

# Evaluation of the agreement among three handheld blood glucose meters and a laboratory blood analyzer for measurement of blood glucose concentration in Hispaniolan Amazon parrots (*Amazona ventralis*)

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**Objective**—To determine the degree of agreement between 3 commercially available point-of-care blood glucose meters and a laboratory analyzer for measurement of blood glucose concentrations in Hispaniolan Amazon parrots (*Amazona ventralis*).

**Animals**—20 healthy adult Hispaniolan Amazon parrots.

**Procedures**—A 26-gauge needle and 3-mL syringe were used to obtain a blood sample (approx 0.5 mL) from a jugular vein of each parrot. Small volumes of blood (0.6 to 1.5  $\mu$ L) were used to operate each of the blood glucose meters, and the remainder was placed into lithium heparin microtubes and centrifuged. Plasma was harvested and frozen at  $-30^{\circ}\text{C}$ . Within 5 days after collection, plasma samples were thawed and plasma glucose concentrations were measured by means of the laboratory analyzer. Agreement between pairs of blood glucose meters and between each blood glucose meter and the laboratory analyzer was evaluated by means of the Bland-Altman method, and limits of agreement (LOA) were calculated.

**Results**—None of the results of the 3 blood glucose meters agreed with results of the laboratory analyzer. Each point-of-care blood glucose meter underestimated the blood glucose concentration, and the degree of negative bias was not consistent (meter A bias,  $-94.9$  mg/dL [LOA,  $-148.0$  to  $-41.7$  mg/dL]; meter B bias,  $-52$  mg/dL [LOA,  $-107.5$  to  $3.5$  mg/dL]; and meter C bias,  $-78.9$  mg/dL [LOA,  $-137.2$  to  $-20.6$  mg/dL]).

**Conclusions and Clinical Relevance**—On the basis of these results, use of handheld blood glucose meters in the diagnosis or treatment of Hispaniolan Amazon parrots and other psittacines cannot be recommended. (*Am J Vet Res* 2009;70:172–175)

In human and small animal medicine, hyperglycemia and hypoglycemia are common hematologic abnormalities. Treatment for either condition requires serial measurements of blood glucose concentrations with near instantaneous results. Therefore, POC devices that require a minimal amount of blood for measurement of glucose concentration are essential. The introduction of reliable, inexpensive, and accurate handheld POC blood glucose meters has greatly facilitated the treatment of humans,<sup>1</sup> cats, and dogs<sup>2</sup> with abnormalities in blood glucose concentrations. The routine use of such meters has been made possible through studies<sup>3–5</sup> in which use of POC instruments has been validated in the relevant species.

Abnormalities in glucose regulation are not believed to be as common in avian species as they are in

## ABBREVIATIONS

|     |                     |
|-----|---------------------|
| CI  | Confidence interval |
| LOA | Limits of agreement |
| POC | Point-of-care       |

mammals.<sup>6</sup> Nevertheless, hyperglycemia secondary to diabetes, stress, and steroid hormones has been reported.<sup>7</sup> Hypoglycemia in many species is associated with starvation, liver disease, aspergillosis, and sepsis.<sup>6,7</sup> The inability to obtain values for blood glucose concentration in a timely, cost-effective manner has made use of blood glucose concentrations problematic in the treatment of pet birds. Typically, measurement of blood glucose concentrations in birds requires sending serum samples to a central laboratory or the use of specialized chemistry analyzers. The associated cost and time have limited the usefulness of measuring blood glucose concentrations in critically ill birds.

Two different technologies are commonly used in POC blood glucose meters: amperometric and colorimetric. With amperometric devices, glucose in the subject's blood reacts with glucose oxidase or glucose

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dehydrogenase to produce an anodic current. The meter measures the resulting current and converts it into a glucose concentration that is displayed on the unit's liquid crystal display. In these units, the correct amount of blood is automatically drawn into the hollow portion of a test strip, where the reaction takes place. With colorimetric devices, the correct amount of blood (typically 1.5  $\mu\text{L}$ ) is automatically drawn into the hollow portion of a test strip, where the blood glucose is oxidized, resulting in a color change. The degree of change is dependent on the concentration of glucose in the blood and is read by use of reflectance photometry. This information is then translated into a glucose concentration that is displayed on the unit's liquid crystal display.

