Journal of Herpetological Medicine and Surgery

Performance of Three Portable Blood Glucose Meters in Inland Bearded Dragons (Pogona vitticeps)

--Manuscript Draft--

Manuscript Number:	JHMS-D-24-00024R1
Article Type:	Original Research
Keywords:	Glucose, glucometer, hyperglycemia, neuroendocrine, Pogona vitticeps
Corresponding Author:	Danielle K Tarbert, DVM, DACZM UTIA: The University of Tennessee Knoxville Institute of Agriculture Knoxville, TN UNITED STATES
First Author:	Danielle K Tarbert, DVM, DACZM
Order of Authors:	Danielle K Tarbert, DVM, DACZM
	Lisa Pacumio, BS
	Mélanie Ammersbach, DVM, DACVP
	Hugues Beaufrère, DVM, PhD
Abstract:	Blood glucose concentration measurement is essential for the diagnosis and management of many bearded dragon (Pogona vitticeps) diseases. Portable blood glucose monitors (PBGMs) are inexpensive alternatives to traditional benchtop analyzers and require whole blood volumes as small as 0.3µL. However, PBGMs should be assessed for analytical and clinical agreement with a reference analyzer prior to use in a new species. The potential effects of variables such as packed cell volume (PCV) should also be evaluated. Using blood samples from 48 bearded dragons, three PBGMs were assessed, including a veterinary PBGM (VPBGM) using the canine and feline settings, a human PBGM (HPBGM), and a human point-of-care analyzer (LDX). Statistical analysis was performed using difference plots and Passing-Bablok regression analysis. Analytical agreement was determined using the bearded dragon-specific inherent imprecision of each analyzer, and clinical agreement was based on mammalian total allowable error (TEa) guidelines. A multiple linear regression model was used to investigate the potential effects of PCV, glucose, total solids (TS), lipemia, and hemolysis. The VPBGM overestimated blood glucose on both settings, while the HPBGM and LDX underestimated blood glucose. These respective discrepancies became more pronounced at higher blood glucose concentrations due to proportional biases. No analyzers had analytical agreement with the reference analyzer, and only the LDX was within acceptable clinical decision limits. However, if correction formulas were applied, all analyzers were in clinical agreement. A higher PCV was overall associated with an increasingly negative constant bias. There was no effect of TS concentration or lipemia. While the VPBGM and HPBGM are inexpensive analyzers compared to the LDX and reference analyzer, additional steps, such as the application of corrective formulas, are necessary to ensure acceptable diagnostic results. Alternatively, as precision was good for all analyzers and correlation to the reference analyzer was strong, method-specific reference intervals could be generated.

Performance of Three Portable Blood Glucose Meters in Inland Bearded Dragons (*Pogona vitticeps*)

Danielle K Tarbert[1,2]*, Lisa Pacumio[1], Mélanie Ammersbach[3], Hugues Beaufrère[1]

[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA

[2]Present address (Tarbert): Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, 37996, USA

[3]Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA

*Corresponding author: dtarbert@utk.edu

Abstract

Blood glucose concentration measurement is essential for the diagnosis and management of many bearded dragon (*Pogona vitticeps*) diseases. Portable blood glucose monitors (PBGMs) are inexpensive alternatives to traditional benchtop analyzers and require whole blood volumes as small as 0.3µL. However, PBGMs should be assessed for analytical and clinical agreement with a reference analyzer prior to use in a new species. The potential effects of variables such as packed cell volume (PCV) should also be evaluated. Using blood samples from 48 bearded dragons, three PBGMs were assessed, including a veterinary PBGM (VPBGM) using the canine and feline settings, a human PBGM (HPBGM), and a human point-of-care analyzer (LDX). Statistical analysis was performed using difference plots and Passing-Bablok regression analysis. Analytical agreement was determined using the bearded dragon-specific inherent imprecision of each analyzer, and clinical agreement was based on mammalian total

allowable error (TE_a) guidelines. A multiple linear regression model was used to investigate the potential effects of PCV, glucose, total solids (TS), lipemia, and hemolysis. The VPBGM overestimated blood glucose on both settings, while the HPBGM and LDX underestimated blood glucose. These respective discrepancies became more pronounced at higher blood glucose concentrations due to proportional biases. No analyzers had analytical agreement with the reference analyzer, and only the LDX was within acceptable clinical decision limits. However, if correction formulas were applied, all analyzers were in clinical agreement. A higher PCV was overall associated with an increasingly negative constant bias. There was no effect of TS concentration or lipemia. While the VPBGM and HPBGM are inexpensive analyzers compared to the LDX and reference analyzer, additional steps, such as the application of corrective formulas, are necessary to ensure acceptable diagnostic results. Alternatively, as precision was good for all analyzers and correlation to the reference analyzer was strong, method-specific reference intervals could be generated.

Key Words: Glucose, glucometer, hyperglycemia, neuroendocrine, *Pogona vitticeps*

Introduction

Blood glucose concentration measurement is essential for the diagnosis of multiple well-described inland bearded dragon (*Pogona vitticeps*) diseases. Hyperglycemia is nearly always present in highly malignant gastric neuroendocrine carcinomas (Ritter *et al*., 2009; Anderson *et al*., 2019) and has been documented in other neoplasms such as lymphoid leukemia and hepatocellular carcinoma in this species (Raiti, 2019; Hepps Keeney *et al*., 2021). In reptile species in general, hyperglycemia may be found in association with conditions such as stress, pancreatitis, and diabetes mellitus (Frye, 1991; Raiti, 2019). Recognition of hypoglycemia in reptiles is of equal importance, as this could indicate hepatic

failure or sepsis (Raiti, 2019). While many reptile species have very low blood glucose concentrations compared to mammals, blood glucose reference values in healthy bearded dragons are higher at 153-308 mg/dL (8.49-17.09 mmol/L) (Heatley and Russell, 2019; Raiti, 2019; Howard and Jaensch, 2021).

