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Accuracy evaluation of two portable blood glucose meters in feline patients using whole blood samples

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ABSTRACT: Using portable blood glucose meters (PBGMs) to measure blood glucose (BG) concentration is a common procedure in veterinary practice. Our objective was to evaluate the analytical and clinical accuracy of a human PBGM (Accu-Chek Performa®), (AC) and a veterinary PBGM (GlucoCalea®), (GC) in feline patients. Central venous blood samples were collected from 48 cats at a Brazilian Veterinary teaching hospital. Two devices from each model were used and compared to a reference method (RM). Analytical accuracy was assessed according to ISO 15197:2013 requirements for human PBGMs. Data were compared using Wilcoxon's nonparametric test and represented by Bland-Altman plots. Hematocrit's effect on BG measurements was evaluated by the Spearman correlation coefficient. Clinical accuracy was determined using error grid analysis (EGA). Values of BG were significantly higher in all PBGMs compared to the RM. Although ISO's analytical accuracy requirements could not be met by any of the devices, AC meters were more accurate than GC meters. All AC measurements - but not GC ones - were within zones A and B of the EGA, meeting ISO requirements for clinical accuracy. Significant hematocrit interference was observed in all devices. Therefore, AC showed greater accuracy compared to GC using feline whole blood samples.
Key words: cats, glycemia, hematocrit, portable blood glucose meters.

Avaliação da acurácia de dois glicosímetros portáteis em amostras de sangue total de pacientes felinos

RESUMO: O uso de glicosímetros portáteis (GPs) para aferição da glicemia é um procedimento comum na rotina clínica veterinária. O objetivo deste trabalho é avaliar a acurácia analítica e clínica de um GP humano (Accu-Chek Performa®), (AC) e um GP veterinário (GlucoCalea®), (GC) em gatos. Amostras de sangue venoso central foram coletadas de 48 gatos atendidos em um hospital veterinário-escola no Brasil. Foram utilizados dois GPs de cada modelo e comparados a um método de referência (MR). A acurácia analítica foi avaliada de acordo com os requisitos estipulados pela ISO 15197:2013 para GPs de uso humano. Os dados foram comparados pelo teste não-paramétrico de Wilcoxon e representados em gráficos de Bland-Altman. O efeito do hematócrito sobre os valores de glicemia foi avaliado pelo coeficiente de correlação de Spearman. A acurácia clínica foi avaliada pela análise da grade de erros (AGE). Em comparação com o MR, os valores de glicemia foram maiores em todos os GPs avaliados. Nenhum deles atendeu aos requisitos da ISO quanto à acurácia analítica, mas o AC mostrou-se mais acurado que o GC. Todos os valores de glicemia obtidos pelos GPs humanos - mas não pelos GPs veterinários - estiveram dentro das zonas A e B da AGE, demonstrando acurácia clínica de acordo com as exigências da ISO. A interferência do hematócrito da amostra mostrou-se significativa em todos os aparelhos testados. Portanto, o AC apresentou maior acurácia quando comparado ao GC em amostras de sangue total em felinos.
Palavras-chave: gatos, glicemia, hematócrito, glicosímetro portátil.

INTRODUCTION

The use of portable blood glucose meters (PBGMs) is common in veterinary clinical practice to determine blood glucose (BG) concentration and to guide decision-making due to their lower blood volume requirements allowing less invasive blood collection and quick results.The PBGMs are especially useful when multiple blood samples must be acquired within a short period, e.g., for obtaining a serial BG curve or diabetic ketoacidosis monitoring (WESS & REUSCH, 2000; COHEN et al., 2009).

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Although, BG values obtained by PBGMs are strongly correlated with measurements obtained by a reference method (RM, i.e., automated chemistry analyzers), its analytical accuracy is still questionable (WESS & REUSCH, 2000; JOHNSON et al., 2009). Variables such as hematocrit, blood type (venous versus capillary), and device enzymatic method can all influence acquired values (STEIN & GRECO, 2002). Therefore, clinicians should be aware of possible sources of error when interpreting the results. Thus, PBGM's accuracy evaluation is necessary owing to the continuing launch of new devices on the market. The objective of this study was to evaluate the analytical and clinical accuracy of a human PBGM and a veterinary PBGM using whole blood samples from feline patients.

