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Blood Glucose Measurement in Rabbits - Performance Evaluation of Portable Glucometers*

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ABSTRACT

***Background*:** Blood glucose level measurement plays an important role in the diagnosis and prognosis of different clinical conditions in rabbits (*Oryctolagus cuniculus*). Portable glucometers offer a quick and affordable tool to measure blood glucose. However, not all commercially available glucometers are suitable for this purpose in different animal species and in different clinical conditions. This study aimed to assess the performance of 4 portable glucometers compared to a biochemical analyzer using blood samples from both healthy and sick rabbits.

***Materials, Methods & Results*:** Blood samples were collected via jugular venipuncture from 51 rabbits. The blood glucose concentration was measured using 3 human (FO= FreeStyle Optium Neo®; AA= Accu-chek® Active; IS= Injex Sens II®) and 1 veterinarian (PGM= Wellion Vet®, Gluco Calea) glucometer, configured for dog (WD), cat (WC), and horse (WH). The results obtained from the glucometers were compared to those from the laboratory biochemical analyzer in wet biochemistry, using Spearman correlation, intraclass correlation coefficient, Bland-Altman analysis, error grid analysis and total error analysis. Blood glucose concentrations were underestimated in the human devices evaluated and overestimated in the veterinary device. Bland-Altman analysis revealed significant systematic differences in 3 of the 4 devices tested. Total error above 20% was observed in normoglycemic samples from 2 devices (FO; and PGM - WC= cat code) and in hyperglycemic samples from 1 glucometer (AA). All glucometers showed glycemic results within Zone A and B of the error grid analysis, although a glucometer (FO) present most of the results (60.8%) in Zone B. Only a single portable glucometer (IS) consistently met the objectives across all analyses.

***Discussion*:** The sample included 26 males and 25 females, with ages ranging from 5 to 96 months. Thirty-five rabbits were healthy, while 16 had various clinical abnormalities. Significant differences in median glycemia were found between the biochemical analyzer and all PGMs, except for the IS glucometer. FO and AA showed significantly lower glycemic values, while WD, WC, and WH showed higher values. The IS glucometer demonstrated the smallest mean variation and highest intraclass correlation coefficient (ICC), indicating excellent agreement. Bland-Altman plots revealed that FO and AA underestimated glycemic values, while WD, WC, and WH overestimated them. Only IS showed agreement with the laboratory analyzer. Error grid analysis indicated no serious errors with any PGMs; however, IS had over 75% of results in Zone A, showing high agreement, while FO had the least favorable results. Total error analysis suggested that only IS consistently met accuracy objectives across various analyses. The study concluded that IS was the most accurate PGM, while others showed varying degrees of inaccuracy. Although PGMs are clinically acceptable based on ISO 15197:2013 standards, accuracy and precision issues remain, particularly for euglycemic and hyperglycemic samples. Although all glucometers showed clinically acceptable results in the error grid analysis, the accuracy varied among the devices. Collectively, the IS glucometer achieved the best results in measuring blood glucose in pet rabbits, demonstrating a better accuracy and lower tendency for systematic error compared to the laboratory analyzer. Despite the lower accuracy, the other evaluated glucometers showed potential for use in rabbits when a laboratory analyzer is not available, but results must be considered with caution, considering the glycemic range presented.

Keywords: glycemia, glycemic range, biochemical analyzer, devices, *Oryctolagus cuniculus*, lagomorphs, PGMs.

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INTRODUCTION

Glucose plays a vital role in energy metabolism. Its metabolism is complex and glycemic levels can be influenced by several factors including stress, pain, age, and different pathological conditions [22]. In rabbits, accurate glucose measurement may aid in treatment, diagnosis, and prognosis of various conditions [8,15,19].

The most reliable method for measuring blood glucose is automated chemical analyzers using the hexokinase or glucose oxidase method [12,22]. However, this requires expensive equipment, larger sample volumes, and more time, making it impractical for rapid response scenarios like fieldwork, anesthetic procedures, or clinical emergencies [26]. Portable glucometers offer a quick, convenient, and cost-effective alternative, requiring only small sample volumes, making them ideal for specific clinical situations and frequent monitoring [4,22,27].