In addition to aiding clinical diagnoses, blood glucose concentration also informs clinical decision-making. For example, accurate blood glucose concentration measurement is important for fluid therapy planning and treatment response monitoring. A recent investigation demonstrated that administration of 2.5% dextrose solution by either subcutaneous or intracoelomic route resulted in significant increases in blood glucose concentration within 5 minutes (Minor *et al*., 2021). In experimentally dehydrated bearded dragons, administration of reptile Ringer solution (1:1 mixture of 5% dextrose and isotonic crystalloid solution), historically recommended over lactated Ringer solution, resulted in severe hyperglycemia and electrolyte changes that persisted at least 24 hours and is therefore not recommended (Parkinson and Mans, 2020). Neither lactated Ringer solution nor Plasma-Lyte A administration resulted in hyperglycemia, electrolyte changes, or increased blood lactate concentration (Parkinson and Mans, 2020).

Blood glucose concentration may also aid in determining prognosis. In chelonians as well as mammals, derangements in blood glucose at the time of hospital presentation are associated with increased odds of death (Harcourt-Brown and Harcourt-Brown, 2012; Colon and Di Girolamo, 2020). In cold-stunned Kemp's ridley turtles (*Lepidochelys kempii*), plasma glucose concentration at initial presentation was similar between survivors and nonsurvivors, but over the first 2-3 days of hospitalization glucose tended to increase in survivors and decrease in nonsurvivors (Keller *et al*., 2012). While similar studies have yet to be conducted in bearded dragons, severe glucose derangements may occur in critical patients, indicating a need for a more aggressive diagnostic and treatment plan.

Portable blood glucose meters (PBGMs) are inexpensive alternatives to traditional benchtop analyzers and can be useful when limited blood volume can be collected or results are needed

immediately. However, prior to use in a novel species, the PBGM should be assessed for analytical and clinical agreement with the reference analyzer (Gerber and Freeman, 2016). In many cases, there is insufficient agreement to recommend the use of the PBGM, as unpredictable variation could cause important clinical errors (Selleri *et al*., 2014; Higbie *et al*., 2015; Capasso *et al*., 2019; Proulx *et al*., 2022). Poor agreement can be due to different analyzer methodologies, such as the enzymes used to react with glucose or the transducers used for measurement (Gerber and Freeman, 2016). Further, some PBGMs have a filter that separates the red blood cells from the plasma, while others do not and measure glucose concentration from whole blood (Gerber and Freeman, 2016). Plasma glucose concentration tends to be higher than whole blood glucose (Gerber and Freeman, 2016). Some PBGMs have built-in algorithms designed to generate a plasma equivalent from a whole blood capillary sample for ease of comparison to benchtop methods; however, these algorithms may be species-specific, taking into account factors such as glucose distribution between plasma and red blood cells and may be affected by hematocrit (Gerber and Freeman, 2016). Thus, agreement should be assessed for a new species, as well as under a range of variables such as packed cell volumes (PCV) (Gerber and Freeman, 2016). Error associated with hemodiluted or hemoconcentrated samples has been documented in species including humans, dogs, cats, and rabbits (Mann *et al*., 2008; Lane *et al*., 2015; Lane and Koenig, 2019; Cutler *et al*., 2020). In rabbits, correction equations accounting for PCV resulted in improved agreement with the reference method for human glucometers, but did not improve agreement for veterinary glucometers (Cutler *et al*., 2020). Thus, in some cases it is possible to determine correction equations, while in other cases the PBGM simply should not be used.

The objective of this study was to evaluate three PBGMs for potential glucose concentration measurement in inland bearded dragons. Two human devices and one veterinary device with two settings (canine and feline) were assessed for agreement with a reference analyzer. Additionally, the potential effects of variables including PCV, total solids (TS), lipemia, and hemolysis were evaluated.

Material and Methods

This research was approved by the University of California-Davis (UC Davis) Institutional Animal Care and Use Committee (protocol #22816).

Animals: Forty-eight captive-born adult inland bearded dragons (23 males and 25 females) originally selected for culling from a breeding facility (Chico, CA, USA) were evaluated. Culling decisions were made by the breeder based on genetics, age, or unspecified health concerns. Ages ranged from 1.5-7 years old, and mean weight was 305 g +/- 79 g. Bearded dragons were fed a combination of crickets, mealworms, and dark leafy greens supplemented with a calcium carbonate powder and provided water in a bowl. Prior to sampling, animals were temporarily housed at the UC Davis – Teaching and Research Animal Care Services headquarters in glass enclosures with mercury vapor bulbs and fasted for 48 hours. Ambient room temperature was maintained between 26.4-30°C (79.5-86°F) during the day (05:00-17:00), with individual heat lamps providing a range up to 33.3°C (91.9°F). Night temperatures were 26.7-28.9°C (80.1-84°F). Humidity was not monitored. Based on intake physical examination, all dragons appeared alert, hemodynamically stable, euhydrated, and free from pain.

Sample collection: Each bearded dragon was sedated with a subcutaneous injection of alfaxalone (10 mg/kg, Alfaxan multidose, Jurox, Kansas City, MO, USA) in the cranial half of the body. Once sedation was achieved, characterized by decreased response to external stimuli, muscle relaxation, and decreased purposeful movement (Shippy *et al,* 2023), the phlebotomy site was prepped with a 70% alcohol swab and allowed to dry. Up to 2.5 ml blood was collected from the caudal tail or jugular vein using a 25-gauge needle and 3 mL syringe.

