High concordance of blood glucose measurement in cats between a beta prototype glucometer device and a reference laboratory standard in a clinical setting

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OBJECTIVE
The objective of this study was to evaluate the accuracy of a beta prototype version of a new portable blood glucose meter in feline patients.

ANIMALS
60 client-owned cats.

METHODS
In this prospective study, 3-mL blood samples were collected from each cat and analyzed in triplicate using a beta prototype device (AlphaTRAK 3 [AT3]) and by a reference lab standard immediately after collection. Accuracy of the AT3 device was determined in accordance with the International Organization of Standardization (ISO) 15197:2013 criteria, including Bland-Altman plotting and consensus error grid analysis. A Passing-Bablok regression analysis was also performed.

RESULTS
96% of feline measurements fell within the ISO accuracy threshold, and 100% of measurements fell within zones A and B of the consensus error grid, meeting the ISO accuracy requirements. There was no significant bias in the data according to the Bland-Altman analysis. Within the full range of glucose concentrations (20 to 750 mg/dL) the correlation coefficient between the AT3 and the reference lab standard was 0.99. There was no significant constant or proportional bias present in the data.

CLINICAL RELEVANCE
The AT3 device met the ISO requirements and is accurate for measurement of blood glucose concentrations in cats.

Keywords: diabetes mellitus, glucometer, glucose, hyperglycemia, accuracy
however, it underestimated blood glucose concentrations in > 66% of cases, with measurement inaccuracies that were clinically relevant in 6.9% of cases.\textsuperscript{15} This underestimation of blood glucose concentrations could lead to underdosing of insulin in some diabetic patients.

The single-use sensors of the continuous interstitial glucose monitors are also not practical for use in all situations. These sensors require frequent replacement if long-term blood glucose monitoring is required, and they may become dislodged, requiring them to be changed out sooner. A recent study\textsuperscript{15} demonstrated that the sensors were functional for a median period of 10 days. Despite the convenience of this device and utility for managing poorly regulated or fractious patients, some owners may find the ongoing expense of regular sensor replacement cost-prohibitive. The lower ongoing costs of portable blood glucose measurement devices may be more suitable for some owners with pets that do not require continuous blood glucose monitoring.

The AlphaTrak 3 (AT3; Zoetis Services LLC) glucometer device is designed to rapidly measure capillary and venous blood glucose concentrations from a small blood sample (≥ 0.3 µL).\textsuperscript{16} The device is portable and simple to use in both the clinical setting and for at-home use by pet owners. This device also has Bluetooth connectivity, allowing for automatic transfer of results to a connected device.\textsuperscript{17} The AT3 device has not yet been independently validated.

The aim of this study was to evaluate the performance of a beta prototype of this device when used to measure blood glucose concentrations in a population of feline clinical patients. The hypothesis was that results from the AT3 device would correlate strongly with the results of a reference laboratory standard.

**Methods**

**Inclusion criteria**

Cats weighing > 1.5 kg were eligible for study inclusion. Animals presenting with persistent signs of diabetes mellitus (polyuria, polydipsia, polyphagia, and weight loss) or a history of poorly controlled diabetes mellitus were encouraged to enroll in the study. Cats with a known normal blood glucose level were eligible to enroll as a control. Patients that made blood collection difficult due to aggression were excluded from the study. Animals requiring > 3 attempts at blood collection were excluded from the study. This study was reviewed and approved by the Zoetis Ethical Review Board, and informed owner consent was obtained for all cats prior to entry into the study. Information regarding patient breed, age, and sex were recorded for each patient.

**Sample collection and analysis**

Blood samples were collected from feline patients at 5 private specialty hospitals, with each hospital having its own internal reference laboratory. A 3.0-ml sample of whole blood was collected from each cat by aseptic venipuncture. Up to 3 independent samples were allowed to be collected from each cat as long as they were separated by at least 1 hour during the same visit. Each cat was only allowed to be entered into the study once. Cats were sedated for blood collection if required. Once collected, the blood sample was divided between 2 K2EDTA lavender top microtainer tubes (1.5 mL each) and gently inverted 10 to 15 times to mix the anticoagulant with the sample. The samples were examined for clots, and if clots were present, a new sample was drawn. Blood tubes were labeled with patient identifiers before testing.

