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Accuracy of a flash glucose monitoring system in cats and determination of the time lag between blood glucose and interstitial glucose concentrations

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Abstract

Background: The FreeStyle Libre (Abbott Laboratories) is a flash glucose monitoring system (FGMS) that measures interstitial glucose concentration (IG). The system is factory-calibrated, easy to use, inexpensive, and could be useful for monitoring diabetic cats.

Objectives: To evaluate the analytical and clinical accuracy of the FGMS in cats and establish the lag-time between IG and blood glucose concentration (BG).

Animals: Twenty client-owned diabetic cats and 7 purpose-bred healthy cats.

Methods: Prospective study. Blood glucose concentration was measured using a portable glucose meter validated for use in cats that served as a reference method for IG, as measured by FGMS. In diabetic cats, data were collected for sensor wearing time with different methods of application and accuracy across glycemic ranges. Accuracy was determined by fulfillment of ISO15197:2013 criteria. In healthy cats, lag-time between IG and BG was established after IV administration of exogenous glucose.

Results: Good agreement between IG and BG was obtained (r = .93). Analytical accuracy was not achieved, whereas clinical accuracy was demonstrated with 100% of the results in zones A + B of the Parkes consensus error grid analysis. In the immediate 30 minutes after an IV bolus of glucose, when BG was increasing rapidly (approximately 2%/min), IG increased slowly, resulting in a difference of as much as 579 mg/dL, and no positive correlation between BG and IG was found.

Conclusions and Clinical Importance: The FGMS did not fulfill ISO requirements but is sufficiently accurate for glucose monitoring in cats, while considering the lag between IG and BG during periods of rapid changes in BG.

KEYWORDS
continuous glucose monitoring system, delay, diabetes mellitus, FreeStyle Libre, insulin

1 | INTRODUCTION

Insulin treatment requires close monitoring in cats. When dysglycemia is controlled effectively, cats are more likely to achieve diabetic remission because glucotoxicity is minimized.[1-3] In cats with newly diagnosed diabetes mellitus, use of a near-euglycemic management paradigm improves remission rate compared to a traditional paradigm.[3] Traditionally, glucose monitoring was aided by measuring blood glucose concentrations (BG) at home or in the clinic[4] using a portable blood glucose meter (PBGM). The need for repeated venipuncture, however, can be stressful, painful and might lead to stress-induced hyperglycemia and erroneous clinical decisions.[5] In addition, the relative invasiveness and intermittent nature of BG monitoring limits the ability to obtain granular data over long period of time at all hours of day, which increases the chance of missing important trends, intra- and inter-day variability and hypoglycemic events.[6]

Continuous glucose monitoring systems (CGMS) have been developed for people suffering from diabetes mellitus (DM), with the goal of overcoming the challenges associated with intermittent BG monitoring. These devices measure interstitial glucose concentration (IG), which correlates well with BG.[7,8] However, a lag-time occurs between changes in BG and IG and the latter also is affected by local factors specific to the tissue in which it is measured.[9] The accuracy of some CGMS units has been described in cats.[10-16] In people, a lag-time of a few minutes was reported between BG change and IG change when BG is fairly static. Awareness of the potential range of lag-time and absolute differences between IG and BG is especially important in cats, considering the proclivity of cats stress-induced hyperglycemia.

The FreeStyle Libre (Abbott Laboratories Ltd, Chicago, Illinois) is a novel flash glucose monitoring system (FGMS) that measures and records IG with several advantages over previously described CGMS units. The disposable sensor is small, easy to apply, lasts up to 14 days and does not require calibration. Data are transmitted from the sensor wirelessly to the reader by "scanning" the sensor. A recent study showed that the FreeStyle Libre is well tolerated and correlates with PBGM data in diabetic cats. However, sensors in that study were secured by skin sutures, limiting the application of the sensor to veterinary professionals. In addition, the accuracy of the FGMS was tested mostly in the hyperglycemic range and the potential lag-time between IG and BG was not described.[16]

Our aim was to describe a practical, easy to use approach to application of the FreeStyle Libre sensor in cats and evaluate its accuracy and potential lag-time compared to BG measurements.