Biochemical analysis: Whole blood drops were immediately applied to three PBGMs: a veterinary PBGM (VPBGM, AlphaTrak 2, Zoetis, Parsipanny, NJ, USA) set to canine (cVPBGM), a second identical VPBGM (AlphaTrak 2) set to feline (fVPBGM), and a human PBGM (HPBGM, Accu-Chek® Guide, Roche Diabetes Care Inc., Indianapolis, IN, USA). PBGMs were checked weekly prior to use with control solution in accordance with manufacturer guidelines. The VPBGM requires 0.3 µL whole blood per test strip and has a detection range of 20-750 mg/dL (1.11–41.6 mmol/L). Proprietary formulas convert the result to a species-specific (canine or feline) plasma equivalent. The HPBGM requires 0.6 µL whole blood, serum, or plasma per test strip, with a detection range of 10-600 mg/dL (0.555-33.3 mmol/L). The HPBGM also has built-in calculations that provide the result as a human plasma equivalent. While both analyzers utilize an enzymatic reaction with glucose dehydrogenase, the VPBGM measures the resultant current using coulometry whereas the HPBGM uses amperometry.

The remaining blood was placed into 0.4 ml lithium heparin collection tubes without plasma separator and 0.5 ml K_2EDTA tubes (BD microtainer, Becton Dickinson and Company, Franklin Lakes, NJ, USA). Blood tubes were placed on ice until further processing for not more than 2 hours after collection. PCV and TS were determined in duplicate from the K_2EDTA samples using microhematocrit tubes without additive. Glucose was opportunistically measured using 40 µL heparinized whole blood with a portable human analyzer (LDX, Cholestech LDX™ Analyzer, Abbott Point of Care Diagnostics, Princeton, NJ, USA) as part of a lipid panel (Cholestech LDX™ Lipid Profile GLU cassette, Abbott Point of Care Diagnostics) being performed for a related study (Beaufrère *et al*., 2024). The Cholestech LDX™ Optics Check Cassette (Abbott Point of Care Diagnostics) was run daily in accordance with manufacturer's instructions to ensure the optical system was functioning appropriately. The LDX determines glucose concentration utilizing reflectance photometry following an enzymatic reaction with glucose oxidase.

Remaining lithium heparin tubes were centrifuged at 3000 x g for 7 minutes. Plasma was removed with plastic micropipettes and stored in 0.5 mL polypropylene tubes (Eppendorf, Hamburg,

Germany) at -80°C (-112°F) until analysis. Samples were collected over the course of 3 weeks, then all heparinized plasma was submitted simultaneously to the University of Miami Miller School of Medicine Avian and Wildlife Laboratory (Miami, FL, USA) for biochemistry panel on a reference biochemistry analyzer (Vitros 5600 dry slide chemistry analyzer, Ortho Clinical Diagnostics Inc., Rochester, NY, USA) which included glucose. Hemolysis was graded on a scale of 0-3+ by visual examination, with 0 indicating a non-hemolytic sample (clear plasma color), and 1+, 2+, and 3+ representing mild, moderate, and marked hemolysis, respectively, as previously described (Stacy *et al*., 2019). Lipemia was similarly graded visually on a 0–4 scale with 0 having no lipemia, 1 being mildly lipemic, and 4 being the most severely lipemic. The reference analyzer was calibrated daily with commercial quality controls in accordance with manufacturer instructions. The reference analyzer utilizes enzymatic reactions with glucose oxidase and peroxidase in the presence of dye, which is measured by colorimetry.

To establish bearded-dragon-specific coefficients of variations (CV) for each analyzer, samples from 5 bearded dragons were run in quintuplicates on the reference analyzer, HPGBM, and LDX, and under both the canine and feline setting for the VPBGMs.

Statistical Analysis: Statistical analysis was performed using conventional units (mg/dL) to remain consistent with values reported by the analyzers. The agreement between the PGBMs and the reference analyzer was investigated using difference plots (Fig. 1) and Passing-Bablok regression analysis (Fig. 2). For the Passing-Bablok regression analysis, the constant bias (meter – reference method) is represented by the intercept of the regression line and should be different from 0 to be significant (0 not included in the 95% confidence interval) whereas the proportional bias is represented by the slope of the regression line and should be different from 1 to be significant (1 not included in the 95% confidence interval). For the difference plot, the bias was plotted against the reference method. The 95% limits of agreement (LOA) were obtained by the following formula: bias ± 1.96 $\sqrt{\sigma^2}$ with σ^2 the variance of the bias.

Acceptance limits, within which the two analyzers were considered analytically identical (hereafter referred to as analytical agreement), were defined as: bias ± 1.96 * CV where CV was the combined coefficient of variation of both techniques [$CV=\sqrt{(CV_1^2+CV_2^2)}$] (Jensen and Kjelgaard-Hansen, 2006). Acceptance limits represent the acceptability based on the inherent imprecision of both methods.

Clinical decision limits, within which discrepancies between the two analyzers would not lead to alteration in clinical decisions (hereafter referred to as clinical agreement), were based on the concept of total allowable errors (TE_a) and set at 20% according to published TE_a values for blood glucose in mammals (Harr *et al*., 2013). The observed total error (TE_{obs}) of the study was calculated as 2CV+Bias% and was used to interpret clinical agreement. If TE_{obs} was lower than the acceptance limits, then analytical agreement was interpreted as acceptable. If TE_{obs} was lower than the decision limits, then clinical agreement was interpreted as acceptable (Harr *et al*., 2013).

