Hypoglycemia in ferrets (Mustela putorius furo) is often secondary to a pancreatic islet β-cell tumor, commonly termed insulinoma. A blood glucose concentration < 70 mg/dL is strongly suggestive of insulinoma, which reportedly comprises approximately 25% of all neoplasms diagnosed in this species. Other causes for hypoglycemia are much less common and include severe liver disease, other types of neoplasia, sepsis, starvation, and heatstroke. Clinical signs of hypoglycemia in ferrets include a so-called stargazing posture (ie, dorsiflexion of the neck), hind limb weakness, ataxia, lethargy, and ptalism secondary to presumed nausea. Seizures are not commonly described in ferrets with insulinoma, unlike dogs with this disease. This disparity is thought to be associated with the sedentary lifestyle and ad libitum feeding of most pet ferrets. Diabetes mellitus leading to hyperglycemia is considered uncommon in ferrets, although individual cases have been reported. Because the clinical signs of hypoglycemia are nonspecific, further diagnostic tests, specifically measurement of blood glucose concentrations, are necessary for diagnosis. A PBGM is often used to obtain rapid test results. The alternative procedure involves the use of an automated chemistry analyzer, which often requires sending samples to a laboratory; this can delay diagnosis and, ultimately, appropriate treatment of affected ferrets.

Investigators in numerous studies have evaluated the efficacy of PBGMs designed for use in hu-
man and veterinary medicine to test samples from various species, including dogs, cats,2,12,13 horses,14,15 and birds.16,17 To our knowledge, no study published to date has evaluated the use of PBGMs in domestic ferrets. The purpose of the study reported here was to evaluate the agreement of 3 commercially available models of PBGMs (2 designed for use with human samples and 1 designed for veterinary use) with a laboratory analyzer (considered the reference standard) for measurement of blood glucose concentrations in ferrets. The PBGM for veterinary use included 2 settings used to specify analysis of canine or feline samples, and thus 2 meters of the same model were included so that each could be set to 1 code for the duration of the study.

Materials and Methods

Animals—Fifty-two adult client-owned ferrets were used for the prospective study, which was performed at Gulf Coast Avian and Exotics from June 21, 2010, to March 8, 2011. The only inclusion criterion was that venipuncture was performed for purposes of hematologic analysis during patient evaluation. Nine of the ferrets had a previous diagnosis of insulinoma and were receiving treatment with orally administered prednisolone, diazoxide, or both at various dosages. Consent forms were signed by all owners prior to enrollment in the study.

PBGMs—Three commercially available models of PBGM were evaluated in the study. Two meters of the same model4 for veterinary use were included; 1 (ATC) was set to the number corresponding to canine sample analysis, and 1 (ATF) was set to the number corresponding to feline sample analysis for the duration of the study. The remaining 2 PBGMs (OTU5 and ACA6) were designed for use with human samples.

A drop of blood was placed onto the corresponding test strip, and via capillary action, the sample was drawn into the reaction chamber. Blood glucose concentration was measured via a colorimetrica,b or amperometricc method. The manufacturer’s instructions were followed for each PBGM with the exception that venous rather than capillary blood samples were tested. Any sample for which ≥ 1 PBGM displayed an error code was eliminated from the study.

Experimental protocol—Blood samples were collected from a jugular, lateral saphenous, or cephalic vein with a 25-gauge needle attached to a 1-mL syringe or with an insulin syringe. No duplicate samples were obtained. A minimum of 0.2 mL of whole blood was collected from each ferret for the study. One drop (approx 0.3 μL) of whole blood was immediately placed on the test strip of each PBGM. The order in which blood was tested on each of the PBGMs was kept constant for the first 40 samples, then randomly assigned for the remaining 11 samples. The order of testing for the initial 40 samples was as follows: ATC, ATF, OTU, and ACA. All whole blood samples were tested on the PBGMs within 2 minutes after collection. The remainder of the blood sample was placed in a lithium heparin microtube4 and centrifuged ≤ 5 minutes at 5,000 X g after collection. The plasma was harvested, and plasma glucose concentration was measured with a laboratory analyzer,7 via a hexokinase reaction, ≤ 4 hours after sample collection. The laboratory was located in the same building where samples were collected; therefore, the samples were briefly stored at room temperature (22°C) until analysis.

