

Evaluation Report

Eurolyser equine Progesterone test kit (VT0250) on solo and CUBE-VET analysers

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Specimens

The specimens for sample correlation were taken from a reference lab/veterinary university from horses 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

Equipment

- Eurolyser CUBE-VET analyser: Ca10615, Ca10910, Ca10911, Cb12910
 - Eurolyser solo: Ae5050, Ae5052, Ae5053, Bc14783

 - Test kits: VT0250: LOT 20171016_2

 - Reagent:
 - R1_90: 600 µl
 - R2 Typ A: 300 µl
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1. Introduction and Scope

During early pregnancy, progesterone is produced in the equine ovary by the corpus luteum (CL). Its concentration remains elevated and peaks between 60 and 120 days of gestation. Circulating progesterone is used diagnostically to evaluate luteal function during early pregnancy. When the circulating progesterone concentration reaches a distinct level, this can be considered adequate to maintain early stage pregnancy. There are a number of reasons for monitoring during pregnancy, such as uterine infections, history of pregnancy loss and luteal insufficiency.

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 a pooled equine serum sample.

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

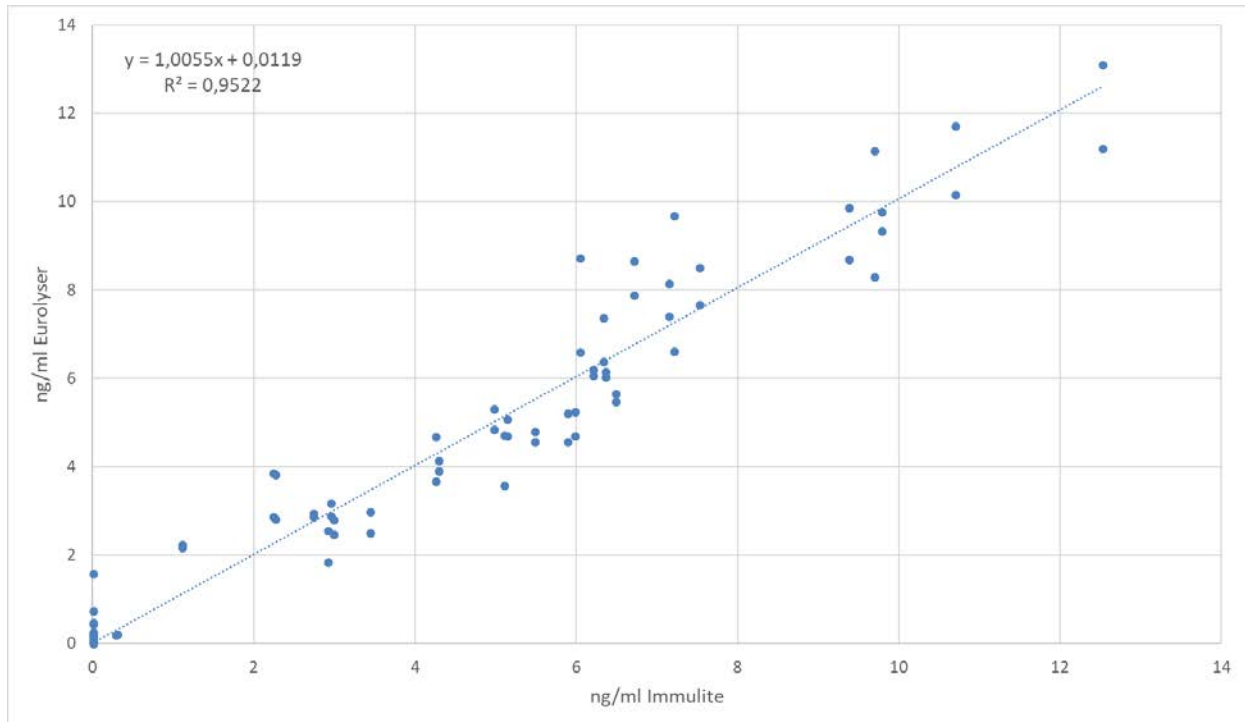
The acceptance criterion for this comparison study (for each species) is a coefficient of determination **$R^2 > 0.90$** obtained from linear regression between the Eurolyser progesterone and the Immulite progesterone.

