Art of measuring progesterone: understanding immunoassays

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Abstract

Measurement of progesterone has become an essential component of canine reproductive medicine. Reliable measurement of steroid hormones is challenging. In veterinary medicine, we depend on immunoassays to determine concentrations of circulating progesterone. Immunoassay is the general term for a diagnostic technique that relies on antibodies to detect a compound within a biologic sample. There are several types of immunoassays available, which have innate differences. Therefore, to correctly apply the results to a clinical scenario, it is essential for the practicing veterinarian to understand the method they are utilizing. Data from development of a new in-house progesterone test are reviewed and a variety of immunoassay methods, assay validation, and future trends within diagnostic industry are discussed. Understanding these principles will help the clinician choose an assay which will best suit their diagnostic needs.

Keywords: Diagnostic testing, progesterone, canine, immunoassay, liquid chromatography-mass spectrometry

Introduction and background

Progesterone is a female reproductive hormone. Measurement of progesterone in blood (plasma or serum) from bitch is an important element to predict and confirm ovulation to determine optimal breeding time and maximize fertility,[1] to predict parturition[2] and to investigate reproductive abnormalities.[3]

Catalyst® Progesterone is a new immunoassay from IDEXX that is designed to provide prompt and reliable in-clinic measurement of progesterone in canine blood samples. It works with both IDEXX Catalyst One® and IDEXX Catalyst Dx® chemistry analyzers. It has a reportable range of 0.6 - 63.6 nmol/l.

In clinical practice, various methods have been used to monitor progesterone in bitch. For many decades, radioimmunoassay (RIA) (Coat-A-Count® radioimmunoassay, Siemens Health Care Diagnostics Inc., Los Angeles, CA) was regarded as a gold standard; however, the originally validated assay was discontinued in 2014[4] and liquid chromatography-mass spectrometry (LC-MS) has been proposed as the gold standard.[5]

Because LC-MS is not typically available in veterinary reference laboratories, chemiluminescent immunoassay (CLIA) (IMMULITE®, Siemens Medical Solutions Diagnostics, Los Angeles, CA) analyzers is more widely used. However, despite strong correlation between CLIA and reference methods,[6] clinically significant bias between methods has also been demonstrated.[4] It is further complicated by differing performance between iterations of the CLIA methodology.[7]

For progesterone assays used with canine samples, it is important to have accuracy and precision in the range associated with ovulation 9.5 - 31.2 nmol/l or lower.[4] For this study, range of clinical interest was defined as 0 - 32.0 nmol/l.

Objectives of the study were to evaluate the performance of Catalyst Progesterone by a method comparison to LC-MS (reference method), evaluate the performance of 2 iterations of the CLIA methodology by a method comparison to LC-MS, and to evaluate precision of Catalyst Progesterone using control fluids (precision study).

Materials and methods

Data were collated in Microsoft Office Excel 2016 before being exported to JMP® 14.0.0 for statistical analysis, where appropriate, using the Method Comparison Add-In from SAS Institute.

Method comparison study

Blood samples were collected from 60 bitches visiting 2 veterinary hospitals for breeding management during September and October of 2018. All samples were collected in the periovulatory period. Some patients were sampled on multiple days (range 1 - 7 venipunctures), allowing 101 comparisons to be made. Samples used for this study were residual blood samples after clinical diagnostic testing was performed and animals were not subjected to additional venipuncture to collect study samples. We complied with the IDEXX reference laboratory sample retention policy. Refer Table for details.

Table. Sample types and handling for the progesterone assays

Hospital	Samples	CLIA	Catalyst® Progesterone	LC-MS
A	32 bitches; 52 comparisons	• CLIA1 (IMMULITE 1, Siemens Medical Solutions Diagnostics, Los Angeles, CA; IMMULITE®/ IMMULITE 1000 Progesterone [catalog number: LKPW1]) • Serum • Gel barrier tubes were not used as the package insert details a time-dependent decrease in progesterone concentrations • Analyzed within 24 hours after collection at IDEXX Reference Laboratories by laboratory technicians	• Catalyst Dx® Chemistry Analyzer • Lithium heparin plasma • Gel barrier tubes were not used as the operator's guide indicates they are not suitable • Analyzed within 4 hours after collection at the hospital by veterinary technicians	• LC-MS at IDEXX R&D • Lithium heparin plasma • Samples stored at 4°C and analyzed in batches within 1 week after collection
B	28 bitches: 49 comparisons	• CLIA2000 (IMMULITE 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA; IMMULITE® 2000 Progesterone [catalog number: L2KPW6]) • Serum • Gel barrier tubes were not used as the package insert details a time-dependent decrease in progesterone concentrations • Analyzed within 24 hours after collection at IDEXX Reference Laboratories by laboratory technicians	• Catalyst Dx® Chemistry Analyzer • Lithium heparin plasma • Gel barrier tubes were not used, as the operator's guide indicates they are not suitable • Samples stored at 4°C and analyzed at IDEXX R & D by laboratory technicians	• LC-MS at IDEXX R & D • Lithium heparin plasma • Samples stored at 4°C and analyzed in batches within 1 week after collection

For each venipuncture, within 30 minutes:

1. Serum was harvested from blood collected in tubes without anticoagulant.
2. Lithium heparin plasma was separated from the red blood cells and divided into 2 aliquots.