2 | MATERIALS AND METHODS

2.1 | Flash glucose monitoring system and portable blood glucose meter device

The FreeStyle Libre FGMS is composed of a small round sensor (35 mm × 5 mm) that measures IG through a small, SC catheter (0.4 mm × 5 mm). It can be worn for up to 14 days. Technical aspects of the device are described in the human and veterinary medical literature.[16,17]

In all cats, the sensor was placed on the dorsal or lateral aspect of the neck using the applicator supplied with the unit and according to the procedures recommended by the manufacturer (Figure 1). If application in the neck area was not possible, the sensor was placed caudally on the dorsum. Before application, the hair was clipped and the skin cleaned with chlorhexidine and alcohol. A drop of tissue glue (3M Vetbond Tissue Adhesive [3M Corp., Minnesota] in purpose bred cats; Mastisol [Ferndale laboratories srl, Soviclle, Italy] in diabetic cats) was placed on the skin-facing surface of the sensor in all purpose-bred cats and in 13 of the 20 diabetic cats. In diabetic cats, after the application, the sensor was fixed with an extra-tape (Pic Solution Soffix Stretch, Pikdare Srl, Como, Italy) and a body bandage (Vetrap, 3M Italia Srl, Milano, Italy). Data were uploaded to the LibreView website at the end of the study.

The accuracy of the FGMS was assessed by comparison to a veterinary portable blood glucose meter (vPBGM; Alphatrak 2, Abbot Laboratories Ltd, Chicago, Illinois; Zoetis srl, Roma, Italy) that was previously validated for use in cats with a BG range 20-750 mg/dL and intra-assay coefficient of variation of 3.8%.[18]

2.2 | Diabetic cats

2.2.1 | Animals

Twenty client-owned diabetic cats were enrolled. Diagnosis of DM was in accordance with the Agreeing Language In Veterinary Endocrinology criteria established by the European Society of Veterinary Endocrinology.[19] Cats were treated with insulin for at least 1 month before enrollment. All cats were cared for by owners able to perform home monitoring using the vPBGM. Eight were neutered females, and 12 were neutered males. Represented breeds included 18 domestic shorthair cats, 1 Birman cat, and 1 Norwegian Forest cat. Mean ± SD and median (range) ages were 12.3 ± 2.3 years and 12 (8-16 years). Median (range) body condition score was 6/9 (2/9-9/9). Mean ± SD and median (range) body weights were 5.9 ± 1.5 kg and 5.6 kg (3.0-9.2 kg). Eighteen cats were treated with insulin glargine 100 U/mL and 2 with insulin glargine 300 U/mL. The median dose of insulin was 2 units (range, 0.5-19). One cat had concurrent acromegaly and was not receiving specific medications except insulin; none of the other cats had concurrent disorders.

The protocol and informed consent forms were approved by the Scientific Ethics Committee of the University of Bologna (protocol number 1147). Recruitment of cats to the study was voluntary and at no cost to the owners. Written informed consent was obtained before enrollment in the study.

2.2.2 | Accuracy of FGMS

To compare IG measured with FGMS to the BG obtained with vPBGM, paired samples were collected and then classified as being in

FIGURE 1   FreeStyle Libre application in a diabetic cat. (1) Freestyle libre sensor pack, applicator and reader with the necessary equipment: alcoholic wipes supplied by the Freestyle libre manufacturer, gauze (3 with chlorhexidine and 3 with alcohol), scissors and forceps, tissue glue, tape, cotton and elastic bandage; (2) the dorsal aspect of the neck is trichotomized; (3) the skin is cleaned with chlorhexidine and alcoholic wipes; (4) the dark mark on the sensor applicator is lined up with the dark mark on the sensor pack and, on a hard surface, is pressed down firmly on the sensor applicator until it comes to a stop; (5) lifting the sensor applicator out of sensor pack, the sensor applicator is ready; (6) a drop of tissue glue is added on the skin-surface of the sensor; (7) the sensor applicator is placed over the site and pushed down firmly in order to apply the sensor; (8) it is ensured that the sensor is secure (if necessary, the forceps can be used to avoid the detachment of the sensor); (9) the sensor is additionally secured by covering it with a patch; (10) the reader is turned on by pressing the home button; (11) touching "start new sensor" and (12) holding the reader within 1.5 in. (4 cm) the sensor is scanned and is ready to measure the glucose concentration after 60 minutes; (13) the sensor is secured with a cotton bandage and (14) with an elastic bandage, and (15) the cat is ready to go home