MATERIALS AND METHODS

Patients

The study was conducted at the Veterinary Clinic Hospital, Federal University of Rio Grande do Sul, Brazil. Forty-eight feline patients were randomly allocated among the general practice population during regular appointments in which blood sampling was indicated. Informed consent was obtained from all cat owners to allow glucose measurement in the blood collected for the study.

Blood collection and BG determinations

Blood samples from the jugular vein were collected as part of the diagnostic workup of each cat after minimal physical restraint using a 21G needle connected to a five-milliliter syringe. Blood was immediately fractionated into tubes (Vacutainer, BD, New Jersey, USA) containing K2 ethylenediaminetetraacetic acid (EDTA) for hematocrit (microhematocrit method at 9520 g for 5 minutes) and hematological evaluations (0.5 mL), sodium fluoride EDTA for BG evaluation by the RM (2 mL), and without anticoagulant for any other serum measurements needed for each specific cat (2 mL). Blood samples were immediately handled by the Hospital's Veterinary Clinical Analysis Laboratory (LACVet). Mean BG concentration by the enzymatic colorimetric glucose oxidase method (Labtest Diagnostica, Lagoa Santa, MG) was obtained in an automatic spectrophotometer (CM 200, Wiener Lab Group, Argentina) as the RM in duplicates.

The remaining blood in the syringe was used to assess BG concentration with both PBGM models. Blood glucose concentration was assessed using four devices: two identical human PBGMs (Accu-Chek Performa®, Roche Diagnostics, Basel, Switzerland; AC1 and AC2), and two identical veterinary PBGMs (Gluco Calea®, Med Trust, Marz, Austria; GC1 and GC2). All exams were performed in duplicate. Both models evaluate BG concentration by the electrochemical method. For the assessment of low BG values, data were obtained from additional 12 blood samples kept for 12 hours in EDTA tubes at room temperature before analysis by PBGMs and the RM (FOBKER, 2014).

Device technical information

According to manufacturers, human AC devices require a minimum blood volume of 0.6 µL and their BG detection limits are 10 to 600 mg/dL. It operates without interference within the 10-65% hematocrit range. The test strip uses an enzymatic reaction of glucose dehydrogenase. The veterinary GC devices require a minimum blood volume of 0.5 µL and their BG detection limits are 20 to 600 mg/dL. It operates without interference within the 35-55% hematocrit range. The test strip uses an enzymatic reaction of glucose oxidase.

Accuracy

Analytical accuracy was assessed according to the INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (ISO 15197:2013) requirements for human PBGMs (ISO, 2013). For a PBGM to be considered accurate, two conditions must be met: 1) when glucose is <100 mg/dL, 95% of its measurements should not differ by more than 15 mg/dL from the RM value, and 2) when glucose is ≥100 mg/dL, 95% of its measurements should not differ by more than 15% from the RM value.

Consensus error grid analysis (EGA) for insulin-dependent diabetic patients was applied to assess the clinical risk of each measure (i.e., clinical accuracy) (PARKES et al., 2000). Error grid analysis compares the BG values from the RM with the PBGM within five error zones associated with the following risk levels: zone A, clinically accurate; zone B, altered clinical action, but with no or minimal effect on clinical outcome; zone C, altered clinical action likely to affect the clinical outcome; zone D, altered clinical action with considerable medical risk; and zone E, altered clinical action with potentially dangerous consequences. For a PBGM to be considered accurate, ISO 15197:2013 stipulates that 99% of values should lie within zones A and B (ISO, 2013).

