Monitoring of blood glucose concentrations is extremely important in veterinary medicine. The accurate and rapid measurement of blood glucose concentrations is not only important for the treatment of patients with diabetes mellitus but also in the diagnosis and management of other disease processes such as sepsis, insulinomas, and hepatic injury or failure. Point-of-care blood glucose measurement devices allow for convenient and accurate measurement of blood glucose concentrations of patients both at home and in the hospital setting.

Home glucose monitoring by owners of diabetic patients using portable glucose monitoring devices can be adopted by most owners of diabetic cats, and studies have found positive outcomes in glycemic control for pets that undergo home blood glucose monitoring. One study surveyed 748 diabetic cat owners and found that 71% of owners performed blood glucose monitoring at home. These studies demonstrate the utility of glucose monitoring devices for owners of diabetic pets.

The use of continuous interstitial glucose monitoring devices has also been described in the veterinary literature. Several studies have assessed the use of the Freestyle Libre device (Abbott) for use in diabetic cats. A 2022 study of 41 diabetic cats evaluated the use of the FreeStyle Libre and found that it did not meet the International Organization for Standardization’s (ISO) requirements for accuracy. The device was useful in that it allowed for accurate measurement of the blood glucose nadir;
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-µL 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>58</td>
<td>+3 mg/dL (–9.0 to +13.0 mg/dL)</td>
</tr>
<tr>
<td>Values &gt; 100 mg/dL</td>
<td>115</td>
<td>–2.3% (–15.0% to +14.7%)</td>
</tr>
<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.
could be due to an issue with the test strips used, de-
by the CEG analysis. Reasons for these discrepancies
result in an inappropriate clinical action, as supported
of these 7 measurements were discrepant enough to
ments fell outside of the ISO accuracy guidelines. None
vice meets the ISO accuracy requirements.
Overall, 96.1% of results measured by the AT3 device
for glucose concentrations that are > 100 mg/dL.
for glucose concentrations < 100 mg/dL and ± 15%
sasured value on the reference laboratory standard
fall within either ± 15 mg/dL of the average mea-
meet the ISO accuracy guidelines, which stipu-
99% of in-
Zones D and E indicate that the results would alter
the clinical action and could have significant medi-
cal 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.

Table 2—Hematocrit and total protein (TP) distributions
for glucose measurements that did and did not meet
accuracy threshold.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Within ISO accuracy threshold</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
<th>P value (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct</td>
<td>Yes</td>
<td>173</td>
<td>40.3</td>
<td>18.8–52.8</td>
<td>.51</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7</td>
<td>33.4</td>
<td>24.0–52.8</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>Yes</td>
<td>173</td>
<td>8.0</td>
<td>5.7–10.0</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7</td>
<td>8.5</td>
<td>6.6–10.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)P values calculated using Mann-Whitney tests.

Discussion

The AT3 device met the ISO guidelines of ac-
curacy 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 stan-
dard, with the Bland-Altman analysis confirming this
agreement and the Passing-Bablok analysis demon-
strating strong linearity.

The results of this study were analyzed in accor-
dance with ISO 15197:2013 guidelines, which stipu-
late that 95% of the measured glucose values must
fall within either ± 15 mg/dL of the average mea-
sured value on the reference laboratory standard
for glucose concentrations < 100 mg/dL and ± 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 de-
vice meets the ISO accuracy requirements.

There were 7 cats whose blood glucose measure-
ments 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, de-
lays 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 indi-
vidual 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. Zones A and
B indicate little or no effect on the clinical outcome.
Zone C indicates the result would alter the clinical
action and be likely to affect the clinical outcome.
Zone D and E indicate that the results would alter
the clinical action and could have significant medi-
cal 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 proto-
type 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. Correla-
tion 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, in-
dicate 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 mea-
surements. 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 mea-
surement 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. This study demonstrated that
neither Hct nor TP, when within the target product
profile (Hct range, 15% to 65%), had a significant im-
 pact 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 glu-
cose 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 confi-
dently 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