EXL, Siemens Healthcare Diagnostics Inc, Tarrytown, NY.
- g. AccuChek Aviva Control Solution, Roche Diagnostics GmbH, Mannheim, Germany.
- h. AlphaTrak 2 Control Solution, Abbott Laboratories, Abbott Park, Ill.
- i. AccuChek Aviva Test Strips, Roche Diagnostics GmbH, Mannheim, Germany.
- j. AccuChek Aviva user guide, Roche Diagnostics GmbH, Mannheim, Germany.
- k. AlphaTrak 2 Blood Glucose Test Strips, Abbott Laboratories, Abbott Park, Ill.
- l. Algorithm property of Abbott, Abbott Laboratories, Abbott Park, Ill.
- m. VetScan Comprehensive Diagnostic Profile, Abaxis Europe GmbH, Darmstadt, Germany.
- n. CHEM 1 Calibrator, Siemens Healthcare Diagnostics Inc, Tarrytown, NY.

- o. MedCalc, version 12.2.1, MedCalc Software bvba, Mariakerke, Belgium.

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