Statistical analysis

Statistical analyses were performed with GraphPad Prism 6 software package (GraphPad

Software Inc., San Diego, USA). Data normality was assessed by the Shapiro-Wilk test. For accuracy assessment, PBGMs (AC1, AC2, GC1, and GC2) and RM values were compared using Wilcoxon's nonparametric test. The difference between these values was represented by the Bland-Altman plot (BLAND & ALTMAN, 1986). The effect of hematocrit on BG measurement in each meter was evaluated by calculating the Spearman correlation coefficient between hematocrit and the difference between PBGM and RM results (DOMORI et al., 2014). Correlation coefficient values were interpreted as follows: 0.9-1, very high; 0.7-0.89, high; 0.5-0.69, moderate; 0.3-0.49, low, and 0-0.29, minimal correlation (JOHNSON et al., 2009; DOMORI et al., 2014; MORI et al., 2016).Differences were considered significant at P-value <0.05.

RESULTS

From the total of 60 samples evaluated, 10 were hyperglycaemic (> 110 mg/dL, mean = 138 ± 20.7 mg/dL, range 122 to 181 mg/dL), 38 normoglycemic (60-110 mg/dL, mean = 79.8 ± 11.1 mg/dL, range 62 to 104 mg/dL), and 12 hypoglycaemic (< 60 mg/dL, mean = 14.4 ± 16.2 mg/dL, range 1 to 40 mg/dL). The mean coefficient of variation (CV%) in the hyperglycaemic, normoglycemic, and hypoglycaemic ranges was respectively 2.62%, 2.22%, and 2.11% for the AC devices while it was respectively 3.23%, 4.79%, and 2.52% for the GC devices. Values of BG concentration obtained with both PBGMs were significantly higher than the reference values for all measurements (P < 0.001). In terms of analytical accuracy, none of the devices has met ISO 15197:2013 requirements regarding the percentage of variation in the RM. However, when compared to each other, human PBGMs were more accurate than the veterinary meters. Meters AC1 and AC2 had 83 and 92% of their measurements within the required limits, respectively (Figure 1A-B). Conversely, meters GC1 and GC2 performed poorly; only four samples for GC1 and two samples for GC2 were within the required limits by ISO (Figure 1C-D). In contrast, the clinical accuracy of PBGMs AC1 and AC2 assessed by EGA met ISO requirements. All their measurements were within the clinically acceptable zone (zones A and B) (Figure 2A-B). However, PBGMs GC1 and GC2 displayed unsatisfactory results, presenting 15 and 21% of their values in

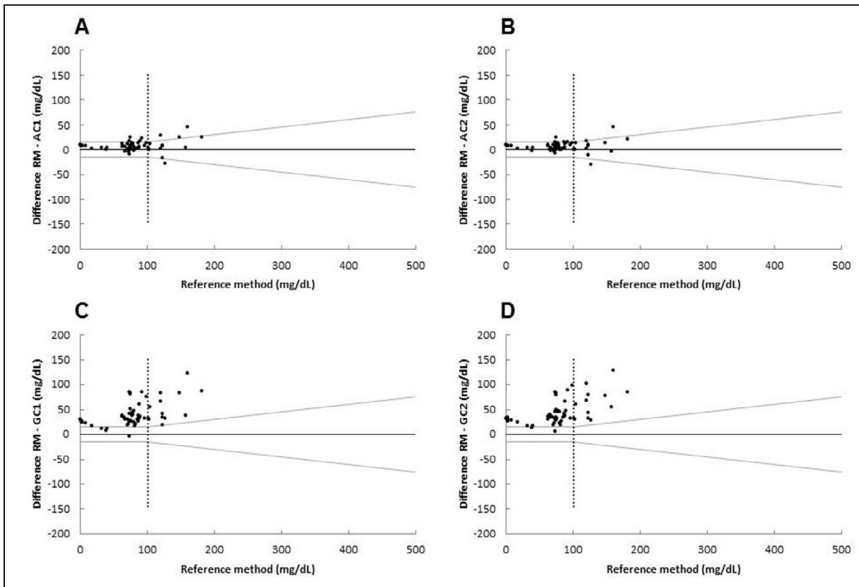

Figure 1 - Bland-Altman plots of four portable blood glucose meters (PBGMs) evaluated in this study. Blood glucose (BG) values determined by the reference method (RM) are represented on the x-axis, while the corresponding differences between BG values determined by the PBGM and the RM are represented on the y-axis. Grey lines express the limits defined by ISO 15197:2013 for analytical accuracy. (A) Accu-Chek Performa® 1 (AC1); (B) Accu-Chek Performa® 2 (AC2); (C) Gluco Calea® 1 (GC1); (D) Gluco Calea® 2 (GC2).

