# Catalyst Progesterone for in-house measurement of progesterone in plasma from bitches

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## Introduction

Progesterone is a female reproductive hormone. Measurement of progesterone in blood plasma (plasma) or serum from the bitch is an important element of:

- Predicting and confirming ovulation to determine optimal breeding time and maximize fertility.[1]
- Predicting parturition.[2]
- Investigating 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 samples. It works with both the IDEXX Catalyst One® and IDEXX Catalyst Dx® chemistry analyzers. It has a reportable range of 0.2–20.0 ng/mL.

In clinical practice, various methods have been used to monitor progesterone in the bitch. For many decades, radioimmunoassay* (RIA) 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) on IMMULITE® 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] The situation 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 3.0–9.8 ng/mL; 9.5–31.2 nmol/L or lower.[4] For this study, the range of clinical interest was defined as 0–10.0 ng/mL

The study objectives were to:

- Evaluate the performance of Catalyst Progesterone by a method comparison to LC-MS (reference method).§
- Evaluate the performance of two iterations of the CLIA methodology by a method comparison to LC-MS.
- Evaluate precision of Catalyst Progesterone using control fluids (precision study).

## Materials and methods

Data was 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 attending two 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. See table 1 for details.

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 two aliquots.

| Hospital | Samples | CLIA | Catalyst® Progesterone | LC-MS |
|---|---|---|---|---|
| A | 32 bitches; 52 comparisons | CLIA1<br>• Serum (per stipulation in relevant package insert)<br>• Gel barrier tubes were not used as the package insert details a time-dependent decrease in progesterone levels<br>• IMMULITE®/IMMULITE 1000 Progesterone (catalog number: LKPW1) run on an IMMULITE 1 at IDEXX Reference Laboratories by laboratory technicians<br>• Analyzed within 24 hours of collection | • Lithium heparin plasma<br>• Gel barrier tubes were not used as the operator's guide indicates they are not suitable<br>• Catalyst Dx® Chemistry Analyzer at the hospital, operated by veterinary technicians<br>• Analyzed within 4 hours of collection | • Lithium heparin plasma<br>• LC-MS§ at IDEXX R&D<br>• Samples stored at 4°C and analyzed in batches within 1 week of collection |
| B | 28 bitches; 49 comparisons | CLIA2000<br>• Serum (per stipulation in relevant package insert)<br>• Gel barrier tubes were not used as the package insert details a time-dependent decrease in progesterone levels<br>• IMMULITE® 2000 Progesterone (Catalog Number: L2KPW6) run on an IMMULITE 2000 at IDEXX Reference Laboratories by laboratory technicians<br>• Analyzed within 24 hours of collection | • Lithium heparin plasma<br>• Gel barrier tubes were not used as the operator's guide indicates they are not suitable<br>• Catalyst Dx® Chemistry Analyzer at IDEXX R&D, operated by laboratory technicians<br>• Samples stored at 4°C and analyzed within 48 hours from collection | • Lithium heparin plasma<br>• LC-MS§ at IDEXX R&D<br>• Samples stored at 4°C and analyzed in batches within 1 week of collection |

**Table 1.** Sample types and handling for the progesterone assays

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 (0.2 ng/mL for all assays) were assigned to 0.2 ng/mL.

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]

The regression analysis was also used to look 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 study

Precision was assessed by repeated analysis of two 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.