New devices continuously need evaluation, especially since most veterinary glucometers are adapted from human models and may not provide reliable results [5]. Inaccurate devices can lead to erroneous diagnoses and inappropriate treatments [4]. Thus, validating these devices is crucial for veterinary practice. Although some studies have assessed portable glucometers' accuracy in various species, information on their use in rabbits is limited.

This study aimed to evaluate the performance of commonly available human and veterinary portable devices in monitoring blood glucose levels in rabbits, considering samples obtained by jugular venipuncture from animals in different clinical conditions.

MATERIALS AND METHODS

Animals

Fifty-one blood samples from rabbits presented to the Veterinary Teaching Hospital, between August and December 2022, regardless of age, sex, breed or health status, were used in an observational prospective study. The blood collection was conducted as part of routine clinical evaluations or preoperative assessments for elective or non-elective surgeries. Animals were not fasted for blood collection.

Blood sample exclusion criteria

According to the specifications provided by the manufacturer of the glucometer manufacturers, blood samples with triglyceride concentrations ≥ 1000 mg/dL and cholesterol concentrations ≥ 500 mg/dL may experience interferences in blood glucose analysis. Same occurs in samples with hematocrit below 20% and above 50%. Thus, rabbits that presented values outside the mentioned ranges would be excluded from the study. Likewise, animals with glycemia readings that were non-quantitative (LOW or HI) on the portable glucometer would be excluded.

Blood collection and procedures

Rabbits were restrained in sternal recumbency, and antisepsis of the jugular vein region was performed with 70% alcohol. Jugular venipuncture was performed always by the same experienced operator, using a 3 mL hypodermic syringe and 25-gauge needle, to collect 2 mL of blood. Blood samples were distributed as follows: 0.5 mL in a tube containing sodium fluoride and ethylenediaminetetraacetic acid (EDTA)[1]; 0.5 mL in tube containing EDTA[1] and 0.5 mL in a clot activator tube[1]. Residual whole blood was immediately used for blood glucose analysis on the portable glucometers [9,22].

Whole blood samples collected in EDTA tubes were used for hematocrit determination. These tubes were centrifuged in a microhematocrit centrifuge (CELM® LS 3 PLUS)[2] for 5 min at 18,275 *g*. Readings were performed using a hematocrit card. Tubes containing sodium fluoride and clot accelerator were centrifuged in a tube centrifuge (HCL-4)[3] for 5 min at 2,100 *g* to obtain plasma and serum, respectively. Fluoride plasma aliquot was used for glucose measurement by glucose oxidase enzymatic colorimetric method (GOD-Trinder), and the serum sample from the tube with clot accelerator was used for analysis of cholesterol and triglyceride concentration, by colorimetric enzymatic method (Enzymatic Trinder). Readings were performed using commercial kits[4] and automated biochemical analyzer (BS-230)[5]. All samples were centrifuged within 20 min after blood collection and refrigerated at 4°C. All measurements were performed on the same day as the blood collection. Blood glucose values obtained by the laboratory biochemical analyzer[5] were considered the reference method for statistical analysis.

Portable glucometer (PGM)

Three human PGMs and one veterinary PGM were evaluated in the study. These PGMs were cho-

sen based on variations in price, design, and their widespread use in different countries. Calibration and operation of PGMs was performed under similar environmental temperature and humidity conditions, following the manufacturer's instructions. The order of measurements was alternated among the different devices for each reading. In case of analytical strip failure, another strip from the same bottle was immediately used.

The human PGMs used for measuring blood glucose in this study included FO (FreeStyle Optium Neo)[6], which is considered suitable for measuring blood glucose in dogs [22]; AA (Accu-chek Active)[6], which is considered suitable for measuring blood glucose in healthy rabbits [9]; and IS (Injex Sens II)[7], which is regularly used in the hospital where the study was performed. Despite the greater range and availability of human PGM models compared to veterinary PGMs, considering the hypothesis that veterinary glucometers are better suited for animals, a veterinary PGM (Wellion Vet)[8] calibrated for dogs (WD), cats (WC) and horses (WH) was also evaluated in this study. This PGM was programmed for dog, cat or horse, according to the manufacturer's own algorithm, using a species-specific chip.

