A novel sandwich ELISA for the measurement of insulin in feline serum and plasma

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CONCLUSION
This novel insulin ELISA provides analytical performance, precision and reliability equal to methods currently used in human clinical research.

Sample analysis using only 10 µL of sample volume confirmed a dynamic range and high sensitivity.

This method enables scientists to perform additional mechanistic investigations, which may contribute to a more complete understanding of the development of feline diabetes.

OBJECTIVE
The objective of this study was to evaluate an ELISA optimized for quantitative measurements of feline insulin. The ELISA assay has recently been developed at Mercodia AB, Uppsala, Sweden.

BACKGROUND
Diabetes mellitus is one of the most common endocrine disorders in cats, with a form that closely resembles human type 2 diabetes. Its incidence rate among cats appears to be increasing, probably due to an increase in obesity and a decrease in physical activity in the cat population. Obesity increases the risk for diabetes 3- to 5-fold. Diabetes occurs in a wide range of cats, but most cats are over six years of age when diagnosed. Diabetic cats may go into remission and studies have shown that different insulin therapy regimens may have an influence on this. The mechanisms underlying the development of insulin resistance are currently unclear and further investigations are warranted.

METHODS
The Feline Insulin ELISA is a simultaneous assay with a required sample volume of 10 µL. The incubation time is 120 minutes plus a 30 minute TMB substrate incubation.

The established ELISA was validated for accuracy, sensitivity, specificity and precision. For validation of the accuracy twelve feline samples were diluted with calibrator 0 and twelve other feline samples were spiked with calibrator insulin. Capability of detection was determined according to the methodology described in ISO11843. Cross-reactivity of different analogues was studied by serial dilution of the analogues with calibrator D. The precision study included both repeatability and reproducibility. The repeatability study was performed with three feline samples, which were analyzed in four replicates at fourteen different occasions in one laboratory. The reproducibility or inter lab comparison was performed at four laboratories, two in Europe and two in North America. One control sample was measured in duplicate in a total of fifteen runs (1 to 5 runs in each laboratory). Samples from apparently healthy cats were analyzed at three different laboratories in North America. The laboratories analyzed their own subset of samples. The samples included were random samples, fasted and postprandial.

There are no conflicts of interest regarding this work.

RESULTS

Accuracy
Recovery upon addition is 93 to 122 % (mean 107%).
Recovery upon dilution is 79 to 113 % (mean 95%)

Sensitivity
The assay range is 5 to 700 ng/L and the capability of detection was determined to 5 ng/L.

Specificity
Cross reactivity
The following cross reactions were found:
Insulin aspart < 0.008 %
Insulin detemir < 0.008 %
Insulin glargine 8.4 %
Insulin lispro < 0.0000002 %
Insulin glulisine < 0.000000 %
Porcine insulin (Vetsulin®, Caninsulin®) 57.4 %

Precision
Reproducibility
Reproducibility, the same sample was run with the same assay, in different laboratories, with different technicians and different equipment.

Reproducibility can only be achieved with a robust assay, designed and optimized to be insensitive to variations in instruments and other run conditions (see table 1 and figure 2).

Repeatability
Repeatability, the same sample is run in the same lab, by the same technician, using the same equipment (see table 2).

Sample analysis
Laboratory #1
Fasting insulin values (n=12) showed an insulin range of 100 to 483 ng/L. Postprandial insulin values ranged from 97 to 1224 ng/L.

Laboratory #2
Fasting insulin values (n=8) showed an insulin range of 55 to 691 ng/L. Postprandial insulin values ranged from 138 to 655 ng/L.

Laboratory #3
Insulin values from random sampling (n=16) showed an insulin range of 45 to 435 ng/L.

Table 1. Each sample was analyzed in 2 replicates on 14 different occasions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value (ng/L)</th>
<th>Coefficient of variation&lt;br&gt;Within assay %</th>
<th>Between assay %</th>
<th>Total assay %</th>
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