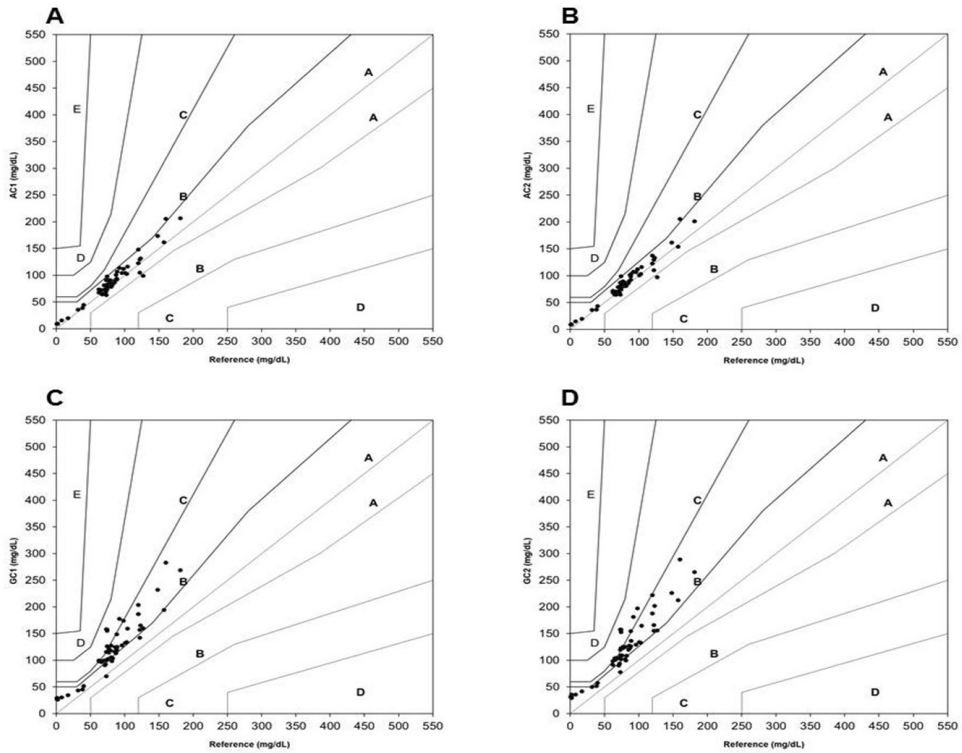

Figure 2 - Error grid analysis of blood glucose (BG) concentrations obtained by four portable blood glucose meters (PBGMs) in 48 feline whole blood samples and 12 hypoglycaemic samples obtained after maintenance in ethylenediamine tetraacetic acid anticoagulant for 12 hours. Values of BG concentration measured with the PBGM are plotted against reference method values. (A) Accu-Chek Performa® 1 (AC1); (B) Accu-Chek Performa® 2 (AC2); (C) Gluco Calea® 1 (GC1); (D) Gluco Calea® 2 (GC2).

zone C, respectively (Figure 2C-D). The median of samples' hematocrit was 28%, ranging from 19 to 40%. Significant hematocrit influence on BG values was observed in all meters. Moderate correlation was detected in AC1 ($r = -0.5782$; $P < 0.001$), AC2 ($r = -0.6045$; $P < 0.001$), GC1 ($r = -0.5871$; $P < 0.001$), and GC2 ($r = -0.5894$; $P < 0.001$).

DISCUSSION

Neither the human nor the veterinary meter here studied has reached the analytical accuracy parameters required by ISO. Surprisingly, we found that the human PBGM (AC) was more accurate than the veterinary device (GC). The reason for such discrepancy; however, remains unclear. Unlike our findings, some studies have shown that veterinary PBGMs are equally or more accurate than those designed for humans (COHEN et al., 2009; ZINI et al., 2009; KANG et al., 2016).