Further, slope has to be within 0.8 and 1.2, and an intercept between -1 and 1 is acceptable.

Correlation equine:

Sample N°	Immulite ng/ml	Eurolyser ng/ml #1	Eurolyser ng/ml #2
1	<0.2	<1.4	<1.4
2	<0.2	<1.4	<1.4
3	<0.2	<1.4	<1.4
4	<0.2	<1.4	<1.4
5	<0.2	<1.4	<1.4
6	<0.2	<1.4	<1.4
7	5.1	5.1	4.7
8	2.9	2.6	1.9
9	<0.2	<1.4	<1.4
10	<0.2	<1.4	<1.4
11	<0.2	<1.4	<1.4
12	<0.2	<1.4	<1.4
13	<0.2	<1.4	<1.4
14	0.3	<1.4	<1.4
15	2.7	3.0	2.9
16	<0.2	<1.4	<1.4
17	<0.2	<1.4	<1.4
18	0.3	<1.4	<1.4
19	9.4	8.7	9.9
20	1.1	2.2	2.3
21	<0.2	1.6	<1.4
22	9.7	8.3	11.2
23	<0.2	<1.4	<1.4
24	5.5	4.6	4.8
25	<0.2	<1.4	<1.4
26	6.2	6.1	6.2
27	0.3	<1.4	<1.4
28	<0.2	<1.4	<1.4
29	2.9	3.2	2.9
30	3.0	2.5	2.8
31	5.0	4.9	5.3
32	4.3	3.9	4.2
33	4.2	3.7	4.7
34	5.1	3.6	4.7
35	6.0	6.6	8.7
36	6.3	6.4	7.4
37	7.1	7.4	8.2
38	7.5	8.5	7.7
39	3.4	2.5	3.0
40	6.0	4.7	5.3
41	10.7	10.2	11.7
42	2.3	3.8	2.8
43	2.2	3.9	2.9
44	12.5	11.2	13.1
45	5.9	5.2	4.6
46	6.5	5.5	5.7
47	6.7	8.7	7.9

48	9.8	9.4	9.8
49	6.4	6.1	6.2
50	7.2	9.7	6.6



Sample correlation:

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

$$y \text{ (Eurolyser)} = 1.0055x \text{ (Immulite)} + 0.0119 \text{ and a } R^2 = 0.9522$$

Based on the correlation the linearity and upper limit of measurement range is defined as 13 ng/ml.

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

Horse: > 2 ng/ml (gestation)

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

3. Reproducibility (within-run precision)

One control and a pool of equine 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	6.0	4.1
2	5.3	4.2
3	5.7	3.3
4	4.6	4.0
5	6.5	4.2
6	5.2	3.9
7	5.3	4.0
8	5.6	3.4
9	5.6	4.2
10	5.1	4.7
11	5.2	4.1
12	5.0	4.2
13	5.3	3.7
14	5.7	4.1
15	5.6	4.1
16	6.5	4.3
17	4.9	3.9
18	4.9	3.2
19	6.1	4.2
20	4.9	4.0
Average	5.4	4.0
Stdev	0.53	0.35
CV	9.69%	8.90%

The CV values are 9.69% for the control and 8.90% for the pooled equine 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	4.6	91.94%	11.9	106.48%
8	4.8	95.33%	12.0	107.47%
15	4.5	89.62%	9.9	88.84%
22	4.7	93.02%	11.0	98.20%
29	5.4	107.76%	11.1	99.30%
50	6.1	122.33%	11.2	99.71%

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	1.82	0.96
stdev	0.26	0.25
CV (%)	14.51%	25.68%

Based on these results the LOQ is set to 1.4 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 equine 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.