Evaluation of a new automated method for rapid measurement of blood progesterone concentration in bitches.

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Introduction: Timing the day of ovulation as accurately as possible is considered by most authors as one of the most important factor in order to determine when to mate or inseminate bitches. Furthermore, measurements of blood progesterone concentrations are commonly used by veterinarians to follow the luteal function during pregnancy (1) or to determine the progesterone drop just before parturition (2). Nowadays, due to multiple interests and uses of progesterone assays, veterinary practitioners are seeking to have the possibility to obtain a progesterone result quickly during or just after a consultation. However, there is controversial data about the accuracy and repeatability of commercial in-house tests (3,4). That is why a comparative study with reference tests such as radioimmuno-assay (RIA) and chemiluminescence-immuno-assay (CLIA) are one of the best ways to confirm the quality of a test and recommend its daily use in veterinary practice.

FUJI DRI-CHEM IMMUNO AU10V (called AU10V hereafter), a new point-of-care immunodiagnostic analyzer has been launched in recent years. This analyzer uses a new technology called Surface Plasmon enhanced Fluorescence (SPF). Through SPF, washing of surplus fluorescent beads and high power irradiation are no longer necessary, enabling the realization of a compact immunodiagnostic analyzer with short measurement time, ideal for point-of-care. But the performance of this new automated method for progesterone measurement has not been evaluated yet.

Objectives: The objectives of this study were to measure progesterone in serum and plasma samples taken in bitches at different stages of the cycle with a new automated method AU10V and to challenge it with two reference methods considered as "gold-standards" (RIA - IM1188 (Beckman coulter) and Immulite 2000® (Siemens, Germany)).

Materials and methods: The study was approved by the ethical committee for clinical studies of the Alfort Veterinary School, France. 110 blood samples collected in reproductive bitches from 36 different breeds coming in consultation for progesterone samplings at different phases of their cycles were included. A written document explaining the aims of the study was given to the owners to get their consent to participate to the study. Blood was collected (cephalic or external saphenous vein) to fill 2 tubes : one dry test tube without gelosis and anticoagulant, and one heparinized test tube (BD Plymouth – United Kingdom). To prepare the serum, the tube without anticoagulant was left to stand at room temperature for 30 minutes prior to centrifugation. The heparinized tube was gently mixed by an oscillator before centrifugation. Centrifugation was then performed at 2500 g, 25°C for 15 minutes. In total, 8 aliquots of the separated supernatants were prepared and identified. The presence of hemolysis, bilirubinemia or lipidemia was registered, if any. One plasma aliquot and one serum aliquot were immediately assayed for progesterone with AU10V. 3 serum aliquots and 3 plasma aliquots were immediately frozen at -20°C for further assays with AU10V, RIA and Immulite®. The 3 assays were performed according to manufacturers' specifications.

Comparison between the methods and statistical analysis were performed using Microsoft Office Excel 2007.

Results: Progesterone values obtained with AU10V showed a good linearity over a wide range with Immulite (0.25 – 39.31ng/ml) and RIA (0.04 – 59.67 ng/ml) in serum samples. AU10V values obtained from fresh and frozen serum/plasma samples showed very good correlation with Immulite®, as well as with RIA (Table 1). AU10V's intra-assay variance was 3.3% at 0.94ng/ml and 3.0% at 28.07ng/ml.

Table 1: Correlation of AU10V with Immulite and RIA.

		Immulite				RIA			
AU10V sample		n	slope	y-intercept	R	n	slope	y-intercept	R
fresh	serum	102	0.76	0.59	0.97	108	0.46	0.52	0.94
	plasma	103	0.77	0.00	0.97	105	0.44	0.01	0.93
frozen	serum	101	0.81	0.75	0.97	107	0.49	0.63	0.95
	plasma	103	0.76	-0.07	0.94	105	0.43	-0.02	0.90

Discussion: The AU10V is highly correlated with both Immulite and RIA, showing coefficients of correlation (R) greater than 0.90 in serum and plasma samples. These results indicate that this new progesterone assay can accurately measure progesterone in dogs.

There was no statistically significant difference in the correlations when either fresh or frozen samples were analyzed with AU10V. This observation is in accordance with Tahir et al. (5) who found no differences in progesterone values in samples handled in different conditions.

Conclusion: AU10V_progesterone has a good correlation with the two "gold-standard" methods which are RIA and Immulite®. It appears as an accurate and reliable in-house progesterone test.

References:

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