

## Evaluation Report

### **Eurolyser canine Progesterone test kit (VT0230) on solo and CUBE-VET analysers**

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Report created on 3<sup>rd</sup> July 2018  
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#### **Specimens**

The specimens for sample correlation were taken from a reference lab/veterinary university from dogs and were fresh serum or li-hep plasma. Samples were aliquoted and tested with the reference method (SIEMENS Progesterone test kit on an Immulite 2000i).

For all other tests the dedicated progesterone controls have been used.

Sample volume: 40 µl

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#### **Equipment**

- Eurolyser CUBE-VET analyser: Ca10615, Ca10910, Ca10911, Cb12910
  - Eurolyser solo: Ae5050, Ae5052, Ae5053, Bc14783
  
  - Test kits: VT0230: LOT 20171016\_1
  
  - Reagent:
    - R1\_90: 600 µl
    - R2 Typ A: 300 µl
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## 1. Introduction and Scope

Progesterone (P4) is an androgen produced by the corpus luteum of bitches. Normally present in low doses in non-pregnant bitches, in dogs, levels of progesterone start to climb around the time of the LH peak. Thus, the production of progesterone by the follicle begins prior to ovulation at the end of proestrus and reaches increased levels in peripheral blood plasma at the time of ovulation. In females, the measurement of progesterone is useful in evaluating the status of ovarian functions, monitoring of progesterone therapy and early stage pregnancy evaluations.

- 1.1 Method comparison  
Testing the correlation between the progesterone measurement results on Eurolyser analysers from serum and li-hep plasma samples and the results of the Siemens progesterone test measured on an Immulite 2000i.
- 1.2 Reproducibility  
Characterisation of the reproducibility of the Eurolyser progesterone test for 1 control and pooled canine serum samples.
- 1.3 Stability testing
- 1.4 Limit of Quantification
- 1.5 Interferences

### **Principle:**

Homogeneous immunoturbidimetric test.

## 2. Comparison Study

Eurolyser vs. reference method (Siemens Immulite 2000i)

The comparison study is based on the correlation between the results of the Eurolyser progesterone test and the Siemens progesterone test measured on an Immulite 2000i.

50 canine samples have been analysed on solo and CUBE-VET analysers. 2 replicates of each sample have been measured.

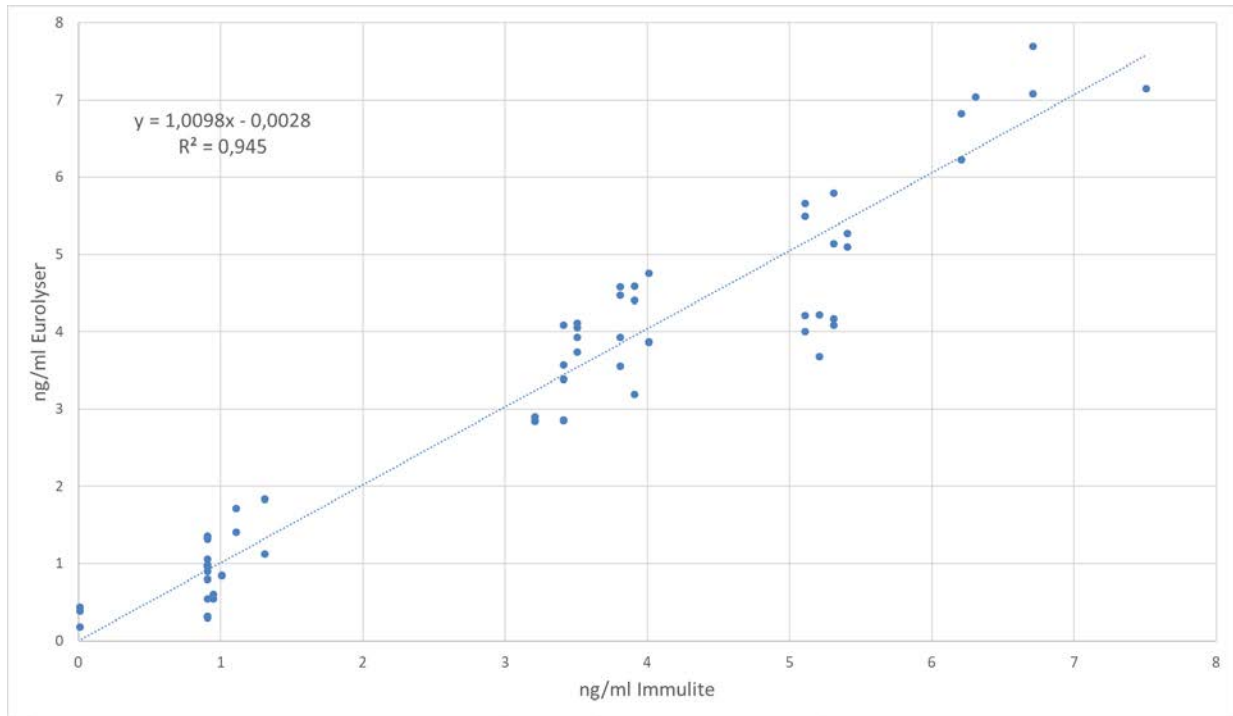
The acceptance criterion for this comparison study is a coefficient of determination  $R^2 > 0.90$  obtained from linear regression between the Eurolyser progesterone and the Immulite progesterone. Further, slope has to be within 0.8 and 1.2, and an intercept between -1 and 1 is acceptable.