FO determines the blood glucose by amperometry assay (glucose dehydrogenase measurement method), with a reported reading range of 20 to 500 mg/dL (1.1 to 27.8 mmol/L). The AA utilizes reflectance photometry (pyrroloquinoline quinone–glucose dehydrogenase reaction) and has a manufacturer-reported range of 10 to 600 mg/dL (0.6 to 33.3 mmol/L) for blood glucose concentration measurements. IS determines the blood glucose by electrochemical biosensor (glucose oxidase reaction), reading results between 10 to 600 mg/dL (0.6 to 33.3 mmol/L). Veterinary PGM (dog, cat and horse) also determines results by electrochemical biosensor (glucose oxidase reaction) and reads blood glucose levels between 20 to 600 mg/dL (1.1 to 33.3 mmol/L). All results analyzed by the PGMs were obtained in approximately five seconds.

Statistical analysis

Descriptive analysis of the quantitative variables is presented as mean and standard deviation for parametric data and median and range for non-parametric data. Data normality was tested using the Shapiro-Wilk test. The significance of differences between glucose values obtained by the reference method and the portable glucometer was evaluated using the Mann-Whitney test. Spearman's correlation coefficient was calculated between the results obtained using the PGMs and the analyzer. Intraclass correlation coefficient (ICC) was used to compare the 2 different diagnostic methods. Bland–Altman plots were used to assess the accuracy of the values generated by the PGMs and reference method. Error grid analysis was performed to assess the clinical relevance of discrepancies between measurements using the PGMs and the analyzer [18]. The error grid system categorized glucose concentrations measured by PGMs and the analyzer into 4 zones: Zone A represented readings that deviated by no more than 20% from the laboratory results, without clinical effects (analytical precision); Zone B represents values that deviated more than 20% from the reference values warranting treatment adjustments, but with minimal impact on the patient; Zone C represents values that can induce unnecessary treatment; Zone D determines values that can lead to significant errors in treatment decisions, and Zone E, indicated errors that could lead to dangerous consequences, such as treatment of hypoglycemia instead of hyperglycemia. Total analytic error was calculated as the sum of random error (imprecision) and systematic error (bias), as described in previous study [9]. According to the guidelines of the American Society for Veterinary Clinical Pathology, the allowable total error for glucose measurement in hypoglycemic, normoglycemic, and hyperglycemic samples was 10%, 20%, and 20%, respectively [6,9]. Statistical analysis and graphs were developed in the R program[9], considering a significance level of 5%.

RESULTS

Blood samples from 51 rabbits were collected to compare blood glucose concentrations between 4 portable glucometers and a biochemical analyzer, from healthy and disease rabbits. Of these samples, 26 (50.98%) were from males and 25 (49.01%) from females. The age of 34 rabbits varied from 5 to 96 months (median 24 months), as the age of 17 animals was unknown. Thirty-five animals (68.6%) were healthy while 16 (31.37%) had some clinical abnormality, including uroliths, lung abscess, pregnancy toxemia, eye, skin or dental diseases and non-specific anorexia.

Hematocrit value ranged from 32 to 50% (mean 42.35 ± 4.02%), triglycerides from 22 to 480 mg/dL

(mean 91.10 ± 77.17 mg/dL) and cholesterol from 15 to 123 mg/dL (mean 46.98 ± 21.79 mg/dL). Considering the values obtained, no sample was excluded from the study.

Significant difference was observed in the median glycemia measured by all portable glucometers (PGMs) compared to the biochemical analyzer, except for the IS ($P = 0.102$) [Table 1]. FO and AA showed significantly lower median glycemic values than the reference method ($P < 0.001$), while WD, WC and WH showed significantly higher values ($P < 0.001$). Considering the physiological glycemia in rabbits from 100 to 200 mg/dL [17] and the biochemical analyzer as a reference method, none of the evaluated animals was hypoglycemic, but seven (13.7%) rabbits had hyperglycemia (ranging from 208 to 439 mg/dL). However, there was variation in the number of euglycemic, hyperglycemic and hypoglycemic samples when using the PGMs compared to the reference method.

The mean variation, as minimum and maximum variations in blood glucose measured by the PGMs in relation to the values obtained by the biochemical analyzer, are also described in Table 1. Negative values indicate variation below the reference (i.e., the PGM obtained glycemic values lower than those determined by the laboratory analyzer), while positive values indicate variations above the analyzer's values. The results reveal that the IS presented the smallest mean variation compared to the gold standard method, followed by AA. However, FO, IS e AA demonstrated the greatest negative variations, while the veterinary PGM (WD, WC and WH) presented the greatest positive variations.