the hypoglycemic (<70 mg/dL), euglycemic (70-180 mg/dL), or hyperglycemic range (>180 mg/dL). All concentration above and below the detection limit of the sensor (≤20 and ≥500 mg/dL) were excluded. During the wearing period of the sensor, each cat was evaluated for 3 time periods, each lasting 12 hours, as follows: 1st day (in hospital), 7th, and 14th day (at home by the owners). On day 1 of the study, cats were hospitalized after food and insulin were administered at home. Sensors were placed immediately after arrival in the hospital, and glucose measurements were started 1 hour later (period of initialization of the sensor). During each evaluation period, BG was measured using the vPBGM and simultaneously (within 1 minute) the sensor was scanned using the FGMS reader. All of the paired results were recorded and then reported in an Excel file. On days 7 and 14, owners were instructed to perform the paired measurements using the same scheme, starting immediately after food and insulin administration. Outside of these evaluation periods and during the entire wearing period of the sensor, owners were allowed to obtain additional paired measurements if they were doubting the accuracy of specific IG results. At the end of the wearing period, sensors were removed by owners at home or by a single clinician in the hospital. If

the sensor was removed at home, owners were asked to photograph the skin at the area where the sensor had been present. The skin at that area was evaluated (either directly or by viewing the photographs) subjectively by a single clinician for the presence of erythema.

2.3 | Purpose-bred healthy cats

2.3.1 | Animals

Seven neutered, purpose-bred, male cats were used in this part of the study. Mean ± SD and median (range) ages were 3.3 ± 0.9 years and 3.5 years (2-4 years). Median (range) body condition score was 7/9 (5/9-7/9). Mean ± SD and median (range) body weights were 5.7 ± 0.8 kg and 5.90 kg (4.4-6.6 kg). Cats were group-housed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All cats were acclimatized and socialized for 1 year before the start of the study, and extensive environmental enrichment was provided. Enrichment included 1 to 3 hours of daily interaction with people and 24-hour access to various

TABLE 1 Mean difference between interstitial glucose concentration and blood glucose concentration and percentages of overestimated and underestimated glucose values

	Mean (±SD) difference between IG and BG	% of underestimated glucose values	% of overestimated glucose values	Identical glucose values %
Overall glucose values	−43 ± 58 mg/dL	77	21.5	1.5
Hypoglycemic range	−1.5 ± 7.1 mg/dL	50	50	/
Euglycemic range	−21.5 ± 28.5 mg/dL	76.5	21.5	2
Hyperglycemic range	−53.6 ± 66 mg/dL	78	21	1

Abbreviations: BG, blood glucose concentration; IG, interstitial glucose concentration.

toys and a climbing apparatus. Cats were deemed healthy based on the absence of clinical signs of disease, routine weekly physical examinations, and a CBC and serum biochemistry panel. Studies were performed at ambient temperatures between 20 and 23°C. The protocol was approved by the Institutional Animal Care and Use Committee (protocol #20539). In each cat, a vascular access port (VAPs; CompanionPort, CP 202K, Norfolk Vet Products, Skokie, Illinois) was surgically placed 11 months before beginning the experiment and maintained as previously described.[20] Cats were fed a commercial dry feline diet (Laboratory Feline Diet 5003, LabDiet, St. Louis, Missouri https://www.labdiet.com/Products/StandardDiets/index.html) at 07:00 every day except on IV glucose tolerance test (IVGTT) days. At 15:00 food bowls were removed. All food was consumed by 15:00 every day. On IVGTT days, food was withheld until after the procedure (cats were fasted from 15:00 the day before).

Intravenous glucose tolerance tests were performed as previously described.[20]

The sensor was scanned during the IVGTT to acquire data. Data were uploaded to the LibreView website at the end of the study. Raw data were extracted from CVS files downloaded from the LibreView website and these raw data were used for statistical analysis.

2.4 | Data analysis

Statistical analysis was performed using 2 commercially available computer software programs (GraphPad Prism 7, Cran R statistical package, R Core Team, 2019. R: A language end environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria).

Normality was assessed using the Shapiro-Wilk test, and nonparametric tests were used accordingly. Normally distributed data are presented as mean ± SD; non-normal data are presented as median and range.