Quality control measures—Manufacturer-supplied control solutions were used to calibrate each respective PBGM weekly. Each meter was also calibrated before a new box of test strips was used. Calibration was performed according to manufacturers’ recommendations. Quality control for the laboratory analyzer was performed 3 times daily and with every chemical supply lot change.

For each PBGM, intra-assay coefficients of variation were assessed by running the same patient sample on 1 PBGM 3 consecutive times. This was repeated with a total of 5 samples on each PBGM.

Data analysis—Bias was defined as the mean difference between glucose concentrations determined with each PBGM and the correlating laboratory analyzer value for the same sample. Bland-Altman plots were created with statistical software1 and used to evaluate agreement between results for each PBGM and the laboratory analyzer. The plasma glucose concentrations from the laboratory analyzer were classified as either hypoglycemic (plasma glucose concentration, < 70 mg/dL), euglycemic (plasma glucose concentration, 70 to 200 mg/dL), or hyperglycemic (plasma glucose concentration, > 200 mg/dL). Bias for hypoglycemic samples, as determined by the laboratory analyzer, was also calculated. The current standard in human medicine is that glucose measurements obtained from a PBGM should not differ by more than 15% from the reference value18; therefore, all results for each PBGM were examined for the percentage of values outside of this range. Linear regression analysis was performed to determine whether the venipuncture site (central [jugular vein] or peripheral [lateral saphenous or cephalic vein]) was significantly associated with blood glucose values.

A validation study was performed to determine whether the order in which the samples were run on the PBGMs was statistically significant, assuming the difference in time (≤ 2 minutes from use of the first PBGM to use of the fourth PBGM) was negligible. Multiple linear regressions were performed, and the P values were deemed nonsignificant (P ≥ 0.48 for all PBGMs). All data were evaluated with standard software,2 and values of P < 0.05 were considered significant.

Results

The range of plasma glucose concentrations, as measured with the laboratory analyzer, was 41 to 160 mg/dL (mean ± SD value, 102.8 ± 31.5 mg/dL) for 51 samples. One sample produced an error code on the ACA and was excluded from further analysis. Ten of the 51 samples had glucose concentrations ≤ 70 mg/dL, and the remaining 41 samples had glucose concentrations within normal range according to the laboratory analyzer (70 to 200 mg/dL). No samples were considered hyperglycemic (glucose concentration > 200 mg/dL in blood or plasma, respectively) on the basis of results from any of the PBGMs or the laboratory analyzer.
When all samples were considered, blood glucose concentrations measured with the ATC had the greatest agreement with the laboratory analyzer values (mean ± SD bias, 1.9 ± 16.1 mg/dL), whereas those for the ACA had the least agreement (mean ± SD bias, –34.0 ± 18.0 mg/dL; Table 1; Figure 1). Agreement with the laboratory analyzer was also poor for the ATF (mean ± SD bias, –16.0 ± 17.0 mg/dL) and the OTU (mean ± SD bias, –22.0 ± 15.6 mg/dL). All PBGMs except the ATC significantly (P < 0.05) underestimated glucose concentrations in the samples. Bias of hypoglycemic samples (as determined with the laboratory analyzer) was also calculated. The ATC was the only PBGM that significantly (P < 0.05) overestimated blood glucose concentration (mean ± SD bias, 11.2 ± 10.1 mg/dL).

