Validation of an in-clinic immunoassay for measurement of canine progesterone

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IDEXX recently introduced an immunoassay to measure canine progesterone on the Catalyst® suite of chemistry analyzers. Objectives were to: (1) validate LC MS for measurement of canine plasma progesterone concentrations; (2) evaluate accuracy of the in-clinic Catalyst® Progesterone test compared to results obtained by LC MS; and (3) evaluate repeatability of the Catalyst® Progesterone test across multiple analyzers. The LC MS progesterone assay was validated in accordance with FDA guidelines for linearity, carryover, sensitivity, selectivity, accuracy, and precision. Chromatographic separation was performed on a Shimadzu Nexera Ultra High-Performance Liquid Chromatograph using Acquity BEH 300 C4 1.7 µm, 2.1 x 100 mm analytical column with analyte detection by a SCIEX API 4000 mass spectrometer with multiple reaction monitoring with progesterone-[$^{13}C_3$] as an internal standard. The dynamic range of the LC-MS assay is 0.2 - 40 ng/ml and accuracy is traceable to the NIST standard (standard reference material 971). The assay exceeded FDA criteria of intra/inter day accuracy/precision, demonstrating imprecision of less than 7% CV and accuracy within 95 - 108% of the expected value over the analyte range. Lower limit of quantification and linearity studies support a dynamic range of 0.2 - 40 ng/ml (S/N e 10; $R^2 = 0.99$). The lower limit of quantitation was 0.2 ng/ml and had a linearity with an R^2 value of 0.99 or better. There was no evidence of carryover or interferences of progesterone/progesterone-[2,3,4-$^{13}C_3$] IS m/z transitions observed. To evaluate the Catalyst® Progesterone test compared to LC MS, 107 blood samples were collected from peri ovulatory bitches presented to 3 veterinary hospitals for breeding management. Within 30 minutes after collection, lithium heparin plasma was separated from erythrocytes. Plasma samples were analyzed both on the Catalyst® Progesterone assay (within 48 hours after collection) and LC MS at IDEXX R&D (within 1 week after collection). Precision was assessed by repeated analysis (80 total replicates each) of 3 control fluids in the range of clinical interest. Statistical analyses were performed using JMP® 14.0.0. Results are reported with 95% confidence limits in parentheses. Mean progesterone obtained by LC MS was 5.6 ng/ml (range: 0.2 to 19.4 ng/ml). Catalyst® Progesterone assay mean progesterone was 5.9 ng/ml (range: 0.2 to 19.3 ng/ml). Passing Bablok regression analysis had an intercept -0.07 ng/ml (-0.2 - 0.02), slope 1.07 (1.02 - 1.12), and Tau 0.89. Pearson's correlation coefficient was 0.98. The mean difference (Bland Altman plot) was 0.27 ng/ml (0.06 - 0.47; SEM 0.1 ng/ml). Increased variation at the higher end of the dynamic range (> 16 ng/ml), where unlikely to impact clinical decisions. In the precision study, mean concentrations of 0.43 ng/ml (± SD 0.07), 3.5 ng/mL (± SD 0.28) and 16.0 ng/ml (± SD 1.35). Catalyst® Progesterone test can be used for in clinic measurement of canine plasma progesterone concentrations, due to its good correlation to the reference method, LC MS, and good precision in the range of clinical interest.

Keywords: Progesterone, canine, LC MS, immunoassay