Table 1. Descriptive statistics associated with the comparison of 51 samples of blood glucose concentrations obtained of 4 portable glucometers (PGMs) and laboratory analyzer from healthy and diseased rabbits.

Variable	Laboratory analyzer	FO	AA	IS	WD	WC	WH
Concentration mg/dL							
Mean ± SD	162.24 ± 68.32	131.86 ± 67.96	132.26 ± 48.79	159.66 ± 84.95	187.96 ± 57.74	185.05 ± 58.79	183.21 ± 55.55
Median (range)	140 (103-439)	102* (78 - 367)	113.5* (86 - 346)	124 (92 - 483)	172.5* (126 - 451)	171* (122 - 433)	169* (121 - 412)
Euglycemic values	44/51 (86.27%)	25/51 (49.02%)	38/51 (74.51%)	37/51 (72.55%)	38/51 (74.51%)	41/51 (80.39%)	39/51 (76.47%)
Hyperglycemic values	7/51 (13.73%)	4/51 (7.84%)	3/51 (5.88%)	10/51 (19.61%)	13/51 (25.49%)	10/51 (19.61%)	12/51 (23.53%)
Hypoglycemic values	0/51 (0%)	22/51 (43.14%)	10/51 (19.61%)	4/51 (7.84%)	0/51 (0%)	0/51 (0%)	0/51 (0%)
Mean variation		21.1%	15.6%	12.2%	23.0%	19.5%	19.2%
Variation's SD		8.2%	7.5%	11.4%	15.8%	14.4%	14.0%
Minimum variation		-34.9%	-32.2%	-29.9%	-12.9%	-11.3%	-14.4%
Maximum variation		11.9%	10.7%	50.9%	68.3%	53.4%	54.1%
Reading below the reference#		94.1%	96.0%	60.8%	4.0%	13.7%	13.7%
Reading above reference#		5.9%	4.0%	35.3%	96.0%	86.3%	86.3%
Reading equal to reference#		0.0%	0.0%	3.9%	0.0%	0.0%	0.0%

SD: standard deviation. #Blood glucose obtained by the laboratory analyzer. (*) indicates statistical difference between laboratory analyzer and portable glucometer ($P < 0.001$).

In terms of the correlation between the portable glucometers (PGMs) and the biochemical analyzer, all correlations were found to be significant ($P < 0.001$). Strong positive association was observed for all PGMs, except for WD, which showed a moderately strong correlation (Table 2). The PGM best associated with the laboratory analyzer was FO, followed by AA and IS.

Intraclass correlation coefficient between 0.75 and 0.90 are considered adequate and values above 0.90 are excellent [14]. All PGMs demonstrated significant results and ICC values greater than 0.75. IS exhibited the highest correlation, surpassing the 0.90 threshold and thus considered excellent in this analysis method (Table 3).

Table 2. Linear correlation coefficient and *P* values associated with Spearman's correlation of blood glucose concentrations obtained of 4 portable glucometers (PGMs) and laboratory analyzer from healthy and diseased rabbits.

PGM	r	*P*
FO	0.907	< 0.001
AA	0.852	< 0.001
IS	0.811	< 0.001
WD	0.729	< 0.001
WC	0.768	< 0.001
WH	0.759	< 0.001

Table 3. Intraclass correlation coefficient (ICC), *P* values and 95% confidence interval (CI) between 4 portable glucometers (PGMs) and laboratory analyzer from healthy and diseased rabbits.

PGM	ICC	*P*	CI95%
FO	0.89	0.025	0.00 - 0.97
AA	0.86	0.017	0.06 - 0.96
IS	0.93	< 0.001	0.88 - 0.96
WD	0.82	0.023	0.01 - 0.95
WC	0.88	0.003	0.36 - 0.96
WH	0.88	0.001	0.45 - 0.96

Bland-Altman plots (Figure 1) revealed quantitative variations between the devices used. FO and AA mostly exhibited underestimated glycemic values in comparison to the laboratory analyzer. On the other hand, IS demonstrated values that were closer to the gold standard method. However, it tended to underestimate the results when the laboratory analyzer indicated lower values, while overestimating higher glycemic values. In contrast, WD, WC and WH generally presented overestimated glycemic values when compared to the laboratory analyzer. IS was the only PGM that showed agreement with the laboratory analyzer in this evaluation. The others PGMs showed significant difference ($P < 0.001$) compared to the reference method (Table 4).

