Evaluation Report

Eurolyser Cortisol test kit (VT0290, VT0291)

for solo and CUBE-VET analysers

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Specimens

The specimens for sample correlation were taken from a reference lab/veterinary university from dogs and were fresh serum. Samples were aliquoted and tested with the reference method (Roche Elecsys Cortisol II Kit on a Cobas 8000 e602).
For all other tests the dedicated cortisol controls have been used.

Sample volume: 40 µl

Equipment

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

- Test kits: VT0290, VT0291: LOT 20181030

- Reagent:
  - R1_90: 800 µl (400 µl R1 + 400 µl dilution buffer)
  - R2 Typ B: 267 µl

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1. Introduction and Scope

Cortisol is a glucocorticoid hormone that affects the metabolism of carbohydrates, proteins, and fats. Chronic, excessive glucocorticoid release leads to hyperadrenocorticism (HAC).

Pituitary-dependent hyperadrenocorticism (PDH), more commonly known as Cushing‘s disease, is caused by a pituitary tumour (mostly adenomas) in the brain. The tumour triggers excessive levels of adrenocorticotropic hormone (ACTH), which stimulates the adrenal glands near the kidneys to produce cortisol. A small percentage of dogs with HAC have a tumour of one of the adrenal glands. This form of HAC is called adrenal-dependent HAC and results from a direct increase in cortisol production by the adrenal gland tumour.

The overproduction of cortisol causes symptoms such as hair loss, pot-bellied appearance, increased appetite, and polydipsia and polyuria. Addison’s disease (hypoadrenocorticism, or underproduction of cortisol) occurs less commonly than the Cushing’s disease in dogs.

Principle:
Homogeneous immunoturbidimetric test.

2. Comparison Study

Eurolyser vs Reference method (Roche Elecsys Cortisol II Kit)

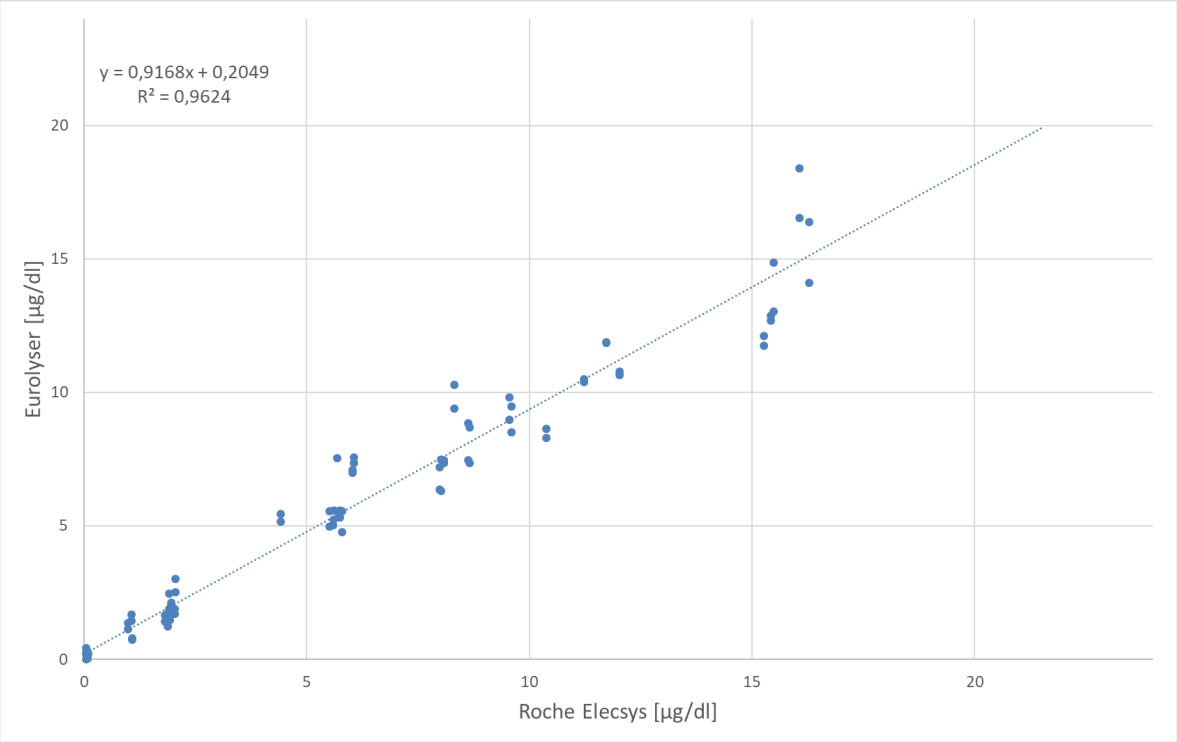
The comparison study is based on the correlation between the results of the Eurolyser cortisol test and the Roche Elecsys Cortisol II Kit measured on a Cobas 8000 e602.