The Mann-Whitney test was used to compare the wearing period of the sensor between diabetic cats in which additional glue was added on the sensor to that of cats in which no additional glue was used. Correlation between the IG measured by FGMS and BG was evaluated using Pearson's correlation. The differences between IG and the BG were plotted against the PBGM results in Bland-Altman plots. The Fisher's exact test was used to compare the number of

underestimated IG readings to the number of overestimated IG readings.

Analytical and clinical accuracy was evaluated by comparing the results of the PBGM measurements to those obtained from the FGMS using the ISO 15197:2013 criteria. Both of the following minimum criteria for acceptable system accuracy had to be met: (a) 95% of the results must be within ±0.8 mmol/L (15 mg/dL) of the BG for a BG <5.5 mmol/L (100 mg/dL) and within ±15% of the BG for a BG >5.5 mmol/L (100 mg/dL) and (b) 99% of the individual BG measured results should fall within zones A and B of the Parkes consensus error grid analysis (EGA) for type 1 DM.[21]

In purpose-bred cats, during each IVGTT, experiment time was recorded (−70 to 180 minutes) as well as clock time when each sample was obtained. Interstitial glucose concentration data were matched to the BG data by obtaining the result recorded with the FGMS at the time point closest to the corresponding BG measurement (with up to 7 minutes discrepancy), based on the time stamp provided in the FGMS CVS file. If no IG measurement was available within 7 minutes of the BG measurement, the BG result was excluded from the analysis. The lag time between BG and IG was calculated as the time from glucose administration until maximal IG and as the time from glucose administration to maximum rate of positive change in IG.[12]

Significance was set at $P \leq .05$.

3 | RESULTS

3.1 | Sensor application and maintenance in client-owned diabetic cats

In diabetic cats, the application of the sensor was successful and easy to perform in 19/20 cats. One cat abruptly moved its head during sensor insertion, leading to insertion failure. This sensor was not included in further analysis. In this cat, a new sensor was easily placed immediately after, without further complications. In 13 cats, additional tissue glue was applied to the skin-facing surface of the sensor. In 2 overweight cats, the sensor was placed caudally on the dorsum because there was not enough space on the neck. The sensor was well tolerated by 17/20 cats. In 3 cats, the sensor was not well tolerated, and was removed by the cat 24 hours (in 1 cat) or 48 hours (in 2 cats) after

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application. In these cats, owners reported signs of stress such as restlessness and attempts to remove the sensor by scratching the neck.

In all 20 cats, the sensor read IG 60 minutes after successful application. The overall median wearing period of the sensor was 5.5 (1–14) days. Sensors that were secured using tissue glue had a median wearing period of 6 days compared to 5 days for sensors in which glue was not used ($P = .5$). In 1 of the 2 cats in which the sensor remained on for 14 days, the sensor stopped recording IG continuously 7 days after application (the reader showed IG alternating between "LO" and "ERR"). After sensor removal, 1 cat had erythema at the application site.

3.2 | Accuracy of the FGMS in client-owned diabetic cats

A total of 283 paired glucose results were obtained, of which 67% (190/283) were in the hyperglycemic range as determined by the PBGM (BG, 356 mg/dL; range, 182-672); 32% (89/283) were in the euglycemic range (BG, 127 mg/dL; range, 76-179) and 1% (4/283) were in the hypoglycemic range (BG, 59.5 mg/dL; range, 50-70).

Paired samples were collected on the following days after sensor placement: day 1 (133/283, 47%), 2 (30/283, 11%), 3 (47/283, 17%), 4 (6/283, 2%), 7 (60/283, 21%), and 10 (7/283, 2%).

Median (range) glucose concentrations in all measured samples was 248 mg/dL (range, 40-491) using FGMS, and 292 mg/dL (range, 50-672) using PBGM ($P = .002$). A strong positive correlation overall was found between IG and BG ($r = 0.90$; 95% confidence interval [CI], 0.88-0.93; $P < .001$) and separately in the hypoglycemic range ($r = 0.85$; 95% CI, −0.6 to 0.99; $P = .15$), in the euglycemic range ($r = 0.66$; 95% CI, 0.52-0.76; $P < .001$) and hyperglycemic range ($r = 0.75$; 95% CI, 0.68-0.80; $P < .001$).

The mean (±SD) difference between IG and BG and the percentages of underestimated and overestimated IG readings in the 3 glycemic ranges are presented in Table 1.