The error grid evaluation revealed that none of the analyzed PGMs presented serious errors compared to the laboratory analyzer, as none of the devices had values falling within Zone C, D and E. Only IS demonstrated more than 75% of the results in Zone A, indicating a high level of agreement with the reference method. AA, WC and WH showed values above 60% in Zone A, while FO presented values above 60% in Zone B, indicating a slightly lower level of agreement and making it the least favorable result among the evaluated devices (Table 5).

The total error for different blood glucose ranges obtained by the laboratory analyzer (euglycemia and hyperglycemia) were summarized in Table 6.

Finally, the results showed that only IS consistently achieved the objective in all performed analyses. WD e WH achieved the objective in 4 out of 6 analyses and FO, AA and WC showed a positive result in 3 out of the 6 analyses (Table 7).

DISCUSSION

In the present study, blood glucose concentrations from sick and healthy rabbits were obtained using 3 human PGMs and 1 veterinary PGM designed for canine, feline and equine species, and the results were compared to a laboratory biochemistry analyzer, considered the reference method.

Blood glucose concentrations obtained by FO e AA were significantly lower compared to the reference method, while the values obtained by WD, WC and WH were significantly higher, demonstrating that, while human PGMs tended to underestimate glycemic levels, veterinary PGM tended to overestimate these values in comparison to the reference method. This observation aligns with findings from previous studies that evaluated portable human glucometers, where the measured blood glucose levels were generally lower than those obtained by reference methods [2,3,24]. This same pattern is not observed in veterinary glucometers, varying depending on the PGM model and the evaluated species [16,24]. Although underestimated values were also observed in the results obtained by the IS, this PGM presented results statistically closer to the values obtained by the biochemical analyzer, demonstrating higher accuracy.