The clinical decision limits were also plotted on the Passing-Bablok plots. Graphically, agreement was considered adequate when 95% of the datapoints were within these limits. Spearman correlation coefficients were also obtained between the analyzers (weak when $\rho \leq 0.3$, moderate when $0.3 < \rho \leq 0.7$, and strong when $\rho > 0.7$). Passing-Bablok regression equations were used to generate corrective formulas, whenever required.

To investigate the potential effect of PCV, glucose, TS, lipemia, and hemolysis on the agreement, the bias was modelled using a multiple linear regression model including the reference method concentrations as a covariable to account for proportional bias and the type of analyzer to control for analyzers. Assumptions of normality of the residuals, homoscedasticity, linearity, and the presence of outliers were checked on residual and quantile plots. R Statistical Software (Version 4.2.2, R Core Team 2022, R foundation for statistical computing, Vienna, Austria. http://www.R-project.org/) was used for statistical analysis. An alpha of 0.05 was used for statistical significance.

Results

One bearded dragon was excluded due to blood glucose levels above the limits of detection for the HPBGM and VPBGM (1577 mg/dL [87.52 mmol/L] via reference method) thus final sample size was 47 bearded dragons. This severely hyperglycemic animal was later euthanized; post-mortem examination revealed a gastric neuroendocrine carcinoma with hepatic metastasis. Blood glucose concentration for the other 47 animals on the reference analyzer ranged from 127-419 mg/dL (7.05-23.3 mmol/L) with a mean of 192 mg/dL (10.7 mmol/L) and a median of 182 mg/dL (10.1 mmol/L). Three samples were hypoglycemic, 13 euglycemic, and 2 hyperglycemic (Howard and Jaensch, 2021). Reliability statistics are reported in Table 1.

Precision was generally good for all analyzers with the LDX having the lowest precision (Table 1). A strong correlation was found for all PBGMs with the reference analyzer.

The VPBGM had significant constant and proportional biases in canine and feline modes (Fig. 1, 2, Table 1). Both the HPBGM and LDX only had significant proportional biases (Fig. 1, 2, Table 1). The VPBGM was found to overestimate the blood glucose on both settings, while the HPBGM and LDX were found to underestimate the blood glucose. These respective discrepancies became more pronounced at higher blood glucose concentrations due to the significant proportional biases.

On the Passing-Bablok plots, only the LDX was found to be in clinical agreement with the reference analyzer (Fig. 2). However, if correction formulas were applied, all analyzers were found to be in clinical agreement (Fig. 2). Correction equations, as generated by Passing-Bablok regression equations, were as follows:

$$\text{Reference analyzer equivalent} = (\text{cVPBGM} + 54\ \text{mg/dL})/1.7$$

fVPBGM

Reference analyzer equivalent = (fVPBGM + 42 mg/dL)/1.5

HPBGM

Reference analyzer equivalent = (HPBGM + 0.5 mg/dL)/0.8

LDX

Reference analyzer equivalent = (LDX – 9 mg/dL)/0.9

No analyzers had TE_{obs} within the analytical acceptance limits. Only the LDX had TE_{obs} within the clinical decision limits. The corrective formula obtained from the Passing-Bablok regression analysis was applied to the PBGM values and the bias recalculated to generate corrected LOA and TE_{obs} (Table 2). When corrective formulas were applied, all analyzers were within clinical decision limits.

A higher PCV was overall associated with an increasingly negative constant bias (-1.03 ± 0.41 mg/dL per PCV unit, $P < 0.004$) controlling for glucometers. There was no effect of TS ($P = 0.22$) and lipemia ($P = 0.23$). No samples had hemolysis and nine samples were lipemic.

Discussion

All PBGMs lacked analytical agreement with the reference analyzer and thus cannot be used interchangeably. Only the LDX demonstrated clinical agreement with the reference analyzer, which may be in part because the LDX utilizes the same glucose oxidase enzymatic reaction as the reference analyzer. Passing-Bablok regression analysis showed proportional bias was of greatest magnitude with the VPBGM. Negative proportional bias was present for the LDX and HPBGM, resulting in a tendency to underestimate blood glucose concentration. Proportional bias was positive for the VPBGM on both

241 settings, resulting in overestimation of blood glucose concentration within the range of values
242 evaluated. However, for both settings on the VPBGM, due to the concurrent presence of negative
243 constant bias, concentrations could be underestimated with severe hypoglycemia (*e.g.*, <77 mg/dL [4.27
244 mmol/L] on reference method; concentrations this low were not assessed in the data set).
245 A similar recently discontinued HPBGM (Accu-Chek® Aviva, Roche Diabetes Care Inc.,
246 Indianapolis, IN, USA) has been evaluated in rabbits and ferrets, with both tending to underestimate as
247 in bearded dragons (Petritz *et al*., 2013; Selleri *et al*., 2014). Similar to bearded dragons, the TE_{obs} in
248 rabbits for the HPBGM was unacceptable but lower compared to the VPBGM (Selleri *et al*., 2014).
249 VPBGM trends were similar to those found for the same analyzer in dogs and rabbits, but not
250 ferrets (Selleri *et al*., 2014; Proulx *et al*., 2022; Wolfenden *et al*., 2022). As in bearded dragons, the
251 VPBGM tended to overestimate blood glucose concentration in rabbits on both the feline and canine
252 setting, and in dogs on the canine setting (Selleri *et al*., 2014; Wolfenden *et al*., 2022). In rabbits, due to
253 positive proportional bias, overestimation increased on both settings at higher blood glucose
254 concentrations as in bearded dragons (Selleri *et al*., 2014). In ferrets, this VPBGM was overall
255 unpredictable but more commonly underestimated blood glucose concentration (Proulx *et al*., 2022).
256 Similar to rabbits, a negative bias was of greater magnitude at higher PCVs for all PBGMs tested
257 in this study (Cutler *et al*., 2020). Correction equations for PCV have been experimentally derived
258 successfully in rabbits for a HPBGM (Accu-Chek® Aviva), but did not improve agreement when applied to
259 the VPBGM (Cutler *et al*., 2020). While insufficient PCV range was present in this data set to derive PCV
260 correction equations, future studies could assess PCV corrections in bearded dragons using
261 experimentally diluted blood samples (Cutler *et al*., 2020).
262 These results demonstrate that trends are not consistent across species, especially for the
263 VPBGM, and should not be extrapolated in the absence of quality assurance data. Additionally, trends
264 may not be conserved even within the same manufacturer and evaluation of new models is necessary.