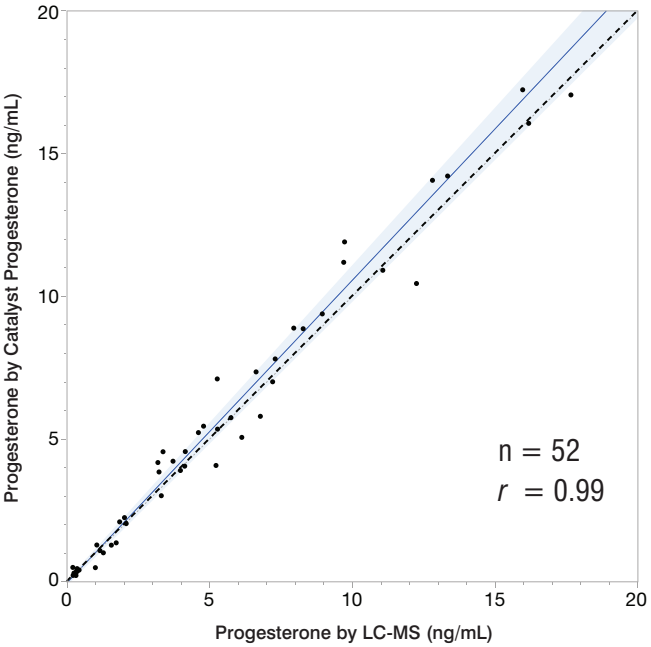
## Results

### Method comparison study

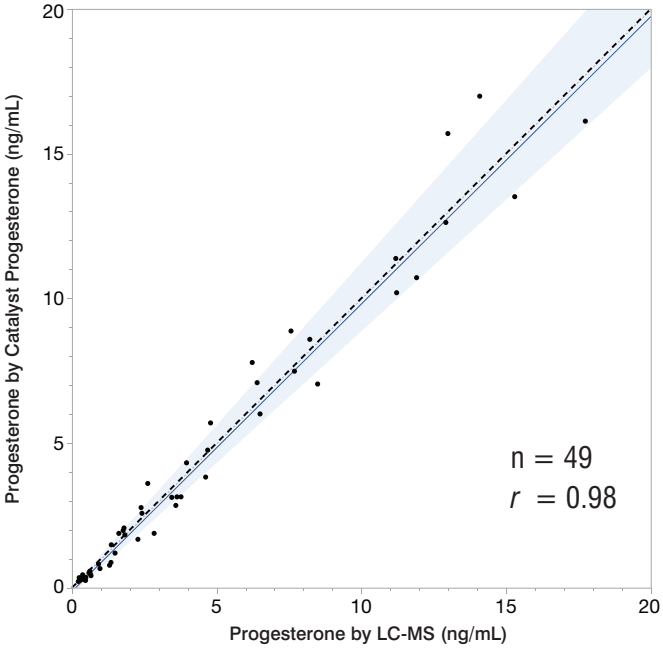
The results are summarized in figure 1. CLIA1, CLIA2000, and Catalyst® Progesterone all demonstrated very high correlation to the reference method. For Catalyst Progesterone, this was shown in both groups of samples.

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 0.12–0.27 ng/mL) and proportional bias (slope = 0.75; with confidence limits of 0.70–0.80).

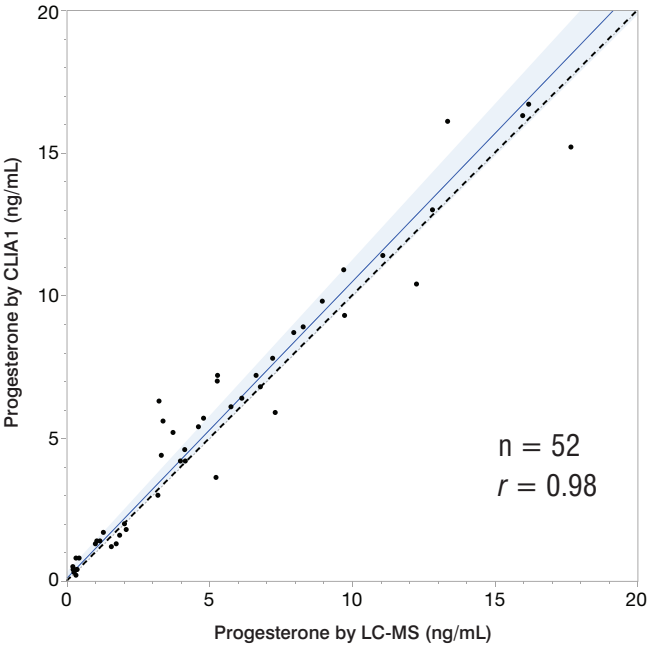
**a. Hospital A:** Comparison of Catalyst Progesterone and the reference method (LC-MS)