One K2EDTA tube was immediately used for testing on the AT3 devices in triplicate. Three distinct AT3 devices and 3 separate test strip lots were used to test each blood sample. The AT3 device employs an enzymatic reaction with glucose oxidase to determine the glucose concentration in a whole blood sample. Blood glucose concentration results from each AT3 device were recorded on a data collection form. Within 10 minutes of collection, the remaining K2EDTA tube and data collection form were then transferred to the internal reference lab where the sample was separated into 2 aliquots. One was used to perform a CBC on an ADVIA 2120i Hematology System (Siemens Medical Solutions USA Inc) to measure the Hct. The second was centrifuged to isolate the plasma, which was then used to measure total protein (TP) via a refractometer according to the manufacturer’s recommendations and blood glucose in triplicate on a Beckman Coulter glucose analyzer, which utilizes hexokinase phosphorylation to measure the glucose concentration from the plasma. The average of the 3 plasma glucose concentrations, Hct, and TP measurements were recorded on the data collection form.

**Statistical analysis**

Data were analyzed using a commercial statistical software package (SAS version 9.4; SAS Institute Inc). Normality was assessed by visualizing Q-Q plots and performing the Shapiro-Wilk test for normality, including following logarithmic transformations. Data were analyzed in accordance with standard ISO 15197:2013 guidelines.\textsuperscript{18} The ISO guidelines require for 95% of the measured glucose values to fall within either ± 15 mg/dL of the average measured values of the reference measurement at glucose concentrations < 100 mg/dL or within ± 15% at glucose concentrations ≥ 100 mg/dL. The guidelines also require that 99% of the individually measured glucose values fall within zones A and B of a consensus error grid (CEG). Additionally, Bland-Altman plotting and a Passing-Bablok regression analysis were performed using pair-wise comparisons between the AT3 beta prototype versus the Beckman Coulter glucose analyzer.

Distributions of Hct and TP between cats whose glucose measurements fell within the ISO accuracy threshold failed to meet normality and were analyzed using nonparametric Mann-Whitney tests. A P value cutoff of .05 was used to determine significance.
Results

A total of 60 feline patients were included in the study. Breeds included domestic shorthair (43/60 cats), domestic medium hair (5/60 cats), domestic longhair (5/60 cats), Russian Blue (1/60 cats), Siamese (1/60 cats), Maine Coon (1/60 cats), and Ragdoll mix (1/60 cats). The breed was unknown in 5 of 60 cats. Thirty-seven cats were neutered males, and 20 cats were neutered females. One cat was an intact male, and 2 cats were intact females.

Twenty-two whole blood samples were collected from client-owned feline patients diagnosed with uncontrolled diabetes mellitus or abnormal blood glucose concentrations (hyperglycemia or hypoglycemia). Another set of 38 whole blood samples collected from normoglycemic feline patients were included as controls. Each of these 60 samples were tested in triplicate, resulting in 180 individual glucose measurements.

Overall, 96.1% (173/180) of feline measurements fell within the ISO accuracy threshold of ± 15% or ± 15 mg/dL of the reference value (Table 1). Bland-Altman plotting is shown (Figure 1). The mean bias was 1.81 (95% CI, –0.35 to 3.97), with the lower limit of bias being –26.95 (95% CI, –30.65 to –23.26) and the upper limit of bias being 30.58 (95% CI, 26.88 to 34.27). A small subset (7/180) of feline measurements fell outside the ISO accuracy threshold.

The glucose measurements were also plotted on a CEG, as shown (Figure 2). It was observed that 100% (180/180) of feline blood glucose measurements fell in CEG zones A and B. No blood glucose measurements fell within zones C, D, or E.

The Passing-Bablok regression plot showing correlation between the AT3 beta prototype device in comparison to the reference lab standard is shown (Figure 3). The regression lines are overlapping with the theoretical line, with a correlation coefficient (R2) of 0.99. The intercept was –2.07 (95% CI, –6.17 to 2.00), and the slope was 1.03 (95% CI, 1.00 to 1.06). There was no statistically significant constant or proportional bias.

The median Hct was 40.1% (range, 18.8% to 52.8%), and the median TP was 8.0 (range, 5.7 to 10.0 g/dL). There were no significant differences in Hct or TP between the cats whose glucose measurements fell within the ISO accuracy threshold and those that did not (Table 2).

Table 1—Glucose measurements within the International Organization of Standardization (ISO) accuracy threshold.

