

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 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 to detect if a bitch is at term or to decide for a Cesarean section (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.

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 the new automated method AU10V and to challenge it with two reference methods considered as "gold-standards": RIA - IM1188 (Beckman coulter) and CLEIA (Immulite 2000® -Siemens, Germany).

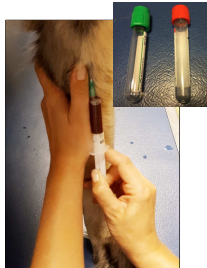
**DRI-CHEM
IMMUNO AU10V**



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.

With AU10V: the progesterone results are obtained in approximately 10 minutes

The study was approved by the ethical committee for clinical studies of the Alfort National Veterinary School, France.



Materials and methods: 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. Blood was collected (cephalic or external saphenous vein) to fill 2 tubes : one dry test tube without gelosin 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. 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.

On a second step, **expected values for different progesterone values (basal, ovulation, pregnancy, pre-parturition) were established in serum samples and correlated with CLEIA (Immulite 2000®)**

Results: Progesterone values obtained with AU10V showed a good correlation over a wide range with Immulite (0.25 – 39.31ng/ml) and RIA (0.04 – 59.67 ng/ml) in serum and plasma samples. AU10V's intra-assay variance was 3.3% at 0.94ng/ml and 3.0% at 28.07ng/ml. (Table 1, Fig 1-2).

AU10V sample	N	Immulite		R	N	RIA		R
		slope	y-intercept			slope	y-intercept	
fresh serum	102	0.76	0.59	0.97	108	0.46	0.52	0.94
fresh 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
frozen plasma	103	0.76	-0.07	0.94	105	0.43	-0.02	0.90

Table 1: Correlation of AU10V with Immulite and RIA.

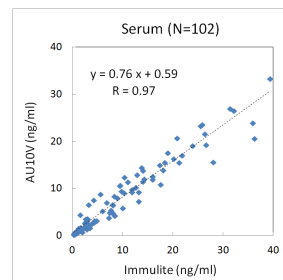


Fig. 1: Serum correlation between AU10V and CLEIA (Immulite®)

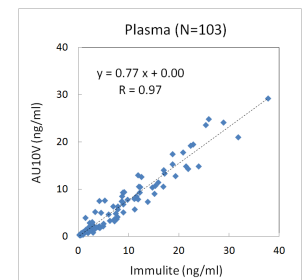


Fig. 2: Plasma correlation between AU10V and CLEIA (Immulite®)

Discussion: 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.

CONCLUSION : AU10V-progesterone has a good correlation with the two "gold-standard" methods which are RIA and Immulite®. **It is an accurate and reliable in-house progesterone test.**

References:

- Günzel-Apel A, Urhausen C, Wolf K et al. Serum progesterone in pregnant bitches supplemented with progesterone because of expected or suspected luteal insufficiency. *Reprod Domest Anim.* 2012 Dec;47 Suppl 6:55-60.
- Kowalewski MP. Luteal regression vs. prepartum luteolysis: regulatory mechanisms governing canine corpus luteum function. *Reprod Biol.* 2014 Apr;14(2):89-102.

Reference ranges (on going study)

Reference ranges are currently being evaluated. Interim results are shown below.

AU10V	N	Reference range
Basal	107	0.94 ± 0.63 (0.31 - 1.57)
Ovulation	65	6.32 ± 2.63 (3.69 - 8.95)
Pregnancy	122	20.40 ± 8.36 (12.04 - 28.76)
Parturition	47	2.39 ± 0.90 (1.49 - 3.29)

Immulite	N	Reference range
Basal	91	0.70 ± 0.36 (0.34 - 1.06)
Ovulation	65	6.95 ± 2.30 (4.65 - 9.25)
Pregnancy	117	20.59 ± 8.39 (12.20 - 28.98)
Parturition	47	2.47 ± 1.11 (1.36 - 3.58)

-Ranges are calculated as average ±SD. Interim results may slightly differ in the final results.