A study<sup>8</sup> revealed that handheld, POC blood glucose meters consistently underestimate blood glucose concentrations in rhinoceros auklets, thus making the meters useful only as a screening device. To the authors' knowledge, no study to date has evaluated the validity of POC blood glucose meters for use in psittacines, which are commonly kept as pets. The purpose of the study reported here was to determine the degree of agreement between 3 commercially available POC blood glucose meters and a laboratory analyzer for measurement of

blood glucose concentrations in Hispaniolan Amazon parrots (*Amazona ventralis*).

## Materials and Methods

**Animals**—Twenty healthy adult Hispaniolan Amazon parrots, which were part of the research flock at the School of Veterinary Medicine, Louisiana State University, were used. Hispaniolan Amazon parrots were selected because their size, dietary requirements, and physiology are comparable to many other popular psittacines kept as pets. The study was coordinated with the annual hematologic analysis and physical examination of each parrot, and each bird was examined to ensure that it was in good health. The study was performed in accordance with the regulations set forth by the Institutional Animal Care and Use Committee of Louisiana State University.

**Sample collection**—A 26-gauge needle and 3-mL syringe were used to withdraw approximately 0.5 mL of blood from a jugular vein of each bird. The blood sample was used to obtain readings with each meter (0.6 to 1.5  $\mu\text{L}/\text{U}$ ). The remainder of each blood sample was placed into a lithium heparin microtube<sup>a</sup> and centrifuged. Plasma was harvested and frozen at  $-30^{\circ}\text{C}$ .

**Blood glucose measurement**—All meters were calibrated in accordance with the manufacturer's specifications and were operated by a person specifically trained in their use (PJS). A colorimetric<sup>b</sup> (meter A) and 2 amperometric<sup>c,d</sup> (meters B and C) POC meters were used immediately after blood sample collection to measure blood glucose concentrations in fresh blood. Within 5 days after collection, plasma samples were thawed and plasma glucose concentrations were determined by means of a chemistry analyzer<sup>e</sup> in the laboratory.

**Statistical analysis**—The distribution of values for blood glucose concentration was evaluated by use of the Kolmogorov-Smirnov test. Agreement between results for pairs of POC meters and between results for each meter and those for the laboratory analyzer was evaluated by use of the Bland-Altman method.<sup>9</sup> Bias was defined as the mean difference between the 2 methods. The LOA were defined as the 95% CI of the mean

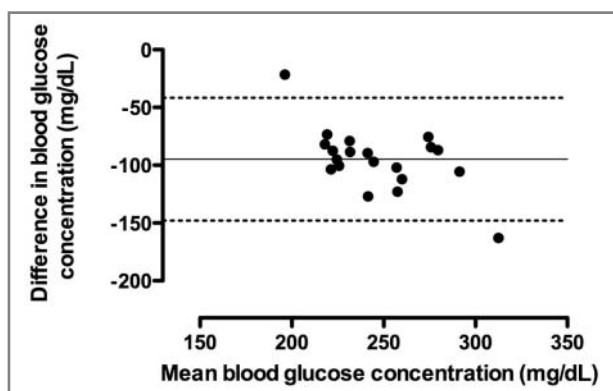


Figure 1—Bland-Altman plot depicting agreement of values for blood glucose concentrations in blood samples obtained from 20 healthy Hispaniolan Amazon parrots (*Amazona ventralis*) and measured by use of 2 devices (colorimetric blood glucose meter A and a laboratory analyzer). Horizontal dotted lines represent 95% LOA values; solid line represents the mean difference.

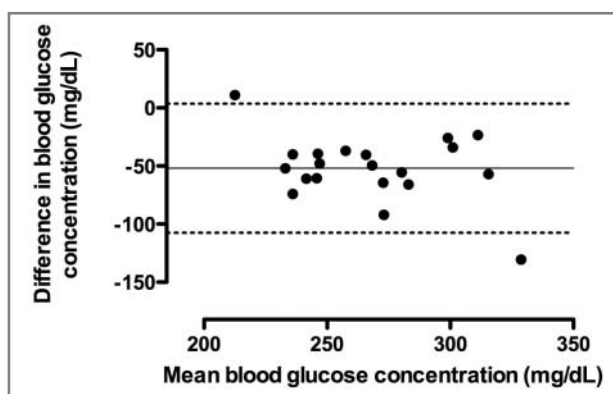


Figure 2—Bland-Altman plot depicting agreement of values for blood glucose concentrations in blood samples obtained from 20 healthy Hispaniolan Amazon parrots and measured by use of 2 devices (amperometric blood glucose meter B and a laboratory analyzer). See Figure 1 for remainder of key.