Recent evaluation of the VPBGM in ferrets gave different results from a study with the original discontinued model (Alpha Trak, Abbott Laboratories, Abbott Park, IL, USA), indicating evaluation of new models is necessary (Petritz *et al*., 2013; Proulx *et al*., 2022). Differing trends are likely multifactorial and associated with variables such as analyzer methodology, inherent algorithms intended to provide a plasma equivalent, species-specific glucose distribution between erythrocytes and plasma, and hematocrit (Gerber and Freeman, 2016).

While the VPBGM and HPBGM are inexpensive analyzers compared to the LDX and reference analyzer, additional steps such as application of corrective formulas are necessary to ensure acceptable diagnostic results. Alternatively, as precision was good for all analyzers and correlation to the reference analyzer was strong, method-specific reference intervals could be generated (Friedrichs *et al*., 2012). Should reference intervals be made for a PBGM, we suggest use of the HPBGM due to cost of the LDX compared to the other analyzers, and greater magnitude of bias with the VPBGM. A new VPBGM model by the same manufacturer was recently released, but resources may be better directed towards a HPBGM.

Precision was lowest for the LDX; pre-analytical error such as presence of air bubbles may have been present during the replication study. Potential sources of preanalytical error were otherwise minimal. Based on chelonian studies, refrigerated heparinized reptilian blood samples have minimal changes in glucose and hemolysis during the first 24 hours even if plasma is in contact with cells (Heatley and Russell, 2019). In future studies, an alternative buffer such as citrate could be considered to improve blood glucose stability (Lippi *et al.,* 2018). For the reference method biochemistry panel, all samples were shipped on the same day, resulting in lack of standard interval between collection and measurement (range 1-21 days). However, samples were kept at -80°C prior to and during shipping; glucose has been shown to be stable in lithium heparinized plasma samples when frozen for up to 12 weeks, with no significant difference in bias between 2 and 4 weeks of storage (Pleus *et al.,* 2022).

Human medicine utilizes Clarke error grid analysis, which categorizes clinical PBGM accuracy on the basis of therapeutic consequences (Proulx *et al*., 2022). For example, region A indicates the 20% TE_a where 95% of the results should fall, and region E indicates the most dangerous scenarios, such as where erroneous reading of hyperglycemia might result in insulin administration (Proulx *et al*., 2022). At this time specific criteria for treating hypo- or hyperglycemia in bearded dragons has not been determined however error grid analysis could be considered in future studies.

Additional point of care analyzers designed for use in reptiles are available, such as the Abaxis Vetscan VS2 (Zoetis, Parsipanny, NJ, USA). Studies in other reptile species such as Hermann's tortoises (*Testudo hermanni*) have found the Abaxis Vetscan VS2 to overestimate glucose concentrations (Di Girolamo *et al.,* 2018). While assessment of the Abaxis Vetscan VS2 was cost-prohibitive for this study, evaluation could be considered in the future. The Vetscan uses a hexokinase-based reaction to measure glucose, which was not utilized by any of the analyzers in the present study and may be worth investigating (Di Girolamo *et al.,* 2018).

Several recent studies have evaluated the effects of alfaxalone in bearded dragons (Perrin and Bertelsen, 2017; Shippy *et al.,* 2023; Webb *et al.,* 2023). In one study, intravenous alfaxalone at 12 mg/kg resulted in rapid anesthetic induction, subsequent intubation, and a surgical plane of anesthesia, with apnea occurring in 25% of dragons (Perrin and Bertelsen, 2017). In another, 15 mg/kg alfaxalone did not cause apnea regardless of route (intracoelomic, subcutaneous, intramuscular, or intravenous) (Webb *et al.,* 2023). While intravenous administration provided the most consistent sedation, no significant differences were found between administration routes for time to loss and recovery of responses and reflexes (Webb *et al.,* 2023). In our study, a lower dose of 10 mg/kg was utilized in an effort to avoid apnea and because a surgical plane was not required. Subcutaneous administration was elected due to large injection volume. Alfaxalone was administered in the cranial half of the body in our study to avoid the renal portal system and liver shunting, as deeper anesthesia is achieved in ball

pythons when alfaxalone is administered cranially instead of caudally (James *et al.,* 2018). However, a recent publication in bearded dragons found no significant differences in plasma concentrations, time to loss of righting reflex, or time to recovery of righting reflex when 10 mg/kg alfaxalone was administered intramuscularly in either the cranial or caudal half of the body (Shippy *et al.,* 2023).

This study confirms the need for assessment of PBGM agreement prior to adoption in a new species, and that trends may not be conserved across species. Use of a VPBGM or HPBGM can be considered in bearded dragons provided correction equations are applied, or analyzer-specific reference intervals are determined.

Acknowledgements: This study was funded by the UC Davis Sue Solomon Reptile Research Fund and the Students Training in Advanced Research (STAR) program, University of California-Davis; School of Veterinary Medicine.

Disclaimer: The authors have no conflict of interests to declare.