No samples were excluded. All results were below the upper limit of the respective dynamic ranges of the assays. Any results below the lower limit of the dynamic range (0.6 nmol/l for all assays) were assigned to 0.6 nmol/l. Passing and Bablok linear regression analysis was completed for various pairs of methodology. Correlation coefficients were interpreted as follows: r = 0.90 - 1.0 defined very high correlation; 0.70 - 0.89, high correlation; 0.50 - 0.69, moderate correlation; 0.30 - 0.49, low correlation; and 0 - 0.29, little, if any, correlation.[8] Regression analysis was used for statistical evidence of systematic error (constant and/or proportional bias). Confidence intervals of 95% for the y-intercept that did not include the value zero were considered evidence of constant bias. Confidence intervals of 95% for the slope that did not include the value 1.0 were considered evidence of proportional bias. Precision was assessed by repeated analysis of 2 control fluids in the range of clinical interest. Each fluid was analyzed 8 times per day (4 in the morning, 4 in the evening) for 10 days to give a total of 80 replicates. Total percentage coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean of the concentration. The greater the CV, the greater the dispersion of results around the mean.

Results

Method comparison study

Results are summarized in Figure 1A - D. CLIA1, CLIA2000, and Catalyst® Progesterone all demonstrated very high correlation to the reference method. For Catalyst® Progesterone, this was shown in both groups of samples. There was no evidence of constant or proportional bias for CLIA1 and Catalyst® Progesterone. For CLIA2000, there was a constant bias (intercept = 0.17 ng/ml; with confidence limits of 0.38 - 0.86 nmol/l) and proportional bias (slope = 0.75; with confidence limits of 0.70 - 0.80). The new method of Catalyst® Progesterone had a total CV of < 10% at both concentration levels.

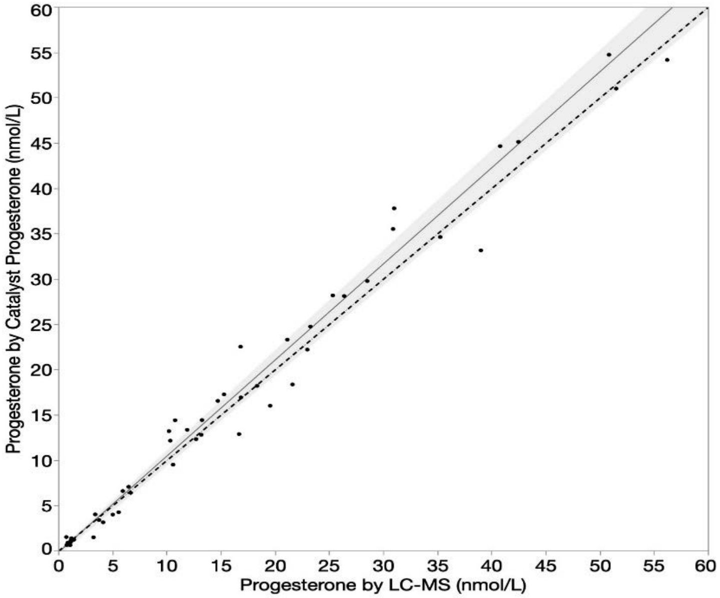

Figure 1A. Passing and Bablok plots for the agreement of progesterone evaluated by two methods in samples collected during the periovulatory period. Dashed line represented the identity line (x = y), solid line represents regression line, and shaded area represents the confidence interval for regression line. Hospital A: Comparison of Catalyst Progesterone and the reference method (LC-MS); n = 52, r = 0.99.