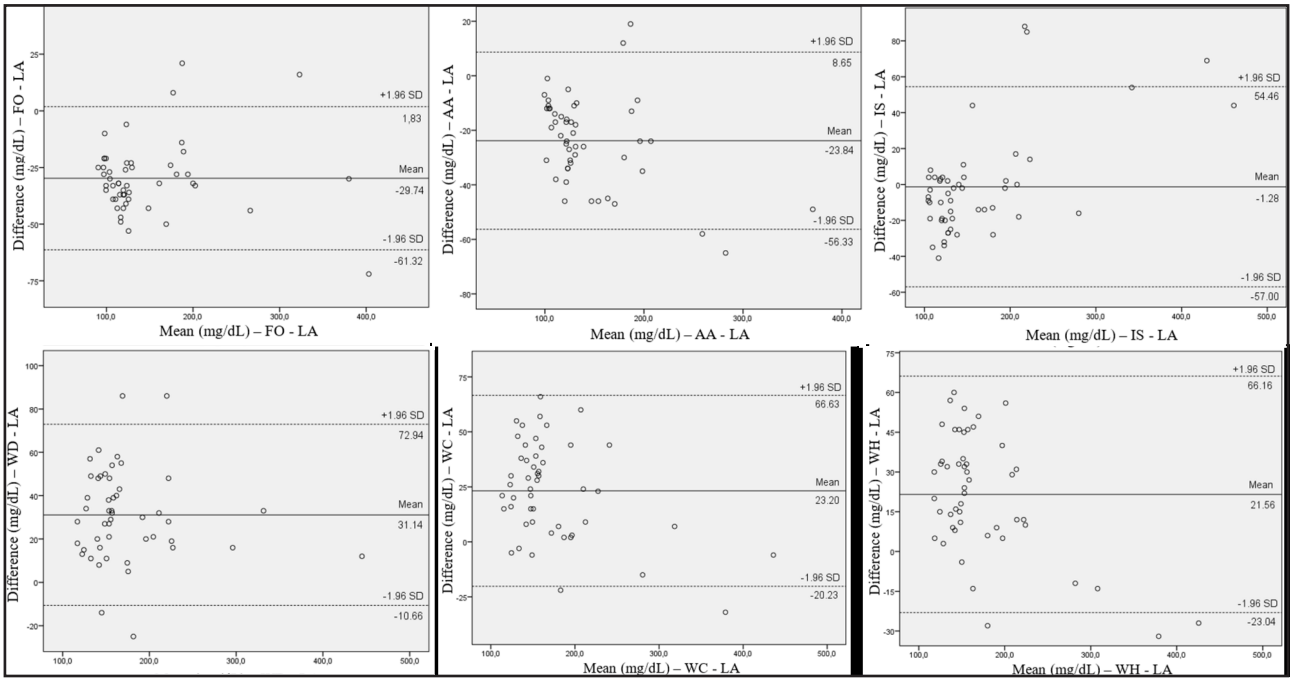

Figure 1. Bland-Altman plot to assess agreement between glucose concentrations of 51 rabbits determined using 4 portable glucometers (PGMs) and the laboratory analyzer (LA). Circles represent individual measurements. The solid horizontal line represents the mean difference between pairs of measurements. The upper and lower horizontal dashed lines represent the 95% limits of agreement.

Table 4. Bland-Altman analysis of the 4 portable glucometers (PGMs) and the laboratory analyzer from healthy and diseased rabbits.

PGM	Mean deviation	Standard deviation of bias	Agreement (CI 95%)		*P*
FO	-29.74	16.11	-61.32	1.83	< 0.001
AA	-23.84	16.58	-56.33	8.65	< 0.001
IS	-1.28	28.44	-57.00	54.46	0.752
WD	31.14	21.32	-72.94	10.66	< 0.001
WC	23.20	22.16	-20.23	66.63	< 0.001
WH	21.56	22.75	-23.04	66.16	< 0.001

Table 5. Results of error grid analysis used to determine the percentage of results that would have potentially led to inappropriate diagnostic or therapeutic decisions with 4 portable glucometers (PGMs) used for determination of blood glucose concentrations in healthy and diseased rabbits.

PGM	Zone A	Zone B	Zone C	Zone D	Zone E
FO	39.2%	60.8%	0.0%	0.0%	0.0%
AA	60.0%	40.0%	0.0%	0.0%	0.0%
IS	76.5%	23.5%	0.0%	0.0%	0.0%
WD	56.0%	44.0%	0.0%	0.0%	0.0%
WC	62.7%	37.3%	0.0%	0.0%	0.0%
WH	62.7%	37.3%	0.0%	0.0%	0.0%

Table 6. Total error of 4 portable glucometers (PGMs) used for determination of blood glucose concentrations in healthy and diseased rabbits at different ranges of glucose concentrations tested.

PGM	Glucose range	
	Euglycemic (n = 44)	Hyperglycemic (n = 7)
FO		
Mean difference (mg/dL)	110.88	265.28
CV (mg/dL)	0.28	0.33
Total error (%)	21.52	11.37
AA		
Mean difference (mg/dL)	119.05	231.00
CV (mg/dL)	0.27	0.27
Total error (%)	15.59	22.80
IS		
Mean difference (mg/dL)	135.38	318.14
CV (mg/dL)	0.30	0.38
Total error (%)	4.09	2.96
WD		
Mean difference (mg/dL)	166.53	289.85
CV (mg/dL)	0.16	0.26
Total error (%)	19.03	2.96
WC		
Mean difference (mg/dL)	172.88	301.65
CV (mg/dL)	0.17	0.29
Total error (%)	23.59	2.04
WH		
Mean difference (mg/dL)	166.56	301.42
CV (mg/dL)	0.15	0.25
Total error (%)	19.04	1.95

CV: Coefficient of variation.

Table 7. Synthesis of the analysis of the effectiveness of portable glucometers in rabbits' blood samples.

PGM	Descriptive/ comparative	Correlation	ICC	Bland-Altman	Error grid	Total error
FO	No	Yes	Yes	No	Yes	No
AA	No	Yes	Yes	No	Yes	No
IS	Yes	Yes	Yes	Yes	Yes	Yes
WD	No	Yes	Yes	No	Yes	Yes
WC	No	Yes	Yes	No	Yes	No
WH	No	Yes	Yes	No	Yes	Yes

No: did not reach the goal. Yes: reached the goal.