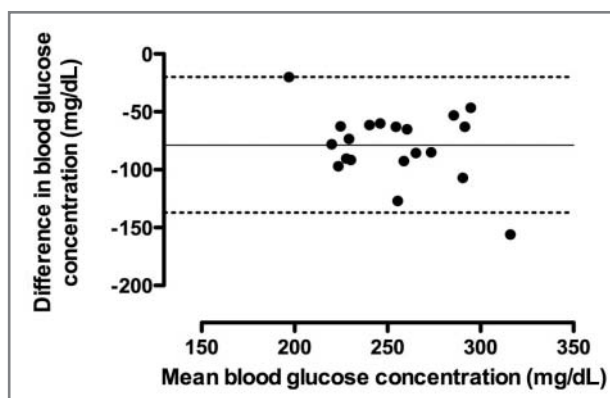


Figure 3—Bland-Altman plot depicting agreement of values for blood glucose concentrations in blood samples obtained from 20 healthy Hispaniolan Amazon parrots and measured by use of 2 devices (amperometric blood glucose meter C and a laboratory analyzer). See Figure 1 for remainder of key.

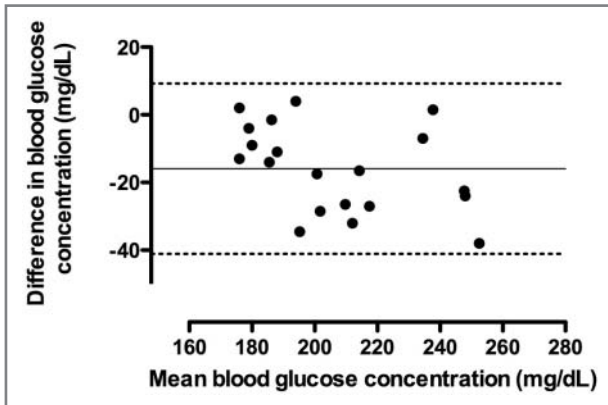


Figure 4—Bland-Altman plot depicting agreement of values for blood glucose concentrations in blood samples obtained from 20 healthy Hispaniolan Amazon parrots and measured by use of 2 devices (colorimetric blood glucose meter A and amperometric blood glucose meter C). See Figure 1 for remainder of key.

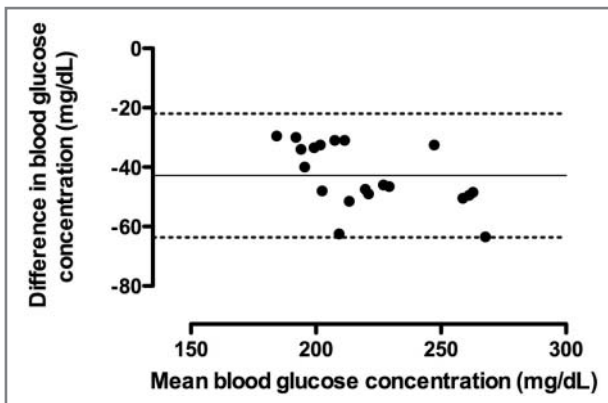


Figure 5—Bland-Altman plot depicting agreement of values for blood glucose concentrations in blood samples obtained from 20 healthy Hispaniolan Amazon parrots and measured by use of 2 devices (colorimetric blood glucose meter A and amperometric blood glucose meter B). See Figure 1 for remainder of key.

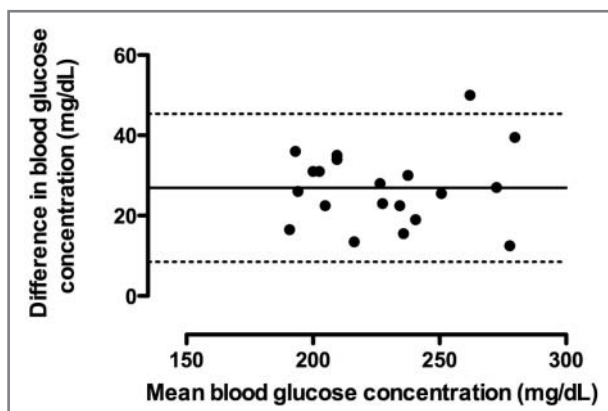


Figure 6—Bland-Altman plot depicting agreement of values for blood glucose concentrations in blood samples obtained from 20 healthy Hispaniolan Amazon parrots and measured by use of 2 devices (amphoteric glucose meters B and C). See Figure 1 for remainder of key.