Considering the ISO 15197:2013 requirements for the range of BG results <100 mg/dL, 47% (9/19) of IG results were within ±15 mg/dL of the BG. For BG >100 mg/dL, 43.6% (115/264) of IG results were within ±15% of the BG. Evaluation of data using the Parkes consensus EGA showed that 100% of the samples fell in the zones A and B.

3.3 | Accuracy and lag time of FGMS in purpose-bred healthy cats

Mean ± SD BG and IG before and during the IVGTT are presented in Figure 2.

In the immediate 30 minutes after an IV bolus of glucose when BG was increasing rapidly (approximately 2%/min), IG increased slowly, resulting in a difference of as much as 579 mg/dL, and no positive correlation between BG and IG was found. Before glucose administration at time zero and between minutes 45-180 of the IVGTT,

when BG was stable or its rate of decrease was slow (approximately 0.5%/min), a strong positive correlation was found between BG and IG ($r = 0.97$; 95% CI, 0.96-0.98) with a consistent bias (across the BG range) toward underestimating BG by the FGMS (mean ± SD bias, 23.3 ± 18.1 mg/dL). The time from IV glucose administration to time of maximum IG was 30 minutes (range, 15-30) with 85% of cats reaching maximum IG at 30 min. The time from IV glucose administration to time of maximum rate of positive change in IG was 5 minutes (range, 5-15).

3.4 | Overall (diabetic cats and purpose-bred healthy cats) analytical and clinical accuracy

Considering 422 paired glucose results obtained from all cats, 52.6% (222/422) were in the hyperglycemic range (as determined by the PBGM), with a median BG of 339 mg/dL (range, 182-672); 46.4% (196/422) were in the euglycemic range with a median BG of 114 mg/dL (range, 76-179) and 1% (4/422) were in the hypoglycemic range with a median BG of 59.5 mg/dL (range, 50-70). The overall correlation coefficient value was $r = 0.93$ (95% CI, 0.92-0.94; $P < .001$).

The Bland and Altman difference plots are shown in Figure 3. The mean (±SD) difference between IG and BG was −36.4 ± 49.7 mg/dL. Considering the ISO 15197:2013 requirements, for the range of BG results <100 mg/dL, 29.2% (14/48) of IG results were within ±15 mg/dL of the BG. For BG >100 mg/dL, 43.8% (164/374) of IG results were within ±15% of the BG. Evaluation of data using the Parkes consensus EGA showed that 100% of the FGMS results fell in zones A and B (Figure 4).

4 | DISCUSSION

We showed that FGMS has sufficient clinical accuracy to be used as an IG monitoring tool in cats, despite falling short of meeting the analytical ISO 2013 requirements. We also report for the first time in cats on the potential for a considerable lag between IG as measured by the FreeStyle Libre and BG during periods of rapid BG changes.

The application of the sensor on the neck was quick, painless, and simple. The neck was chosen as primary site of application because it allowed for easy application of a protective bandage. In our experience and that of others, when placed on the neck, the sensor also can be protected by placing a collar (eg, KittiKollar) which further facilitates sensor application by owners at home. Furthermore, a previous study showed better accuracy of a CGMS when placed on the neck compared to other locations.[22] In 2 overweight cats, however, the sensor had to be placed caudally over the dorsolateral aspect of the thorax as previously described.[23]

