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VALIDATION OF A NEW POINT -OQ-CANE IMMUNOASSAY FOR SERUM CORTISOL MEASURMENT IN CANINE SERUM SAMPLES

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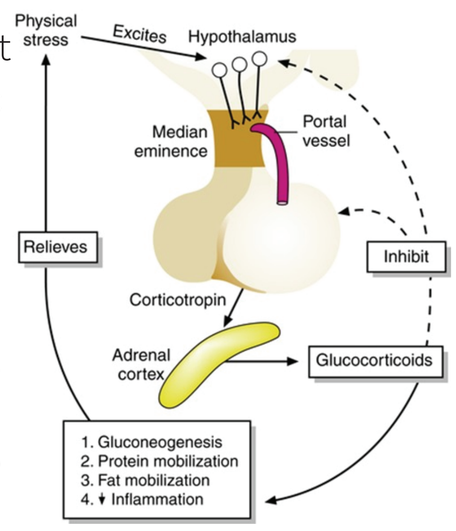
INTRODUCTION

- Cortisol is analysed for the diagnosis of hyper and hypoadrenocorticism, with Hyperadrenocorticism as the most common adrenal disorder in dogs;
(Gilor & Graves, 2011)
- The use of in-house analysers represents a point of success for clinics or veterinary hospitals;
(Rishniw, Pion, & Maher, 2012; Services, 2015)
- It is crucial that the equipments are in accordance with the established prerequisites in terms of precision, accuracy, detection limit and quantification



OBJECTIVE

- Validate the performance of a new in-house immunoassay based on Surface Plasmon enhanced Fluorescence method for canine cortisol measurement in serum.

COMPARED

Another validated method
Immulite, Siemens

FUJI DRI-CHEM Immuno AU10V

MATERIALS AND METHODS

- Serum Samples from 59 Clinical cases
- Dogs of diferent age, breed and gender
- With enough volume

Analytic Validation
- Intra -Assay
- Inter -Assay
- Limit of Detection
- Linearity under **Dilution** Study
- Overlap Performance

RESULTS AND DISCUSSION

Table 1 Cortisol concentration obtained in Dri-Chem Immuno AU10V

		Mean (µg/ml)	SD	CV(%)
Intra-assay	High	17.3	0.3	1.7
	Medium	8.9	0.2	1.9
	Low	1.3	0	0
Inter-assay	High	27.8	0.5	1.7
	Medium	13.4	0.25	1.9
	Low	3.6	0.05	1.4

Intra and Inter-assay CV was below 2%

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Lower than 15%

↓

High Precision

Detection limit was 0

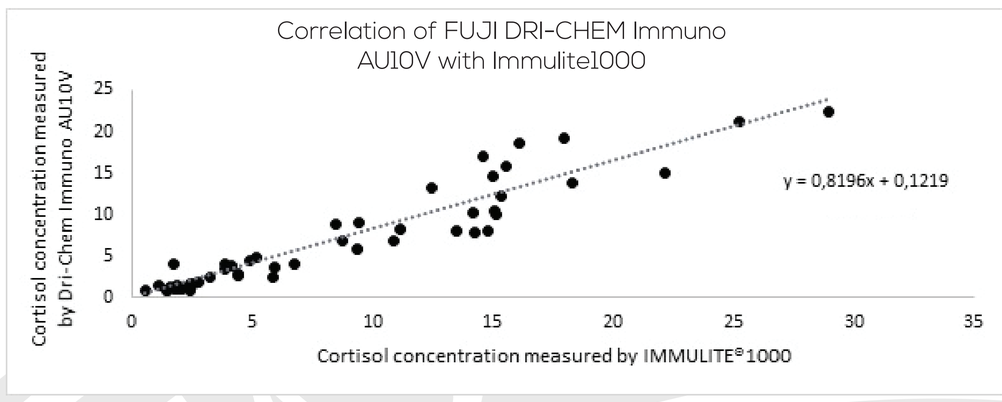

Figure 1 . Regression equation of all samples measured with the two methods (n=59)

- Spearmen correlation revealed a strong positive correlation between the two methods.

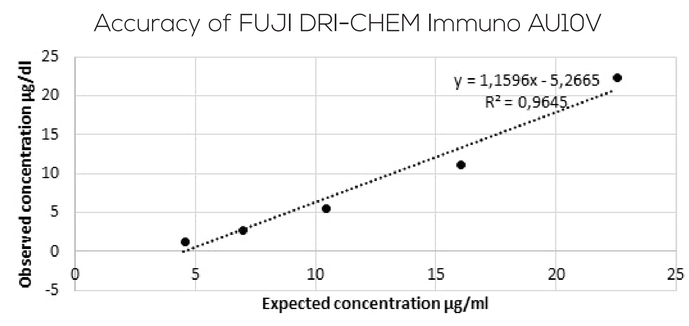

Figure 2. Representative graph of linearity under dilution of a canine serum sample

Linearity under dilution confirmed the accuracy of the method

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Correlation coefficients close to 1

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Recovery range between 91,3 an 117,2%

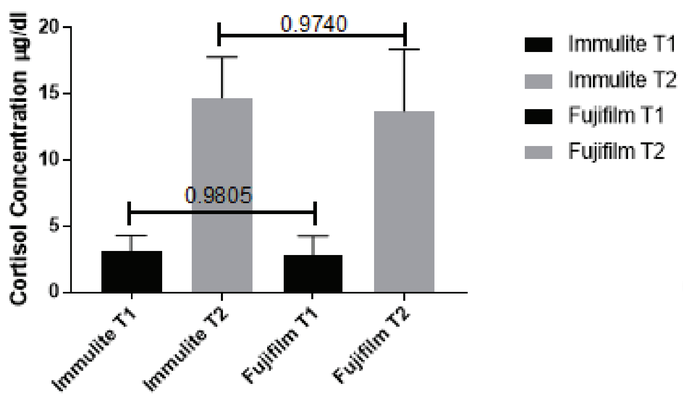

Figure 3. Median and inter-quarterly range concentration of cortisol before (T1) and after (T2) stimulation with ACTH.

Samples from pre and post stimulation with ACTH test from 11 dogs were used to evaluateif the results interpretation depend on the method used.
The interpretation of the results differed in one case, where hyperadrenocorticism was confirmed with Immulite (post-ACTH cortisol: 25.2 µg/dL), while with AU10V's result was considered doubtful (post-ACTH cortisol: 21.5 µg/dL) when considering laboratory cutoff of 22.0 µg/dL for hyperadrenocosticism.

CONCLUSION

Overall, the validated method meant to be quick (approximately 10 min), precise and accurate when measuring cortisol in canine serum samples. It is important to note that there are slight method discrepancies in measuring cortisol thus diagnosis for hyperadrenocorticism should not be based solely on the cortisol results but as well as with the symptoms and other tests such as imaging.

References: Gilor, C., & Graves, T. K. (2011). Interpretation of laboratory tests for canine cushing's syndrome. Topics in Companion Animal Medicine, 26(2), 98–108.
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Services, M. (2015). UK Standards for Microbiology Investigations. Bacteriology, B 55(5.2), 1–21.