Comparison of Whole Blood and Plasma Glucose Concentrations in Green Turtles (*Chelonia mydas*) Determined Using a Glucometer and a Dry Chemistry Analyzer

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ABSTRACT: We compared glucose concentrations in whole blood and plasma from green turtles (*Chelonia mydas*) using a glucometer with plasma glucose analyzed by dry chemistry analyzer. Whole blood glucose (glucometer) and plasma glucose (dry chemistry) had the best agreement (r_s=0.85) and a small negative bias (–0.08 mmol/L).

Glucose is an important diagnostic analyte in sea turtles and, when deranged, can be a useful indicator for stress, nutritional status, disease, or iatrogenic alterations (Stacy and Innis 2017). Glucose concentrations can be determined using clinical chemistry analyzers at diagnostic laboratories at a considerable cost (US $10–20/sample). Point-of-care devices are being increasingly used in wildlife diagnostics due to low costs, small required sample volume, immediate sample analysis, and improved logistics in the field (Wolf et al. 2008; Stoot et al. 2014). In sea turtles, glucose has been measured using handheld glucometers manufactured for human glucose monitoring (Alberghina et al. 2015a, b). While generating blood glucose by glucometer is inexpensive (US $0.18–0.30/sample and the cost of the glucometer is as low as US $10–20), they have not been validated in sea turtles using traditional clinical chemistry analyzers (Stoot et al. 2014). The objective of this study was to compare three different analytical methods for determining glucose concentrations: whole blood (WB^{ET}) and plasma (P^{ET}) using a point-of-care glucometer and in plasma (P^{DCA}) using a dry chemistry analyzer to identify if glucometers manufactured for human glucose monitoring are suitable for use in sea turtles.

Following dip-net capture and immediate release, a total of 31 juvenile green turtles (*Chelonia mydas*) were sampled as part of a larger study on fibropapillomatosis. The venipuncture site was cleaned, disinfected with alcohol and iodine, and blood was collected using 22-ga needles and lithium heparin Vacutainers® (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Samples were insulated and placed on wet ice in a cooler for up to 8 hr in the field. Upon return to the laboratory, the whole blood samples were well-mixed and analyzed for WB^{ET} using the EasyTouch® glucose monitoring system (MHC® Medical Products, Fairfield, Ohio, USA) based on glucose oxidase and potentiometry with test strips for use in capillary whole blood. The test strips were adequately filled with whole blood per manufacturer's recommendations. Plasma was then immediately harvested after centrifugation of the whole blood sample for 10 min at $1,318 \times G$ and analyzed for P^{ET}. Plasma was transferred into cryovials and frozen at –80 C for about 50 d, after which P^{DCA} was determined with a dry chemistry analyzer (Vitros 250®, Ortho-Clinical Diagnostics, Rochester, New York, USA) using glucose oxidase, peroxidase, and colorimetry. Hemolysis was absent in 30 of the 31 samples (score=0 out of 3+); the one hemolyzed sample had a hemolysis score of 1+ (Stacy and Innis 2017), which was not considered to be sufficient to affect glucose measurements.

All results were analyzed using statistical software (MedCalc® version 17.6, Ostend, Belgium). Passing-Bablok regressions were used to determine the Spearman correlation coefficient (r_s) and the intercept and slope

TABLE 1. Agreement between glucose in whole blood using a point-of-care glucometer (WB^{ET}) and plasma determined by dry chemistry analyzer (P^{DCA}), glucose in plasma using a point-of-care glucometer (P^{ET}) and P^{DCA}, and glucose in WB^{ET} and P^{ET} in green turtles (*Chelonia mydas*). Intercepts closest to 0, slopes closest to 1, and biases closest to 0 indicate the fewest systematic and proportional differences between methodologies. WB^{ET} and P^{DCA} had the best level of agreement. Methodology for glucose analysis included the point-of-care glucometer EasyTouch® glucose monitoring system (MHC Medical Products) and a dry chemistry analyzer (Vitros 250, Ortho-Clinical Diagnostics). CI = confidence interval.