The Libre sensor is designed for a 14-day wearing period but in our cohort of diabetic cats, the sensor was worn for only 5.5 days. Application of tissue glue did not significantly increase wearing time. These results are in contrast to those observed in dogs in which the
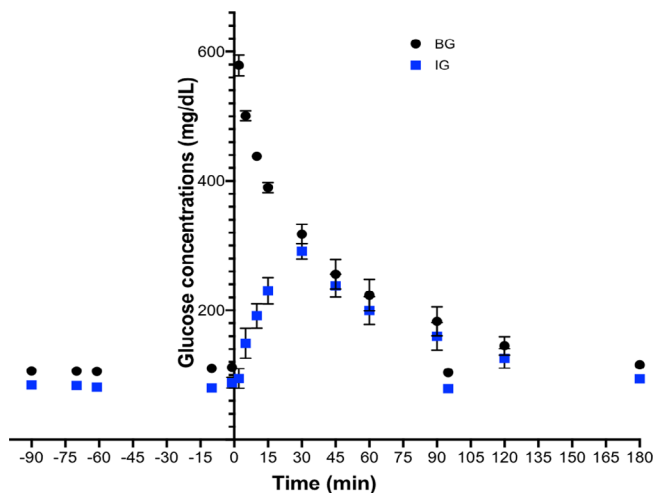
FIGURE 2   Mean ± SD of blood glucose concentrations (BG, black circles) and interstitial glucose concentrations (IG, blue squares) in seven purpose-bred laboratory cats, before and after administration of 0.5 g/kg of glucose IV (time 0)
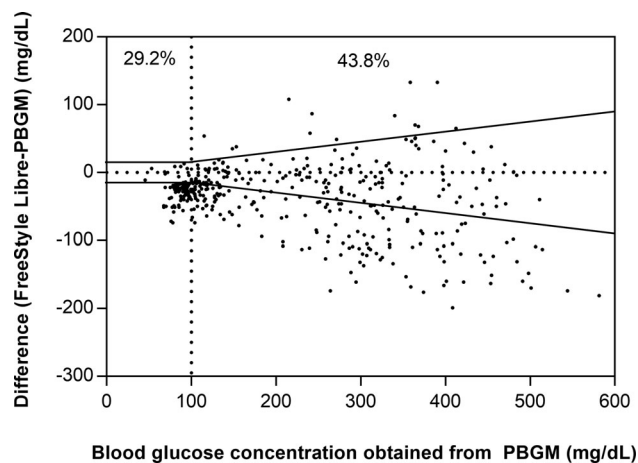

FIGURE 3   Bland–Altman plots represent the differences between the glucose concentrations obtained by the use of the FGMS versus those obtained using the PBGM in all cats (diabetic and purpose-bred healthy cats). The PBGM glucose values plotted against absolute errors for each corresponding value are on the x-axis. The standard required limits are defined by the black symmetric line: at ±15 mg/dL from the reference value for glucose determinations <100 mg/dL and ±15% from the reference value for glucose determination >100 mg/dL. Percentages express the number of samples within the limits when the reference determination was < or >100 mg/dL, and for the total number of measurements (central % value)
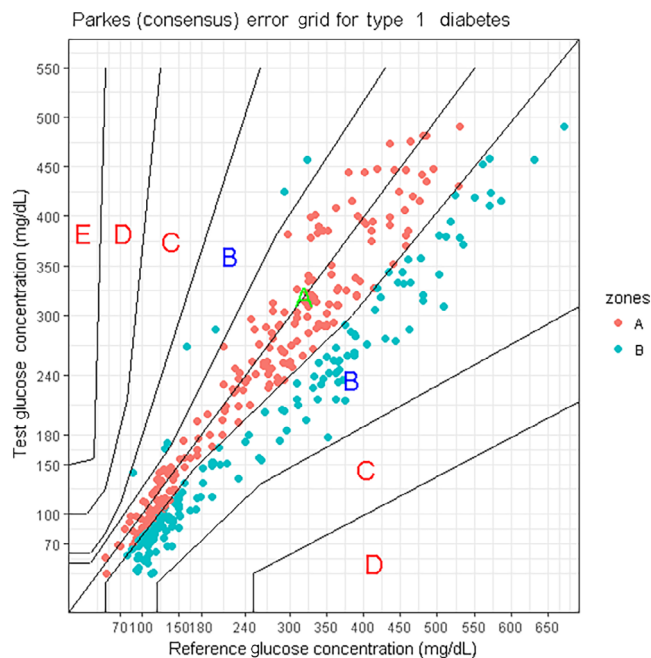

FIGURE 4   Parkes consensus error grid analysis (EGA) representation with the percentage of values within different zones. The reference glucose values (blood glucose obtained by a portable glucometer), on the x-axis, are plotted against the interstitial glucose measurements obtained by the flash glucose monitoring system, on the y-axis. The different zones designate the magnitude of risk: no effect on clinical action (zone A), altered clinical action—little or no effect on the clinical outcome (zone B), altered clinical action—likely to affect the clinical outcome (zone C), altered clinical action—could have a significant medical risk (zone D), and altered clinical action—could have dangerous consequences (zone E). ISO 15197:2013 requires that 99% of the values fall within zones A + B for a device to be considered accurate

maximal duration of the sensor (14 days) was reached in 70% of cases[24] and compare favorably with those recently obtained in cats, using a more invasive method of securing the sensor.[16] Using multiple skin sutures to secure the sensor and protecting with a bandage, previous investigators reported a median wearing time of 8 days[16] for the Libre sensor. Interestingly, another recent study showed that 61% of sensors remained attached and functional in cats for the full