Despite the availability of veterinary devices, the use of human PBGMs is still common in small animal settings. Given the continuous launch of new devices on the market, assessment of their accuracy in clinical practice and validation for the target species are considered a priority in veterinary medicine (BRITO-CASILLAS et al., 2014; CAPASSO et al., 2019). Few studies have evaluated the accuracy of PBGMs exclusively in cats (WESS & REUSCH, 2000; ZINI et al., 2009; DOBROMYLSKYJ & SPARKES, 2010). The models in this study were chosen because AC had a very good performance in dogs compared to other human PBGMs (BRITO-CASILLAS et al., 2014). Conversely, GC was introduced in the Brazilian market and has been only preliminary evaluated in cats (MALERBA et al., 2018).

In our study, the medians of BG values obtained with both human and veterinary PBGMs were higher than the values obtained by the RM.

Several studies have shown that some devices consistently overestimate while others underestimate BG values in small animals (WESS & REUSCH, 2000; JOHNSON et al., 2009; ZINI et al., 2009; BRITO-CASILLAS et al., 2014). Errors in BG measurements may have clinical repercussions or dangerous effects on therapeutic conduct, e.g., iatrogenic hypoglycemia due to insulin overdosing or unnecessary glucose supplementation. In this sense, clinical accuracy assessment of PBGMs should be used in conjunction with analytical accuracy assessment to provide complementary information (WESS & REUSCH, 2000; DOBROMYLSKYJ & SPARKES, 2010; BRITO-CASILLAS et al., 2014; KANG et al., 2016; COSTA et al., 2021).

Recent studies in feline patients evaluating the clinical accuracy of human PBGMs by EGA have obtained all values within zones A and B (DOMORI et al., 2014; MORI et al., 2016), including one study with AC (COSTA et al., 2021). Similarly, our study found 100% of the values for the AC meter within zones A (95%) and B (5%). However, according to EGA, GC presented 15% of the values in zone C, (29% within zone A and 56% within zone B), compromising its clinical accuracy. Therefore, evidence suggested its unsuitability for BG measurement in this species as previously suggested in a research abstract (MALERBA et al., 2018).

Such difference in clinical performance could be partially explained by the interference of the samples' low hematocrit values with BG measurements since GC operates without interference in a much more restricted hematocrit range (35-55%) than AC (10-65%). A wider hematocrit range would be desirable for a veterinary PBGM. The larger the number of erythrocytes in a whole blood sample, the lower the volume of plasma that penetrates the test strip reagent layer, leading to inaccurate results. Thus, hemoconcentration leads to lower BG values, while haemodilution produces higher BG values (RAMLJAK et al., 2013) as observed in our study. However, the correlation between hematocrit and both PBGM analytical accuracy was similar among the four devices studied. Despite this influence, both devices showed acceptable CV% (below 5%) in the different glycemic ranges studied.

CONCLUSION

Accuracy evaluations according to the ISO 15197:2013 criteria before using human or veterinary PBGMs in cats are strongly recommended. Although, none of the devices reached analytical accuracy using feline whole blood samples, Accu-Chek Performa® is a better option than GlucoCalea®. The former has shown acceptable clinical accuracy in the cat and can be safely adopted in clinical routine without compromising clinical conduct. The latter, in turn, has produced clinical accuracy errors that could result in mistaken conduct and unnecessary medical risks. Haematocrit interference on both PBGMs' analytical accuracy was documented, showing a negative relationship with BG measurements.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

FSMM, GLCC, and AGP outlined the study; FSMM, GLCC, and GCS collected the samples; NJSN and SFV were responsible for laboratory determinations; MBM, NJSN, and AGP were responsible for data curation. MBM was responsible for statistical analysis and manuscript draft writing. VCM was involved with writing, reviewing, and translating. AGP was responsible also for writing and reviewing the definitive version. All authors critically review the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The authors declare this research was not evaluated by the Ethics Committee from the Universidade Federal do Rio Grande do Sul; however, the authors are aware of the Conselho Nacional de Controle de Experimentação Animal – CONCEA resolutions. In this way, the authors themselves assume total responsibility for the data presented and are open to any query in case of questions by competent organs.

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