In addition to poor agreement with laboratory analyzer values, results for the ATF, OTU, and ACA frequently differed from the laboratory analyzer value by more than 15%, most often as a result of being too low. For the ATF, OTU, and ACA, 24 of 51 (47%), 37 of 51 (73%), and 47 of 51 (92%) values, respectively, were too low (ie, were less than the corresponding laboratory analyzer value minus 15%), and 1 of 51 (2%) values for each of these units was too high (ie, was greater than the corresponding laboratory analyzer value plus 15%). The ATC had results more similar to those of the laboratory analyzer, with 12 of 51 (24%) values too high and 5 of 51 (10%) values too low according to this same criterion. The ATC, ATF, OTU, and ACA results incorrectly indicated hypoglycemia in 2 (4%), 2 (4%), 7 (14%), and 16 (31%) of the 51 samples, respectively. The ATC and ATF failed to detect hypoglycemia in 4 (8%) and 1 (2%) of the 51 samples, respectively. Intra-assay coefficients of

<table>
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<th>Sample type</th>
<th>PBGM</th>
<th>Euglycemic (n = 41)</th>
<th>Hypoglycemic (n = 10)</th>
<th>All (n = 51)</th>
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<td>11.2 ± 10</td>
<td>1.9 ± 16</td>
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<tr>
<td>ATF</td>
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<td>−3.4 ± 10</td>
<td>−16.0 ± 17</td>
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<td>OTU</td>
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<td>−9.8 ± 10</td>
<td>−22.0 ± 18</td>
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<tr>
<td>ACA</td>
<td>−38.6 ± 16</td>
<td>−15.3 ± 13</td>
<td>−34.0 ± 18</td>
<td></td>
</tr>
</tbody>
</table>

Samples were classified as hypoglycemic or euglycemic on the basis of plasma glucose concentrations (<70 mg/dL and 70 to 200 mg/dL, respectively) as measured with the laboratory analyzer.

Table 1—Mean ± SD bias (mean difference between methods; mg/dL) for blood glucose concentrations obtained for venous samples from 51 ferrets (Mustela putorius furo) by use of PBGMs, compared with plasma glucose concentrations obtained from the same samples with a laboratory analyzer.
variation were 3.6% for the ATC, 5.8% for the ATF, 4.4% for the OTU, and 5.2% for the ACA.

Twenty-four blood samples were classified as central, and the remaining 27 samples were peripheral. There was no significant (P > 0.17 for all comparisons) difference between results for central and peripheral blood samples for any of the PBGMs or the laboratory analyzer.

Discussion

In the present study, PBGMs designed for use with human samples (OTU and ACA) repeatedly underestimated blood glucose concentrations in samples from ferrets; all samples were classified as euglycemic or hypoglycemic on the basis of laboratory analyzer (plasma glucose) values. The PBGM designed for veterinary use (for which 2 meters [ATC and ATF] were evaluated) had the greatest agreement with laboratory analyzer values, and the ATC had the least amount of bias (i.e., mean difference between values determined with the PBGM and the laboratory analyzer). In contrast to the other PBGMs, the ATC indicated glucose concentrations higher than laboratory analyzer values for both euglycemic and hypoglycemic samples. These results are consistent with a previous study\(^8\) in which 43% of canine blood samples tested with the same model of ATC had reported blood glucose concentrations higher than the reference analyzer value. In that study,\(^8\) PBGMs designed for analysis of human samples also indicated blood glucose concentrations lower than the reference analyzer values.

These discrepancies could have a dramatic impact on clinical decision making, if the glucose concentration obtained with a PBGM was not subsequently verified by a reference laboratory. Samples from ferrets in a clinical setting with blood glucose concentrations approaching the lower limit of the reference interval (70 mg/dL) would potentially be misclassified as hypoglycemic if the current acceptability criterion (i.e., that values should not be more than 15% lower than the laboratory analyzer value) were applied.\(^8\) In the present study, all of the PBGMs inaccurately indicated blood glucose values in the hypoglycemic range for some or several samples; this occurred in testing of 2 of 51 (4%) samples each for the ATC and ATF, compared with 7 (14%) and 16 (31%) for the OTU and ACA, respectively. Although the ATC and ATF had the least amounts of bias, these were also the only meters to misclassify samples that were deemed hypoglycemic (on the basis of laboratory analyzer results) as being euglycemic (4 [8%] and 1 [2%] of the 51 samples, respectively). The plasma glucose concentrations detected by the laboratory analyzer for these samples ranged from 66 to 69 mg/dL. If sample analysis had not been repeated with the laboratory analyzer, these results may have delayed treatment for those patients. This emphasizes the importance of confirmatory testing, especially if values obtained with a PBGM are close to the lower limit of the reference interval for a ferret that has clinical signs consistent with hypoglycemia.