Although WD showed only moderately strong correlation, the positive correlation observed in the results obtained by all PGMs indicated that the glycemic values measured by these devices generally move in the same direction as the reference method [28]. This positive correlation was further supported by the ICC, which evaluates the reliability of measurements obtained by different methods of glycemic measurement [14]. All PGMs showed good agreement, with the exception of IS, which showed excellent association with the reference method, indicating superior performance within this analysis.

Significant correlation is a commonly observed result in various studies evaluating PGMs, suggesting an association between the methods, however, it does not rule out the possibility of systematic differences between the tested methods [3,16,22-24]. Despite the correlation being adequate for the different PGMs, descriptive analysis of the data and results from the Bland-Altman concordance analysis showed that only

IS met the expected accuracy in these tests. This finding emphasizes that IS not only exhibited correlation but also demonstrated better agreement with the results obtained by the laboratory analyzer.

Although 3 of the 4 glucometers evaluated did not demonstrated adequate accuracy, error grid analysis provides insight into the clinical acceptability of measurements. However, there is no universally standardized criterion for defining clinical acceptability, as variations exist between countries [26]. One widely referenced standard for this assessment is ISO 15197:2013 [11]. This standard recommends that 99% of the results obtained by the error grid stay between zones A and B. Based on this criterion, all PGMs assessed in this study were considered suitable, as the observed discrepancies in relation to the reference method were considered clinically insignificant and unlikely to impact clinical decision-making [9].

However, it is ideal for a glucometer to not only meet ISO 15197:2013 but also exhibit high accuracy and precision (i.e., low bias and low CV, respectively) in total error analysis. Allowable total error for glucose measurement should be 10% for hypoglycemic samples, 20% for normoglycemic samples, and 20% for hyperglycemic samples [9]. Nevertheless, there was variation in the number of euglycemic, hyperglycemic and hypoglycemic samples when the measurement was performed with PGMs compared to the reference method, demonstrating that all PGMs misclassified the glycemic status of patients at some point. Therefore, considering only euglycemic and hyperglycemic values (as there were no hypoglycemic samples determined by the laboratory analyzer), only FO and WD were considered inadequate for normal glycemic samples, while AA was deemed inadequate for glycemic samples above the reference range for the species. Regardless of the glycemic value, IS showed better accuracy compared to the biochemical analyzer.

A study evaluating 3 human PGMs in dogs, including the FO PGM, demonstrated that this glucometer obtained the best results, being, therefore, the best option among the devices evaluated in the study for the canine species [22]. However, in our experiment, FO presented the poorest performance, especially in relation to the error grid analysis, where approximately 60% of the measurements fell within Zone B, contrasting with other studies that reported higher values falling within Zone A [1,22]. It has been suggested that the methodology used in test strips to separate erythrocytes from plasma might impact in the measurement by PGMs. Nevertheless, studies have shown that the size of erythrocytes between dog and rabbit is similar [10,21,29].

A study using a similar device (same brand, but different model) to the AA in dogs, demonstrated that despite the significant correlation between the glucometer and the reference method, 99% of the glucose concentrations obtained were lower when compared to the laboratory analyzer [3]. Likewise, this similar device consistently underestimated glucose concentrations in studies conducted with horses [10], alpacas [25], ferrets [20] and rabbits [24]. Similar findings were observed in our study, where approximately 96% of the glycemic values measured by the AA were lower than those determined by the reference method. A possible explanation for these results is that human PGMs are designed for self-monitoring of glycemic concentration in humans with diabetes, who adjust their insulin dosage based on the PGM readings. This way, glycemic values slightly lower than actual glucose concentrations would avoid hypoglycemia, since the individual would inject smaller doses of insulin. However, although this PGM also exhibits bias compared to the reference analyzer in humans, the underestimation of glycemia is not as consistent as observed in animals [23].

Hematocrit can affect the accuracy of blood glucose measurement, as the volume of red blood cell can change the glucose ratio in plasma and whole blood [13]. Thus, considering that the hematocrit levels of the animals in this study were within the reference range for the species and within the limits suggested by the manufacturers, the exact cause of this underestimation is remains unknown.