Literature Cited

Anderson KB, Meinkoth J, Hallman M, Bailey K, Brandão J. 2019. Cytological diagnosis of gastric neuroendocrine carcinoma in a pet inland bearded dragon (*Pogona vitticeps*). J Exot Pet Med, 29: 188–193.

Beaufrère H, Pacumio L, Susta L, Tarbert DK, Ammersbach M, Keel K. 2024. Hepatic lipid accumulation is associated with multiple metabolic pathway alterations but not dyslipidemia and insulin resistance in central bearded dragons (*Pogona vitticeps*). Am J Vet Res, 85(6):1-10.

337 Capasso M, Di Girolamo N, Silvestre P, Laricchiuta P. 2019. Performance of two portable blood glucose
338 meters for measuring blood glucose concentration in tigers (*Panthera tigris*) and lions (*Panthera*
339 *leo*). J Am Vet Med Assoc, 254(3):399-408.
340 Colon VA, Di Girolamo N. 2020. Prognostic value of packed cell volume and blood glucose concentration
341 in 954 client-owned chelonians. J Am Vet Med Assoc, 257(12):1265–1272.
342 Cutler DC, Koenig A, Di Girolamo N, Mayer J. 2020. Investigation for correction formulas on the basis of
343 packed cell volume for blood glucose concentration measurements obtained with portable
344 glucometers when used in rabbits. Am J Vet Res, 81(8):642–650.
345 Di Girolamo N, Ferlizza E, Selleri P, Nardini G, Isani G. 2018. Evaluation of point-of-care analysers for
346 blood gas and clinical chemistry in Hermann's tortoises (*Testudo hermanni*). J Small Anim Pract,
347 59(11):704–713.
348 Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. 2012. ASVCP
349 reference interval guidelines: determination of de novo reference intervals in veterinary species
350 and other related topics. Vet Clin Pathol, 41(4):441-453.
351 Frye F. 1991. Biomedical and Surgical Aspects of Captive Reptile Husbandry, 2nd ed. Krieger Publishing,
352 Malabar, FL, USA.
353 Gerber KL, Freeman KP. 2016. ASVCP guidelines: Quality assurance for portable blood glucose meter
354 (glucometer) use in veterinary medicine. Vet Clin Pathol, 45(1):10–27.
355 Harcourt-Brown FM, Harcourt-Brown S. 2012. Clinical value of blood glucose measurement in pet
356 rabbits. Vet Rec, 170(26):674.
357 Harr KE, Flatland B, Nabity M, Freeman KP. 2013. ASVCP guidelines: allowable total error guidelines for
358 biochemistry. Vet Clin Pathol, 42(4):424–436.
359 Heatley JJ, Russell KE. 2019. Clinical chemistry. *In* Divers SJ, Stahl SJ (eds.): Mader's Reptile and
360 Amphibian Medicine and Surgery. Elsevier, St. Louis, MO, USA:319–332.

Hepps Keeney CM, Intile JL, Sims CS, Harrison TM. 2021. Lymphoid leukemia in five bearded dragons (*Pogona vitticeps*). J Am Vet Med Assoc, 258(7):748–757.

Higbie CT, Eshar D, Bello NM. 2015. Evaluation of three point-of-care meters and a portable veterinary chemistry analyzer for measurement of blood glucose concentrations in black-tailed prairie dogs (*Cynomys ludovicianus*). Am J Vet Res, 76(6):532–539.

Howard JG, Jaensch S. 2021. Haematology and plasma biochemistry reference intervals in wild bearded dragons (*Pogona vitticeps*). Aust Vet J, 99(6):236–241.

James LE, Williams CJ, Bertelsen MF, Wang T. 2018. Anaesthetic induction with alfaxalone in the ball python (*Python regius*): Dose response and effect of injection site. Vet Anaesth Analg, 45(3):329-337.

Jensen AL, Kjelgaard-Hansen M. 2006. Method comparison in the clinical laboratory. Vet Clin Pathol, 35(3):276–286.

Keller KA, Innis CJ, Tlusty MF, Kennedy AE, Bean SB, Cavin JM, Merigo C. 2012. Metabolic and respiratory derangements associated with death in cold-stunned Kemp's ridley turtles (*Lepidochelys kempii*): 32 cases (2005–2009). J Am Vet Med Assoc, 240(3):317–323.

Lane SL, Koenig A. 2019. Development and evaluation of a formula to correct blood glucose concentration measurements in hemodiluted and hemoconcentrated feline blood samples tested by use of a veterinary point-of-care glucometer. J Am Vet Med Assoc 254(10):1180–1185.

Lane SL, Koenig A, Brainard BM. 2015. Formulation and validation of a predictive model to correct blood glucose concentrations obtained with a veterinary point-of-care glucometer in hemodiluted and hemoconcentrated canine blood samples. J Am Vet Med Assoc, 246(3):307–312.

Lippi G, Nybo M, Cadamuro J, Guimaraes JT, van Dongen-Lases E, Simundic AM. 2018. Blood glucose determination: Effect of tube additives. Adv Clin Chem, 84:101–123.

Mann EA, Salinas J, Pidcoke HF, Wolf SE, Holcomb JB, Wade CE. 2008. Error rates resulting from anemia can be corrected in multiple commonly used point-of-care glucometers. J Trauma, 64(1):15–20.

Minor RL, Doss GA, Mans C. 2021. Evaluation of glucose absorption rates following intracoelomic or subcutaneous administration in experimentally dehydrated inland bearded dragons (*Pogona vitticeps*). Am J Vet Res, 82(11):920–923.

Parkinson LA, Mans C. 2020. Evaluation of subcutaneously administered electrolyte solutions in experimentally dehydrated inland bearded dragons (*Pogona vitticeps*). Am J Vet Res, 81(5):437–441.