Correlation:

Sample N°	Immulate ng/ml	Eurolyser ng/ml #1	Eurolyser ng/ml #2
1	10.5	7.5	>8.0
2	11.1	>8.0	>8.0
3	11.9	>8.0	>8.0
4	12.0	>8.0	>8.0
5	12.2	>8.0	>8.0
6	15.6	>8.0	>8.0
7	15.9	>8.0	>8.0
8	17.8	>8.0	>8.0
9	19.2	>8.0	>8.0
10	30.1	>8.0	>8.0
11	30.9	>8.0	>8.0
12	6.3	>8.0	7.1
13	6.7	7.1	7.7
14	3.8	4.6	3.9
15	7.5	>8.0	7.2
16	9.9	>8.0	>8.0
17	5.3	4.1	5.8
18	3.5	4.1	3.8
19	5.1	5.7	5.5
20	5.2	3.7	4.2
21	3.8	4.5	3.6
22	3.9	3.2	*
23	3.9	4.6	4.4
24	4.0	4.8	3.9
25	3.4	3.4	2.9
26	8.8	7.5	>8.0
27	8.8	7.9	>8.0
28	6.7	>8.0	>8.0
29	3.2	2.9	2.9
30	3.5	4.1	4.0
31	0.9	0.8	0.6
32	0.9	1.4	1.1
33	1.0	0.9	0.9
34	1.1	1.4	1.7
35	1.3	1.9	1.2
36	6.2	6.9	6.2
37	5.3	4.2	5.2
38	5.1	4.0	4.2
39	3.4	4.1	3.6
40	0.9	1.3	0.9
41	0.9	1.0	1.0
42	9.5	>8.0	>8.0
43	0.9	0.3	0.3
44	5.4	5.1	5.3
45	<0.2	0.0	0.0
46	<0.2	0.5	0.0
47	<0.2	0.2	0.0

48	0.9	0.6	0.6
49	<0.2	0.0	0.4
50	<0.2	0.0	0.0

\* not enough sample material for replication



Sample correlation:

The result for the correlation between the Immulite Progesterone test and Eurolyser Progesterone test is the linear regression function:

$$y \text{ (Eurolyser)} = 1.0098x \text{ (Immulite)} - 0.0028 \text{ and a } R^2 = \mathbf{0.945}$$

Based on the correlation the linearity and upper limit of measurement range is defined as 8 ng/ml.

Based on the excellent correlation data the decision limits of the reference method will be used:

Dog: 4 – 8 ng/ml (Ovulation)

Nonetheless, it is recommended that each laboratory establishes its own decision limits.

### 3. Reproducibility (within-run precision)

One control and a pool of canine serum samples have been tested 20 times each and the CV values were calculated (tested with solo and CUBE-VET analysers):

Sample #	Control ng/ml	Sample pool ng/ml
1	3.8	4.5
2	3.4	4.0
3	3.7	4.9
4	2.9	4.3
5	4.1	4.4
6	3.3	4.3
7	3.4	4.7
8	3.6	4.5
9	3.6	4.0
10	3.2	4.6
11	3.4	5.2
12	3.2	4.8
13	3.4	4.3
14	3.6	5.3
15	3.6	4.2
16	4.2	4.1
17	3.1	4.3
18	3.1	5.7
19	3.9	4.5
20	3.2	4.7
<b>Average</b>	<b>3.5</b>	<b>4.6</b>
<b>Stdev</b>	<b>0.33</b>	<b>0.44</b>
<b>CV</b>	<b>9.57%</b>	<b>9.66%</b>

The CV values are 9.57% for the control and 9.66% for the pooled canine serum samples.

### 4. Stability Test

An accelerated stability test was performed. Reagent stability was recorded over 7 weeks, during this time cuvettes were stored at room temperature.

Cuvettes prepared on: day 0  
 Measurement date: day 1 – day 50

2 control levels have been used.

The recovery of control low and high has to be within 25% of the target value.

Recovery:

day	Control low		Control high	
	ng/ml	% recovery	ng/ml	% recovery
1	2.9	92.15%	7.1	98.27%
8	3.0	95.45%	7.0	96.72%
15	2.8	89.76%	6.9	96.13%
22	2.9	93.19%	6.9	95.26%
29	3.4	107.67%	7.5	104.14%
50	3.9	121.77%	7.9	109.49%

The reagent shows good stability in case of storage at room temperature over 7 weeks, therefore, real time stability can be assumed to be good as well and a 9 months expiry date are implemented.

## 5. Limit of Quantitation (LOQ)

LOQ is determined as the lowest sample run that displays a CV value < 20%.

	Control dilution 1	Control dilution 2
average	1.63	0.74
stdev	0.17	0.21
CV (%)	10.11%	28.55%

Based on these results the LOQ is set to 1.2 ng/ml.

## 6. Interferences:

The test system has been analysed for various interferences. Criterion was the recovery within 15% of initial values.

Haemoglobin	525 mg/dl
Human albumin	12 g/dl
Bilirubin (conjugated)	72 mg/dl
Bilirubin (unconjugated)	72 mg/dl
Cholesterol	620 mg/dl
Rheumatoid factor	1080 IU/ml
Triglycerides	835 mg/dl
Uric acid	30 mg/dl

## 7. Summary

The progesterone test kit designed for solo and CUBE-VET analysers has a good correlation to the progesterone test from Siemens measured on an Immulite 2000i.

The reproducibility and stability of the test are very good.