In previous study, an identical device to the AA was evaluated in healthy rabbits, demonstrating underestimated values compared to the reference method. Despite this, Bland-Altman analysis in that study did not indicate a systematic difference between this glucometer and the biochemical analyzer, and 100% of the measurements fell within Zone A of the error grid. These findings indicated that AA performed an accurate analysis of blood glucose in normoglycemic rabbits [24]. In our study, we also observed that AA underestimated glucose values when compared to the reference method. However, we found a systematic dif-

ference between AA and the laboratory analyzer in the Bland-Altman analysis. Furthermore, although 100% of the measurements performed by AA fell within Zones A and B of the error grid, only 60% were located in Zone A This suggests a certain bias in the use of this glucometer for rabbits. This difference compared to the previous study [24] could be attributed to the clinical condition of the animals, as our study included a wider range of glycemic variations, not limited to healthy animals. This observation is supported by the fact that AA presented an error above 20% in the analysis of hyperglycemic samples in our study. Thus, while AA is suitable for euglycemic patients, it may lead to errors in hyperglycemic patients.

The WC PGM has previously been tested in cattle alongside 2 others human PGMs and it displayed a significant difference in glycemic results compared to the reference method. The authors attributed this discrepancy to calibration of the device for different species than the one being studied [16]. As of the conclusion of this study, there is no glucometer specifically produced for rabbits. However, the development of a chip specifically for this species could be tested to validate this hypothesis.

In another study, a veterinary PGM model designed for dogs and cats substantially overestimated blood glucose levels in rabbits [23]. However, this same device did not demonstrate the same alteration in horses [7] and ferrets [20], highlighting the importance of assessing the accuracy of glucometers for different species. Furthermore, in this same study, the canine and feline configurations incorrectly indicated normoglycemic blood samples as hyperglycemic, with an incidence of 9% and 5.6%, respectively. Due to this variation, as well as the results observed in the error grid analysis and total error, the authors concluded that this PGM was clinically inappropriate [23]. Considering that in our study, the veterinary PGM, in all the configurations used, demonstrated an even greater error than in the previous study, it is reasonable to exercise caution when using this device in normoglycemic rabbits, particularly in the context of feline design, that consistently produced higher values than those obtained by the laboratory analyzer.

Until the completion of this study, there was no publication related to the validation of PGM similar to IS in rabbits or other animal species. Therefore, despite the IS demonstrated better agreement and accuracy compared to the other glucometers tested, there is a lack of available data for direct comparison of the results obtained with this particular PGM.

This study had limitations that need to be emphasized. One limitation was the use of a single device for each PGM. Evaluating multiple devices for each PGM, considering the 6 different PGMs (including the 3 veterinary codifications), was not feasible due to resource constraints. Additionally, various factors can potentially interfere with the accuracy of the analyses, such as sample volume, analysis time, test strip stability, altitude, presence of hemolysis, blood temperature, humidity, partial pressure of oxygen in the blood samples, and the prandial (feeding) state of the animals. Although efforts were made to minimize these variations by obtaining and processing the samples under consistent environmental conditions, and randomizing the glucometers to avoid bias in sample volume and analysis time, these factors could still have some influence on the results. Furthermore, the absence of hypoglycemic samples in this study prevented the assessment of the accuracy of the glucometers for this specific glycemic range. Further research with larger sample sizes and consideration of these limitations would be beneficial to gain a more comprehensive understanding of the accuracy and reliability of PGMs in rabbits.

CONCLUSIONS

All PGMs evaluated in this study demonstrated at least one satisfactory result when considering the different analyzes performed. However, when considering the overall data, is the evident that the IS PGM achieved the best results for measuring blood glucose in pet rabbits, both euglycemic and hyperglycemic samples. These findings suggest that IS can be satisfactorily implemented in rabbit medicine. On the other hand, based on the observation of wide limits of agreement, presence of bias and high total error, FO performed the poorest among the evaluated PGMs in this study, followed by AA and the veterinary PGM in different configurations, with the cat configuration (WC) showing the largest analytical error. Despite the lower accuracy, the other evaluated glucometers still showed potential for use in rabbits when a laboratory analyzer is not available. However, results must be considered with caution when using these glucometers, considering the glycemic range presented.

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