Perrin KL, Bertelsen MF. 2017. Intravenous alfaxalone and propofol anesthesia in the bearded dragon (*Pogona vitticeps*). J Herp Med Surg, 27(3), 123-126.

Petritz OA, Antinoff N, Chen S, Kass PH, Paul-Murphy JR. 2013. Evaluation of portable blood glucose meters for measurement of blood glucose concentration in ferrets (*Mustela putorius furo*). J Am Vet Med Assoc, 242(3):350–354.

Pleus S, Freckmann G, Baumstark A, Haug C. 2022. Stability of glucose concentrations in frozen plasma. J Diabetes Sci Technol, 16(5):1096–1100.

Proulx MP, Vergneau-Grosset C, Ebert JH, Edard CB, Maccolini E. 2022. Comparison of a portable blood glucose meter analyzer with a benchtop point-of-care chemistry analyzer for measurement of blood glucose concentration in client-owned ferrets (*Mustela putorius furo*). J Exot Pet Med, 43:22–28.

Raiti P. 2019. Endocrinology. *In* Divers SJ, Stahl SJ (eds.): Mader's Reptile and Amphibian Medicine and Surgery. Elsevier, St. Louis, MO, USA:835-848.

Ritter JM, Garner MM, Chilton JA, Jacobson ER, Kiupel M. 2009. Gastric neuroendocrine carcinomas in bearded dragons (*Pogona vitticeps*). Vet Path, 46(6):1109-1116.

Selleri P, Di Girolamo N, Novari G. 2014. Performance of two portable meters and a benchtop analyzer for blood glucose concentration measurement in rabbits. J Am Vet Med Assoc, 245(1):87–98.

Shippy S, Allgood H, Messenger K, Hernandez JA, Gatson B, Martin de Bustamante MG, Alexander AB, Wellehan JF, Johnson A. 2023. Pharmacokinetics and pharmacodynamics of intramuscular alfaxalone in central bearded dragons (*Pogona vitticeps*): Effect of injection site. Vet Anaesth Analg 50(3):280–288.

Stacy NI, Chabot RM, Innis CJ, Cray C, Fraser KM, Rigano KS, Perrault JR. 2019. Plasma chemistry in nesting leatherback sea turtles (*Dermochelys coriacea*) from Florida: Understanding the importance of sample hemolysis effects on blood analytes. PLoS One, 14(9):e0222426.

Webb JK, Keller KA, Chinnadurai SK, Kadotani S, Allender MC, Fries R. 2023. Use of alfaxalone in bearded dragons (*Pogona vitticeps*): Optimizing pharmacodynamics and evaluating cardiogenic effects via echocardiography. J Am Vet Med Assoc, 261(1):126–131.

Wolfenden G, James FE, Hung LHT, Bruce M, Thompson M. 2022. Comparative accuracy of two veterinary-calibrated point-of-care glucometers for measurement of blood glucose concentration in dogs. J Small Anim Pract, 63(7):512–519.

Table 1: Results from methods comparison analysis evaluating analytical and clinical agreement of three portable glucose meters (PBGMs) with a reference analyzer in inland bearded dragons (*Pogona vitticeps*). PBGMs assessed included a veterinary PBGM (VPBGM) using the canine and feline settings, a human PBGM (HPBGM), and a human point-of-care analyzer (LDX). Passing-Bablok regression analysis was used to identify constant and proportional bias. Difference plots were used to determine bias based on mean differences and calculate 95% limits of agreement (LOA). Observed total error (TE_{obs}) was calculated using bearded dragon-specific coefficients of variation (CV) for each analyzer and bias as determined by the difference plots. Acceptance limits (analytical agreement) were calculated using the combined CV of both analyzers. Clinical decision limits (clinical agreement) were based on mammalian total allowable error (TE_a) guidelines. No analyzers had TE_{obs} within the analytical acceptance limits and only the LDX had TE_{obs} within the clinical decision limits. CI = confidence interval.

	N	CV (%)	ρ	Constant bias (95% CI ; mg/dL)	Proportional bias (95% CI ; mg/dL)	LOA (mg/dL)	TEobs (%)	Acceptance limits (%)	Clinical decision limits (%)
cVPBGM (AlphaTrak 2, canine setting)	47	2.1	0.90	-54 (-114; -7)*	1.7 (1.4;2.0)*	54.4	42.8	4.2	20
fVPBGM (AlphaTrak feline setting)	47	2.6	0.85	-42 (-105;-5)*	1.5 (1.2;1.8)*	55.0	31.0	5.2	20

HPBGM (Accu-Chek® Guide)	47	1.5	0.90	-0.5 (-29.1;18.5)	0.8 (0.7;0.9)*	31.0	24.1	3.0	20
LDX (Cholestech LDX™ Analyzer)	38	5.0	0.92	9 (-6;24)	0.9 (0.8;0.98)*	38.5	16.3	9.8	20
Reference method	47	0.4	NA	NA	NA	NA	NA	NA	NA

Table 2: Results following application of corrective formulas to methods comparison analysis evaluating analytical and clinical agreement of three portable glucose meters (PBGMs) with a reference analyzer in inland bearded dragons (*Pogona vitticeps*). PBGMs assessed included a veterinary PBGM (VPBGM) using the canine and feline settings, a human PBGM (HPBGM), and a human point-of-care analyzer (LDX). The corrective formulas were obtained from Passing-Bablok regression analysis and applied to the PBGM values. Bias was recalculated to generate corrected 95% limits of agreement (LOA) and observed total error (TE_{obs}). When corrective formulas were applied, all analyzers were within the 20% total allowable error (TE_a) clinical decision limit.