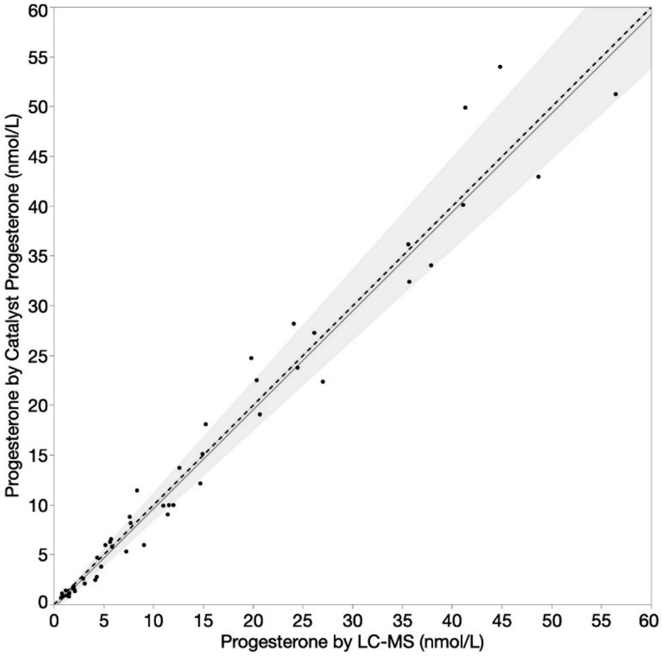

Figure 1B. Passing and Bablok plots for the agreement of progesterone evaluated by two methods in samples collected during the periovulatory period. Dashed line represented the identity line (x = y), solid line represents regression line, and shaded area represents the confidence interval for regression line. Hospital B: Comparison of Catalyst Progesterone and the reference method (LC-MS); n = 49, r = 0.98.

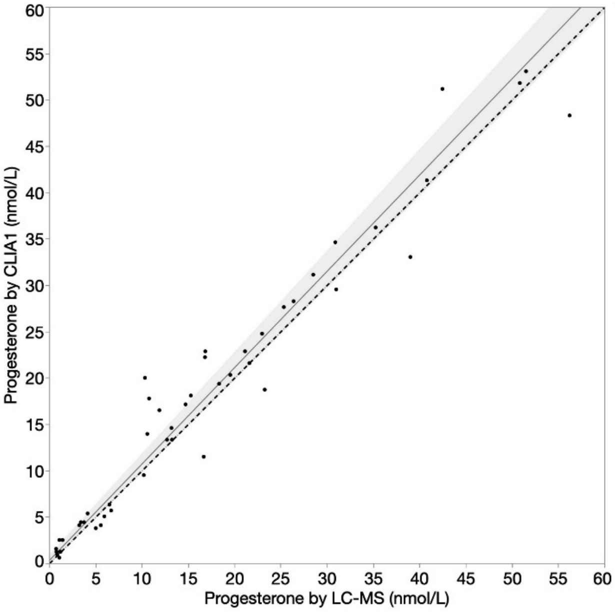

Figure 1C. Passing and Bablok plots for the agreement of progesterone evaluated by two methods in samples collected during the periovulatory period. Dashed line represented the identity line (x = y), solid line represents regression line, and shaded area represents the confidence interval for regression line. Hospital A: Comparison of CLIA1 and the reference method (LC-MS); n = 52, r = 0.98.

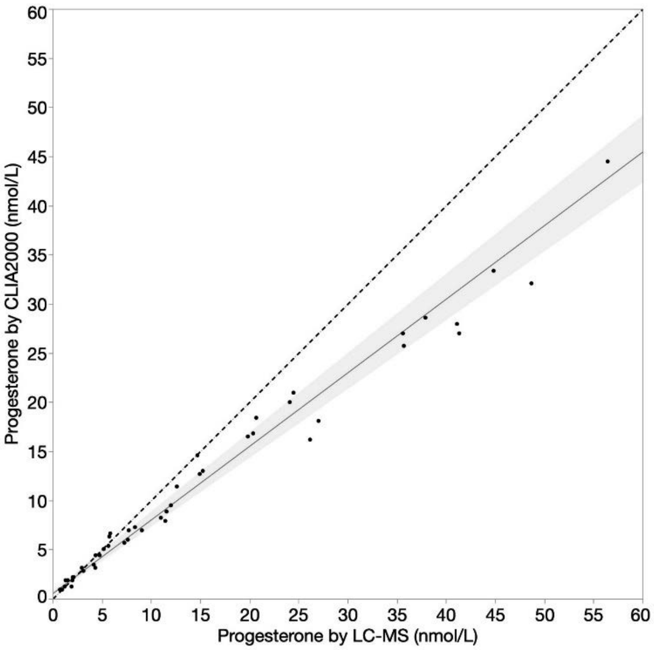

Figure 1D. Passing and Bablok plots for the agreement of progesterone evaluated by two methods in samples collected during the periovulatory period. Dashed line represented the identity line (x = y), solid line represents regression line, and shaded area represents the confidence interval for regression line. Hospital B: Comparison of CLIA2000 and the reference method (LC-MS); n = 49, r = 0.99.

Conclusion

Catalyst Progesterone demonstrated very good correlation (r = 0.98; r = 0.99) to the study reference method of LC-MS and good precision in the range of clinical interest. Both iterations of the CLIA demonstrated very good correlation to the reference method (CLIA1: r = 0.98; CLIA2000: r = 0.99). However, for CLIA2000, there was a marked proportional bias (slope = 0.75) to the reference method. This does not imply that CLIA2000 is unsuitable for clinical use; rather, it emphasizes the need for consistent analytical methodology and sample type when trending progesterone concentrations. Catalyst Progesterone produces accurate and precise results when used to quantify progesterone in plasma samples from bitches. This new immunoassay provides a reliable and convenient option to measure canine progesterone in-house.

Conflict of interest

Authors are employed by IDEXX Laboratories Inc., Westbrook, ME.

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