difference between the 2 methods. Standards in human medicine recommend that the accuracy of a POC meter be within 15% of the reference value<sup>10</sup>; therefore,

the results for the POC meters were considered to be in agreement with results for the laboratory analyzer when the LOA were within 15% of the laboratory values. Statistical analysis was performed by use of commercially available statistical software.<sup>18</sup>

## Results

Values for plasma glucose concentrations as measured by means of the laboratory analyzer were normally distributed and ranged from 207 to 394 mg/dL (mean  $\pm$  SD value,  $297.3 \pm 39.0$  mg/dL). Results for POC meter A (the colorimetric meter) agreed least with results for the laboratory analyzer (bias,  $-94.9$  mg/dL; LOA,  $-148.0$  to  $-41.7$  mg/dL; Figure 1). However, agreement between results of POC meter B (an amperometric meter) and the laboratory analyzer (bias,  $-52$  mg/dL; LOA,  $-107.5$  to  $3.5$  mg/dL) and between results of POC meter C (an amperometric meter) and the laboratory analyzer was also poor (bias,  $-78.9$  mg/dL; LOA,  $-137.2$  to  $-20.6$  mg/dL; Figures 2 and 3, respectively).

All POC blood glucose meters greatly underestimated blood glucose concentrations. On average, POC meters A, B, and C underestimated blood glucose concentrations by 32%, 17%, and 27%, respectively. Only POC meters A and C produced results that were within 15% of each other (Figures 4–6).

## Discussion

All 3 POC blood glucose meters greatly and inconsistently underestimated blood glucose concentrations. Results from all of the POC meters disagreed with those of the laboratory analyzer according to the predetermined definition of agreement (LOA within 15% of the laboratory values). One possible explanation for the underestimation of blood glucose concentrations by use of the POC meters is that avian species tend to have higher Hct values than their mammalian counterparts. Readings from blood glucose meters have a negative bias when used in humans with an abnormally high Hct value.<sup>11</sup> However, the Hct values for the birds in the present study (range, 37% to 51%) were within the specifications for each meter.

Another possibility is that blood glucose concentrations in psittacines are too high for handheld blood glucose meters to accurately measure; however, all blood glucose concentrations reported in the present study were also within the specifications of each meter. An alternative explanation is that the structural differences between mammalian and avian RBCs (eg, existence of a nucleus in avian RBCs) reduce the current flow needed for accurate amperometric measurement of blood glucose concentration. That explanation is challenged by the finding that, although the 2 amperometric blood glucose meters used in the present study were inaccurate, the colorimetric blood glucose meter was also inaccurate.

When results for each glucose meter were compared with each other, only meters A and C (1 colorimetric and 1 amperometric meter) yielded results that were within 15% of each other. The degree of agreement between the 2 amperometric units (meters B and C) was lower. The lowest degree of agreement was

evident between meters A and B, which represented 1 amperometric and 1 colorimetric unit. These findings suggested that the degree of agreement between POC meters was not solely dependent on the underlying technology.

In rhinoceros auklets, POC blood glucose meters consistently underestimate blood glucose concentrations by 33%.<sup>8</sup> Results of the present study suggested that handheld blood glucose meters also underestimated blood glucose concentrations in psittacines. The degree of underestimation was not consistent and varied greatly between the meters. Therefore, no recommendation can be made as to the interpretation of results yielded by use of POC meters.

Abnormalities in glucose metabolism are commonly diagnosed in cats and dogs with endocrine diseases as well as in the critical care setting. These conditions require cage-side serial measurements of blood glucose concentration. The availability of accurate handheld blood glucose meters has facilitated the diagnosis and treatment of cats and dogs with these disorders. The prevalences of hyperglycemia and hypoglycemia in psittacines are believed to be lower than those in cats and dogs; however, the lack of accurate POC blood glucose meters has been an obstacle in identifying affected birds. In addition, serial measurements are significantly more expensive and cumbersome because of the lack of POC devices. The results of the study reported here do not support the use of POC blood glucose meters in psittacines.

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- a. Microtainer, Becton-Dickinson, Franklin Lakes, NJ.
  - b. Accu-Check, Roche Diagnostics, Mannheim, Germany.

- c. OneTouch Ultra, LifeScan Inc, Milpitas, Calif.
- d. Reli On Ultima, Solartek Products, Alameda, Calif.
- e. Olympus AU640e chemistry analyzer, Olympus America, Center Valley, Pa.
- f. MedCalc, version 9.3.9.0, MedCalc Software, Mariakerke, Belgium.
- g. GraphPad Prism, version 5.0 for Macintosh, GraphPad Software Inc, San Diego, Calif.

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