Methods compared	r_s	Intercept with 95% CI	Slope with 95% CI	Bias with 95% limits of agreement
WB^{ET}/P^{DCA}	0.85	0.26 (−0.90 to 0.82)	0.92 (0.79–1.18)	−0.08 (−0.87 to 1.03)
P^{ET}/P^{DCA}	0.93	0.76 (0.10–1.22)	0.58 (0.50–0.70)	−1.70 (−0.42 to −2.98)
WB^{ET}/P^{ET}	0.89	0.62 (−0.14 to 1.24)	0.62 (0.51–0.75)	1.61 (0.16–3.07)

with a 95% confidence interval. Bland-Altman biases with 95% limits of agreement were reported. Data were tested for normality using the Shapiro-Wilk statistic. Differences in glucose concentrations between the three methods (WB^{ET}, P^{ET}, P^{DCA}) were determined using a Welch analysis of variance because the variances between each method were unequal as determined by a Levene's test. A Games-Howell post hoc test was used to determine which methods were different from the others.

The two methods with the smallest bias (−0.08 mmol/L) and a good line of identity closest to $y=x$ (Table 1 and Fig. 1) were WB^{ET} and P^{DCA}. These two methods also showed a good correlation score ($r_s=0.85$). Although this correlation coefficient was lower than the correlation between P^{ET} and P^{DCA} ($r_s=0.93$), the P^{ET} was lower than P^{DCA} by 1.70 mmol/L on average (Table 1).

Welch's analysis of variance indicated that glucose measurements differed significantly by method (Welch's $F_{2,58.01}=19.51$; $P<0.001$). Games-Howell post hoc comparisons showed that WB^{ET} (mean±SD=4.2±0.8 mmol/L) were significantly lower ($P<0.001$) than P^{ET} (5.8±1.4 mmol/L) but were not significantly different ($P=0.922$) from P^{DCA} (4.1±0.8 mmol/L). Games-Howell post hoc comparisons also indicated that glucose data in P^{ET} were significantly higher ($P<0.001$) than P^{DCA}.

The results of this study provided new information on the validity of a point of care glucometer in a nonmammalian species and documented its suitability in field and clinical settings in a sea turtle species. Our results are similar to those presented by Wolf et al. (2008) in that the methods using glucose oxidase methodology with whole blood (WB^{ET}) resulted in lower concentrations when compared to regular chemistry analyzers using plasma (P^{DCA}). Glucose analysis by some glucometers is based on electromechanical methodology (Gerber and Freeman 2016), as in the glucometer used in this study. Compared to other available glucometer methods, electromechanically based glucose analysis reportedly provides more-accurate results for smaller volume samples and sustains fewer effects for samples from species with lower body temperature and packed cell volumes than for humans (Gerber and Freeman 2016). Although lesser but possibly considerable effects are expected from these factors in ectothermic sea turtles with naturally lower packed cell volume, additional considerations affecting this methodology include the presence of nucleated and larger erythrocytes and higher sodium and chloride plasma concentrations in sea turtles compared to human blood. These differences likely explain the variations of glucose results in whole blood and plasma using the glucometer (i.e., from interference with the enzymatic reaction and potentiometry and species-specific differences in glucose bound to erythrocytes).

Our goal was method comparison. Reference intervals for the methodologies are not reported herein because many of the sampled
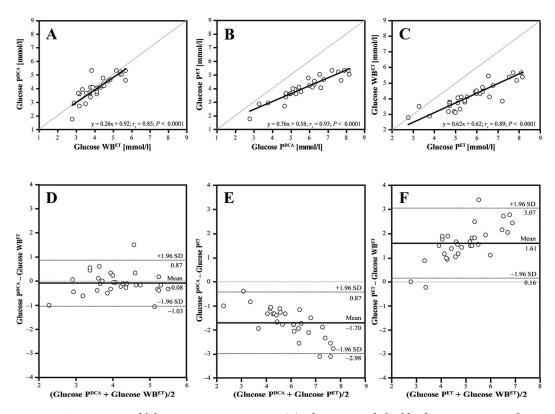
FIGURE 1. Passing-Bablok regressions comparing (A) glucose in whole blood using a point-of-care glucometer (WB^{ET}) and plasma determined by dry chemistry analyzer (P^{DCA}); (B) glucose in plasma using a point-of-care glucometer (P^{ET}) and P^{DCA}; and (C) glucose in WB^{ET} and P^{ET} in green turtles (*Chelonia mydas*). Bland-Altman difference plots comparing (D) glucose in WB^{ET} and P^{DCA}; (E) glucose in P^{ET} and P^{DCA}; and (F) glucose in WB^{ET} and P^{ET}. In A–C, the dashed gray lines indicate $y=x$ (i.e., the lines of identity) and the solid black lines are the lines of best fit. In D–F, the dashed gray lines indicate the lines of identity, the solid black lines indicate the mean difference between the methods, and the longer gray hatched lines indicate the 95% limits of agreement. WB^{ET} and P^{DCA} had the best level of agreement. Methodology for glucose analysis included the point-of-care glucometer EasyTouch® glucose monitoring system (MHC Medical Products) and a dry chemistry analyzer (Vitros 250, Ortho-Clinical Diagnostics).