	LOA corrected (mg/dL)	**TEobs corrected (%)**
cVPBGM (AlphaTrak 2, canine setting)	29.0	13.9
fVPBGM (AlphaTrak 2, feline setting)	40.4	11.0
HPBGM (Accu-Chek® Guide)	25.3	4.2
LDX (Cholestech LDX™ Analyzer)	39.1	10.4

Figure Legends

Figure 1: Difference plot of the bias on y-axis against the reference method concentrations of blood glucose for portable blood glucose meters (PBGMs) in inland bearded dragons (*Pogona vitticeps*). The plain line represents the line of perfect agreement, the dotted line represents the mean bias and the dashed lines present the 95% limits of agreement. Upward trends seen with increasing glucose values are compatible with a positive proportional bias (VPBGM [AlphaTrak 2]). Downward trends seen with increasing glucose values are compatible with a negative proportional bias (HPBGM [Accu-Chek® Guide]; LDX). A) cVPBGM, AlphaTrak 2, canine setting; B) fVPBGM, AlphaTrak 2, feline setting; C) HPBGM, Accu-Chek® Guide; D) LDX, Cholestech LDX™ Analyzer. POC = point of care analyzer

Figure 2: Plot of the glucose concentrations obtained by portable blood glucose meters (PBGMs) and a reference analyzer in inland bearded dragons (*Pogona vitticeps*). The dashed line represents the line of perfect agreement, the dotted line is the Passing-Bablok regression line, the grey shaded area represents the clinical decision limits based on total allowable error limits centered around the Passing-Bablok regression line. If the line of perfect agreement is not within the clinical decision limits, a correction formula is necessary. If correction formulas are applied, the two analyzers are within clinical agreement with 95% of the datapoints within the clinical decision limits.

Figure 1

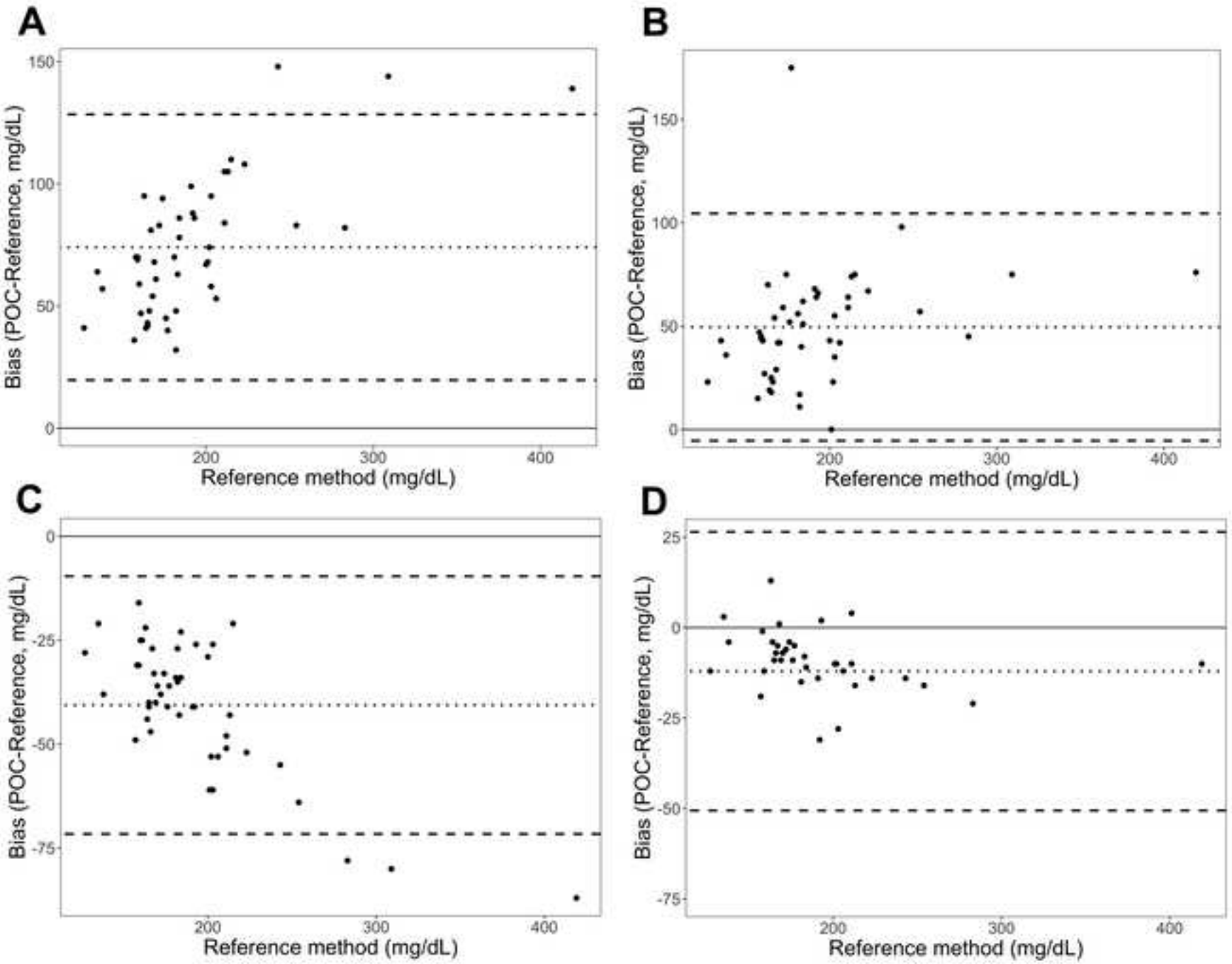

Figure 2