14 days.[23] In that study, sensors were applied on the neck as described here, but the majority of sensors were applied without additional tissue glue and without securing them with a bandage. It is possible that bandaging confers no advantage to maintaining the sensor on the cat and might even cause irritation and shorten wearing time. Also, in that study, sensors sometimes were placed more than once on each cat. It is possible that with repeated sensor applications, the cat becomes accustomed to wearing the sensor, resulting in a longer sensor lifespan. In our study, the only cat in which the sensor functioned for 14 days was a cat that was accustomed to wearing the sensor before inclusion in the study. Based on our findings and those of a previous study, securing the sensor with tissue glue might be advisable although doing so would need to be further examined in future studies.

Dermatologic complications associated with the use of FGMS in cats are reported in 18% of cases and can be mild (erythema, mild crusting, abrasion, mild pruritus, or discomfort) or severe (erosions, ulceration, abscessation, severe pruritus).[23] In our study, mild dermatologic changes (erythema at the site of sensor application) were detected only in 1 cat. In this cat, additional glue was applied to the

skin-facing surface of the sensor. The sensor's built-in adhesive is known to cause allergic contact dermatitis in some people[25,26] and combining it with additional glue might have contributed to the inflammatory response noted in this cat. Of note, these mild dermatologic changes usually are of little clinical consequence and do not preclude placement of subsequent devices.[23]

We found a strong positive correlation between IG and BG in diabetic cats, similar to a recent report ($r = 0.9$).[16] We found an even stronger correlation in purpose-bred cats, but only after excluding results corresponding the time period in which BG was changing rapidly (approximately 2%/min). It is possible the weaker correlation between BG and IG in diabetics cats is the result of including some data that represent time intervals with rapidly changing BG. Because BG was measured relatively infrequently in the diabetic cats (every 2 hours at the most, compared to every 5 minutes in purpose-bred cats), it was not possible to establish the rate of BG change and exclude data from time intervals with rapid change in BG, nor did we consider it important. One can assume that, in the clinical setting in diabetic cats, CGM data would be collected both at times when BG changes rapidly as well as at times when BG is relatively stable.[27] The relative proportion of each would depend on factors such as the type of insulin used, stressful events, and other factors. Stress hyperglycemia is a well-recognized phenomenon in cats. Under controlled conditions in healthy cats, BG can increase by 150 mg/dL within a few minutes of stress induction.[5] In diabetic cats, especially those that are insulin dependent (and therefore are monitored using CGM), the ability to self-regulate BG after activation of the sympathetic system by a stressful event is decreased or absent, and therefore stress hyperglycemia might be more substantial. Some discrepancies between BG and IG in the monitored diabetic patient are to be expected, and a lag time for equilibration between BG and IG might be an important reason for these discrepancies. In addition, we found a systematic bias toward underestimation of BG by IG, regardless of BG range and lag time. It is possible that the cause of this bias is the algorithm used by the Libre which, like other PBGMs used in people, tends to underestimate glucose concentrations. This is in contrast to the veterinary PBGM we used as a reference method here that does not have this built-in bias. Regardless of the cause of this systematic bias, it does not seem to be clinically important, as demonstrated by the results of EGA, in which all readings would have led to clinically correct treatment decisions.