Several types of error grids have been developed to evaluate the potential clinical relevance of erroneous glucometer results in comparison with the reference value.\(^9,20\) These are currently considered standard in studies of human patients\(^18\) and have been used in several veterinary studies.\(^10,12–14\) However, error grid analysis was not used in the present study because of the small sample size and lack of diversity among blood glucose concentrations. This method of analysis should be considered in future studies evaluating the use of PBGM for glucose determination in ferrets.

There are several factors that can adversely affect blood glucose concentrations reported by PBGMs. Glucose is unstable in whole blood; erythrocytes metabolize glucose at a rate of 6 to 10 mg/dL/h at 25°C in human blood.\(^21\) To prevent artifactual hypoglycemia caused by glycolysis, separation of the plasma should be performed within 15 to 30 minutes of sample collection.\(^22\) All samples in the study reported here were evaluated on each PBGM ≤ 2 minutes after collection, and the remaining sample was centrifuged to separate the plasma ≤ 5 minutes after collection. As this was well within the allotted time frame, it is unlikely glycolysis of the sample affected concentrations detected by the PBGMs or laboratory analyzer.

Although the same blood sample was tested with all PBGMs and the laboratory analyzer, whole blood was used for the former and plasma for the latter. Plasma has a higher water content and subsequent higher glucose concentration (approx 11% to 12%), compared with whole blood with a mean Hct of 45%.\(^3\) This inherent difference will be encountered in any comparison study of PBGM in any species when whole blood is compared with plasma. Many commercially available PBGMs have calibrations in place to correct this incongruity, assuming the patient has an Hct within a given reference interval.\(^22\) All samples in the study reported here were venous, results for each meter should have been equally affected.

The PBGMs used in the present study are marketed for evaluation of capillary blood samples. Because of the small size of the ferrets, an adequate amount of blood could not be obtained from a capillary sample; therefore, venous blood samples were used. Differences in blood glucose concentrations between capillary and venous samples have been described, particularly in postprandial samples.\(^21\) Because all samples in the study were venous, results for each meter should have been equally affected.

Intra-assay coefficients of variation were small for all PBGMs; therefore, it is unlikely that the detected bias for each of the glucometers was attributable to a defect in the device. All PBGMs were purchased new at the start of the study, along with the appropriate test strips. All efforts were made to decrease the effect of mechanical error by performing quality control measures and calibration of each machine according to the
manufacturers’ instructions. No calibration problems were detected for any of the PBGMs, and the 1 sample for which an error code was produced was excluded from analysis.

As in many studies, sample size was a limiting factor in the study reported here. Future studies to evaluate efficacy of PBGMs would ideally have a greater number of samples to increase statistical power. Ideally, the order in which blood samples were tested on the PBGMs would have been randomly assigned for each patient. This was instituted for the last 11 samples, but the first 40 samples were run in the same order for each patient. Interestingly, the degree of bias increased from the first (ATC) to the last (ACA) PBGM tested. Because all samples were tested ≤2 minutes after collection, it is extremely unlikely the change in bias was attributable to glycolysis of the sample. To ensure order selection did not affect the results, a validation study was performed, and sample order was determined to be nonsignificant.

Various models of PBGMs are widely used in small animal practice because of their low cost and ability to provide immediate results. There is a misconception that results obtained with these devices correspond well with the results of laboratory analyzers, and their use in several species, including ferrets, has yet to be fully evaluated.25,27 To our knowledge, the present report is the first to critically examine the use of PBGMs designed for analysis of human, canine, and feline samples to measure blood glucose concentrations in ferrets. Our results indicated that blood glucose concentrations should be confirmed with a validated laboratory analyzer prior to making treatment recommendations for ferrets.

References