animals were variably affected by fibropapillomatosis and not considered healthy. In addition, glucose should be analyzed as soon as possible to avoid consumption by erythrocytes during prolonged contact of plasma with erythrocytes, as glucose reportedly decreases by 7% within 24 h (Eisenhawer et al. 2008). Despite this reduction in glucose concentration over several hours, sample analysis and processing was performed at the same time point. Although plasma was frozen for analysis by dry chemistry analyzer, the effect of freezing was not of concern given the stability of glucose for extended time periods (Cray et al. 2009).

From a clinical perspective, our results indicated that the glucometer that we used for our study produced results for whole blood glucose that were acceptable overall in comparison to results from a traditional plasma chemistry analyzer, considering that the glucometer tended to produce comparatively higher glucose data. However, in 16% (5/31) of the samples, the difference was greater than 0.6 mmol/L, with the greatest discordance at 1.50 mmol/L. Such discordance has the potential to affect clinical decisions (i.e., possible difference in fluid treatment). Thus, a low glucose concentration using the glucometer interpreted in context of

clinical findings indicates the presence of hypoglycemia in the sea turtle patient and should alert the clinician to consider appropriate treatment options. Methodology differences in plasma chemistries, specifically of whole blood and plasma glucose, from any species are of clinical importance when reporting and interpreting results. The establishment of methodology-specific reference intervals is warranted.

LITERATURE CITED

Alberghina D, Marafioti S, Spadola F, Panzera M, Piccione G. 2015a. Influence of short-term storage conditions on the stability of total protein concentrations and electrophoretic fractions in plasma samples from loggerhead sea turtles, *Caretta caretta*. *Comp Clin Path* 24:1091–1095.

Alberghina D, Panzera M, Maccarrone V, Spadola F, Insacco G, Piccione G. 2015b. Study of some blood parameters in *Caretta caretta* during a recovery period. *Comp Clin Path* 24:193–195.

Cray C, Rodriguez M, Zaias J, Altman NH. 2009. Effects of storage temperature and time on clinical biochemical parameters from rat serum. *J Am Assoc Lab Anim Sci* 48:202–204.

Eisenhawer E, Courtney CH, Raskin RE, Jacobson E. 2008. Relationship between separation time of plasma from heparinized whole blood on plasma biochemical analytes of loggerhead sea turtles (*Caretta caretta*). *J Zoo Wildl Med* 39:208–215.

Gerber KL, Freeman KP. 2016. ASVCP guidelines: Quality assurance for portable blood glucose meter (glucometer) use in veterinary medicine. *Vet Clin Pathol* 45:10–27.

Stacy NI, Innis CJ. 2017. Clinical pathology. In: *Sea turtle health and rehabilitation*, Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA, editors. J. Ross Publishing, Plantation, Florida, pp. 147–207.

Stoot LJ, Cairns NA, Cull F, Taylor JJ, Jeffrey JD, Morin F, Mandelman JW, Clark TD, Cooke SJ. 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: A review. *Conserv Physiol* 2:cou011.

Wolf KN, Harms CA, Beasley JF. 2008. Evaluation of five clinical chemistry analyzers for use in health assessment in sea turtles. *J Am Vet Med Assoc* 233:470–475.