<table>
<thead>
<tr>
<th>Glucose range</th>
<th>n</th>
<th>Median inaccuracy from reference standard (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values within ISO threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Values &lt; 100 mg/dL</td>
<td>58</td>
<td>+3 mg/dL (–9.0 to +13.0 mg/dL)</td>
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<tr>
<td>Values ≥ 100 mg/dL</td>
<td>115</td>
<td>–2.3% (–15.0% to +14.7%)</td>
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<tr>
<td>Values outside of ISO threshold</td>
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<td></td>
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<tr>
<td>Values &lt; 100 mg/dL</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Values ≥ 100 mg/dL</td>
<td>7</td>
<td>–16.7% (–19.6% to +19.7%)</td>
</tr>
</tbody>
</table>

N/A = Not applicable.

Figure 1—Bland-Altman plot of glucose measurements. Overall, 96.1% of measurements fell within the International Organization of Standardization accuracy threshold, with a small subset (3.8%) falling outside the accuracy threshold. BC = Beckman Coulter.

Figure 2—Consensus error grid plot of glucose measurements. In total, 100% of blood glucose measurements fell in consensus error grid zones A and B. BC = Beckman Coulter.
Discussion

The AT3 device met the ISO guidelines of accuracy and has been proven to deliver accurate blood glucose readings that do not negatively affect clinical decision-making. Furthermore, the readings closely agreed with a recognized reference lab standard, with the Bland-Altman analysis confirming this agreement and the Passing-Bablok analysis demonstrating strong linearity.

The results of this study were analyzed in accordance with ISO 15197:2013 guidelines, which stipulate that 95% of the measured glucose values must fall within either ±15 mg/dL of the average measured value on the reference laboratory standard, or ±15% for glucose concentrations that are >100 mg/dL. Overall, 96.1% of results measured by the AT3 device fell within these ranges, confirming that the AT3 device meets the ISO accuracy requirements.

There were 7 cats whose blood glucose measurements fell outside of the ISO accuracy guidelines. None of these 7 measurements were discrepant enough to result in an inappropriate clinical action, as supported by the CEG analysis. Reasons for these discrepancies could be due to an issue with the test strips used, delays in the time between blood glucose measurement on the glucometer and the reference lab standard, or other sample handling issues.

The mean bias from a Bland-Altman analysis would be considered significant if its 95% CI did not include zero. The 95% CI for the mean bias reported in this study included zero in its range, indicating that there was not significant bias present in the data.

The ISO guidelines also state that 99% of individual glucose measured values must fall within zones A and B of a CEG. The CEG is divided into 5 zones, which are defined by estimated risk to the patient if a result falls in each zone.19 Zones A and B indicate little or no effect on the clinical outcome. Zone C indicates the result would alter the clinical action and could have significant medical risk for Zone D or dangerous consequences for Zone E. Within this study, 100% of the measured values fell within zones A and B as required by the ISO guidelines.

Data were also analyzed using a Passing-Bablok regression model to demonstrate the correlation of AT3 device measurements with reference Beckman Coulter measurements. Overall, the AT3 beta prototype measurements correlated extremely well with the reference Beckman Coulter results for glucose concentrations in the full dynamic range of 20 to 750 mg/dL, with a correlation coefficient of 0.99. Correlation coefficients can range from –1 to +1 and quantify the degree of association between the 2 variables. Values closer to +1, as demonstrated in this study, indicate a near perfect positive linear relationship.

The intercept in the Passing-Bablok analysis represents the constant bias. If zero is not included in the 95% CI, it indicates a significant constant bias between the 2 methods across the range of measurements. The slope in the analysis represents the proportional bias. If 1 is not included in the 95% CI, it indicates a significant proportional bias between the 2 methods across the range of measurements. This means the discrepancy between the 2 methods is not constant but varies proportionally with the measurement values. There was no significant constant or proportional bias in the data from this study.

It has previously been shown that elevated Hct as well as elevated TP can lead to falsely low glucose measurements.5,20,21 This study demonstrated that neither Hct nor TP, when within the target product profile (Hct range, 15% to 65%), had a significant impact on glucose measurements in this group of cats with the AT3 device.

In summary, the analytical performance of the AT3 device is comparable to the reference Beckman Coulter glucose analyzer results in measuring glucose concentration of feline blood samples. Overall, 96.2% of results fell within the ISO accuracy threshold and 100% of results fell within zones A and B of the CEG. The above outcome clearly confirms that feline results measured by the AT3 device passed the ISO success criteria and the device can be used confidently in clinical practice.
Acknowledgments

None reported.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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References

17. AlphaTrak 3 User Guide. Zoetis; 2023