Based on the IV administration of a glucose bolus in purpose-bred cats, our data suggest that large and rapid changes in BG are not reflected by change in IG completely or in a timely manner, thus making the device less accurate during periods of rapidly changing BG. Indeed, a lag between maximal IG and maximal BG of approximately 30 minutes was detected. In a previous study, a 35-minute lag was reported when using a glucose dosage of 1 g/kg (twice the dose used here) for the IVGTT, likely resulting in higher peak in BG.[12] It is likely that with changes of smaller magnitude in BG, as is more commonly seen in the clinical setting, this lag time would be smaller. Of note, the lag time between maximal BG and IG represents time to equilibrium between the 2 compartments. Before achieving this equilibrium however, after a change in BG, a change in IG might already be detected within a few minutes, as represented by the time from IV glucose administration to the time of maximally increasing slope of IG. This lag in detection of concentration change likely represents a sum of 2 phenomena: the lag between the actual change of BG and the time this change is reflected in the interstitium, related to factors such as the movement of glucose across endothelia, the diffusion distance from blood to the sensor, and the concentration gradient. This lag time might be affected by physiological conditions related to blood flow alterations (eg, anesthesia), the rate of glucose consumption by the tissue, and the time it takes for the CGMS itself to respond to this change considering the frequency of IG sampling (once every minute in the Libre) and the unique algorithm that is used. These factors might differ among CGMSs. In the previous study, healthy cats were anesthetized during the IVGTT and the CGMS used was different (Guardian REAL-Time) than the device used in our study.[12] Still, the reported lag time to maximal positive percent change observed previously (11.4 minutes; range, 8.8-19.7 minutes) is similar to the lag time we report here.[12]

Other factors that were not studied here should be taken into account when interpreting CGM data in diabetic cats. Diabetic cats are usually older and overall less well hydrated compared to healthy cats. In people, there is less delay between BG and IG in adolescents relative to adults, and this delay correlates positively with the age of the subjects.[28] This might be related to decreased microcirculation in the subcutaneous tissue with age, leading to slower equilibration of glucose between blood and interstitial fluid. Similarly, an equilibrium between BG and IG also might be reached later in older diabetic cats.

Only 42.2% of all data points included in our study were within the limits of analytical accuracy based on ISO 15197:2013 requirements. In contrast, a previous study using the hexokinase method as a reference found better results, with 67.7% of FGMS measurements within the ISO 15197:2013 requirements.[16] However, Parkes EGA showed good clinical accuracy with 100% of readings in zone A and B. The ISO 15197:2013 standards require comparison of BG meter measurements with the results of a standard reference method. However, these standards are designed for comparisons of results from a single compartment, typically the blood, and comparisons between 2 different compartments (blood and interstitial fluid) may be inappropriate because of the physiological differences between these compartments. In the absence of established standard criteria for evaluation of the accuracy of glucose measurements in the interstitial fluid, the ISO criteria for the evaluation of PBGMs provide a relevant substitute to identify devices that are as close as possible to meeting accuracy criteria and that are not dangerous for the animal's health. Currently, studies in human and veterinary medicine adopt this approach. With this caveat, the FGMS can be considered acceptable for clinical use, despite analytical accuracy requirements not being met. An important limitation of our study and a persistent gap in knowledge in the veterinary literature in general is the scarcity of correlation and accuracy data in the hypoglycemic range. Considering the importance of accuracy in the low BG range to clinical decision making, further studies are needed.

Another limitation of our study is inability to assess precision and compare ideal location for sensor placement because only a single sensor was placed in each cat. Moreover, because of the relatively short lifespan of the sensor, accuracy over time was not investigated. However, in humans[29,30] and dogs,[24] the accuracy of FGMS remains stable over 14 days of use.

In conclusion, the FGMS provides clinically accurate measurements in the euglycemic and hyperglycemic ranges. The clinical accuracy was difficult to determine in the hypoglycemic range. In the latter case, it may be advisable to assess BG using a validated PBGM to confirm the FGMS results. The FGMS is also less accurate during periods of rapid change in BG, especially when the change in BG is of large magnitude. Finally, using a method of application that does not require suturing or even the addition of tissue glue, FGMS sensors can be applied by the owners at home and enhance monitoring frequency and quality. In human medicine, the use of FGMS significantly improves glycemic control.[31] In diabetic dogs, a recent study showed that use of FGMS allowed better identification of BG nadirs and hypoglycemic episodes compared to the use of a PBGM.[32] The FGMS also allows the assessment of BG variations during consecutive days, enabling the clinician to make a more informed decision about the appropriate insulin dose, taking into account day-to-day variations in glycemic control.[32] Although the sensor might not remain on the cat for the full 14 days, using a simple application method, in most cats data can be obtained over a few days and nights. Additional studies are needed to investigate whether long-term use of FGMS during follow-up examination improves glycemic control in diabetic cats.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The protocol and informed consent forms were approved by the Scientific Ethics Committee of the University of Bologna, Italy (protocol number 1147). The protocol also was approved by the IACUC (protocol #20539).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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