**b. Hospital B:** Comparison of Catalyst Progesterone and the reference method (LC-MS)




**c. Hospital A:** Comparison of CLIA1 and the reference method (LC-MS)




**d. Hospital B:** Comparison of CLIA2000 and the reference method (LC-MS)

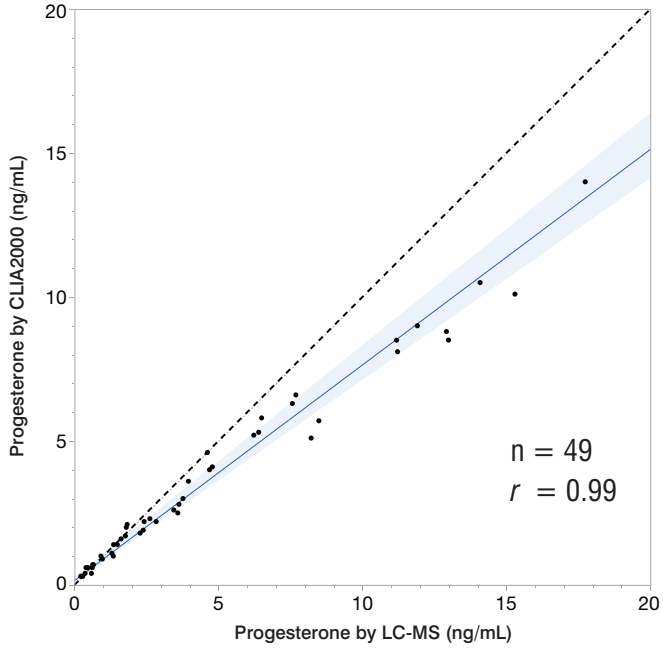



**Figure 1.** Passing and Bablok plots for the agreement between progesterone evaluated by two methods in samples collected during the periovulatory period of the bitch. The dashed line represents the identity line (x = y), the solid blue line represents the regression line, and the blue area represents the confidence interval for the regression line.

### Precision study

The results of the precision analysis are shown in table 2. The new method of Catalyst® Progesterone had a total CV of <10% at both concentration levels. For comparison, the product insert¶ for the CLIA method showed a total CV at 21.7%, 12.5%, 10.5%, and 10.6% at a mean concentration of 0.46 ng/mL, 1.38 ng/mL, 3.92 ng/mL, and 11.6 ng/mL, respectively.

| Fluid | Catalyst Dx | Mean progesterone (ng/mL) | Observations | Standard deviation (ng/mL) |
|---|---|---|---|---|
| A | X | 1.13 | 80 | 0.13 |
| | Y | 1.10 | 80 | 0.11 |
| | Z | 1.13 | 80 | 0.08 |
| B | X | 5.79 | 80 | 0.41 |
| | Y | 5.41 | 80 | 0.58 |
| | Z | 6.02 | 80 | 0.57 |

Average total CV for Fluid A was 9.6%. The average total CV for Fluid B was 9.1%.

**Table 2.** Results from the Catalyst Progesterone precision study

## 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; multiplying the CLIA2000 result by 1.25 will provide an estimate of the result from the reference method, e.g., 2.0 ng/mL x 1.25 = 2.5 ng/mL. 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.

*125-I radioimmunoassay (Coat-A-Count® radioimmunoassay; Siemens Health Care Diagnostics Inc., Los Angeles, California, USA)

†Siemens Medical Solutions Diagnostics, Los Angeles, California, USA.

‡CLIA is used for canine samples at IDEXX Reference Laboratories.

§The LC separation was achieved using Acquity UPLC BEH300 C4 1.7 µm, 2.1 x 100 mm column with gradient Mobile Phase A (0.1% formic acid in water) and Mobile Phase B (0.1% formic acid in methanol). The API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) was operated in multiple reaction monitoring (MRM) mode with positive electrospray interface. The MRM transitions for progesterone were observed at m/z 315.2 → 109.1 (quantifier) and m/z 315.2 → 97.2 (qualifier).

¶IMMULITE 2000 Progesterone (PIL2KPW-21, 2013-12-17).



## References

1. Concannon P, Hansel W, McEntee K. Changes in LH, progesterone and sexual behavior associated with preovulatory luteinization in the bitch. *Biol Reprod*. 1977;17(4):604–613.
2. De Cramer KGM, Nöthling JO. The precision of peri-oestrous predictors of the date of onset of parturition in the bitch. *Theriogenology*. 2017;96:153–157.
3. Meyers-Wallen VN. Unusual and abnormal canine estrous cycles. *Theriogenology*. 2007;68(9):1205–1210.
4. Nöthling JO, De Cramer KGM. Comparing the values of progesterone in the blood of bitches as measured with a chemiluminescence immunoassay and a radioimmunoassay. *Reprod Dom Anim*. 2018;53(5):1136–1141.
5. Stanczyk FZ, Lee JS, Santen RJ. Standardization of steroid hormone assays: why, how, and when? *Cancer Epidemiol Biomarkers Prev*. 2007;16(9):1713–1719.
6. Kutzler MA, Mohammed HO, Lamb SV, Meyers-Wallen VN. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. *Theriogenology*. 2003;60(6):1187–1196.
7. Schmicke M, Urhausen C, Wolf K, Schmidt S, Günzel-Apel AR. Evaluation of the blood progesterone concentration in the bitch measured by chemiluminescence immunoassay at the day of ovulation. *Tierärztl Prax Ausg K Kleintiere Heimtiere*. 2016;44(5):317–322.
8. Zady MF. Z-12: Correlation and simple least squares regression. Westgard QC website. www.westgard.com/lesson42.htm. Accessed January 15, 2019.