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

Correlation:

Sample N°	Elecsys µg/dl	Eurolyser µg/dl #1	Eurolyser µg/dl #2
1	0.16	< 0.8	< 0.8
2	0.16	< 0.8	< 0.8
3	1.07	< 0.8	< 0.8
4	0.13	< 0.8	< 0.8
5	0.07	< 0.8	< 0.8
6	0.09	< 0.8	< 0.8
7	0.06	< 0.8	< 0.8
8	0.07	< 0.8	< 0.8
9	0.05	< 0.8	< 0.8
10	0.05	< 0.8	< 0.8
11	15.49	13.0	14.9
12	8.07	7.4	7.5
13	8.66	8.7	7.4
14	15.42	12.9	12.7
15	8.02	7.5	6.3
16	9.59	9.5	8.5
17	8.62	7.5	8.9
18	11.22	10.4	10.5
19	10.38	8.3	8.6
20	7.99	6.4	7.2
21	15.26	12.1	11.7
22	16.28	14.1	16.4
23	9.55	9.8	9.0
24	8.31	9.4	10.3
25	4.41	5.4	5.2
26	0.98	1.1	1.4
27	1.06	1.4	1.7
28	2.03	1.9	1.7
29	2.05	2.5	3.0
30	1.93	1.5	1.7
31	6.05	7.6	7.4
32	1.81	1.6	1.4
33	1.88	1.2	1.4
34	5.58	5.0	5.0
35	5.74	5.6	5.3
36	16.06	16.5	18.4
37	6.03	7.0	7.1
38	5.68	5.5	7.5
39	1.95	2.0	2.1
40	1.96	2.0	1.8
41	1.90	2.5	1.8
42	12.02	10.6	10.8
43	5.51	5.5	5.0
44	1.91	1.9	1.6
45	5.61	5.6	5.2
46	5.79	4.8	5.6
47	11.72	11.9	11.9



Sample correlation:
The result for the correlation between the Eurolyser cortisol test and the Roche Elecsys cortisol test is the linear regression function:

y (Eurolyser) = 0.9168x (Roche Elecsys) - 0.2049 and a **R^2 = 0.9624**

3. Reference Ranges

The following reference ranges are suggested.
Nonetheless, it is recommended that each laboratory establishes its own reference ranges.

I. ACTH Stimulation Test

Pre-ACTH	Post-ACTH	Interpretation
< 2 µg/dl	< 2 µg/dl	Consistent with hypoadrenocorticism
	2-6 µg/dl	Inconclusive
2-6 µg/dl	6-18 µg/dl	Normal
	18-24 µg/dl	Equivocal, Cushing's syndrome possible
> 24 µg/dl	>24 µg/dl	Consistent with Cushing's syndrome

II. Low-dose dexamethasone suppression test

4-hour cortisol level	8-hour cortisol level	Interpretation
< 1 µg/dl	< 1 µg/dl	Normal
1 – 1.5 µg/dl	1 – 1.5 µg/dl	inconclusive
> 1.5 µg/dl and > 50% of baseline	> 1.5 µg/dl and > 50% of baseline	Consistent with Cushing's syndrome
< 1.5 µg/dl or < 50% of baseline	> 1.5 µg/dl and > 50% of baseline	Consistent with PDH
> 1.5 µg/dl or > 50% of baseline	> 1.5 µg/dl and < 50% of baseline	Consistent with PDH

III. High-dose dexamethasone suppression test

4-hour cortisol level	8-hour cortisol level	Interpretation
< 1.5 µg/dl or < 50% of baseline	> 1.5 µg/dl and > 50% of baseline	Consistent with PDH
> 1.5 µg/dl and > 50% of baseline	< 1.5 µg/dl or < 50% of baseline	Consistent with PDH
< 1.5 µg/dl or < 50% of baseline	< 1.5 µg/dl or < 50% of baseline	Consistent with PDH
> 1.5 µg/dl and > 50% of baseline	> 1.5 µg/dl and > 50% of baseline	Consistent with PDH or ATH

IV. Post ACTH Stimulation Mitotane (Lysodren®) Dosing and Monitoring

Loading Dose	Interpretation	Maintenance Dose	Interpretation
< 1 µg/dl	Discontinue mitotane	< 1 µg/dl	Discontinue mitotane
1 – 5 µg/dl	Begin maintenance mitotane dosing	1 – 5 µg/dl	Maintain current dosage
> 5 µg/dl	Continue mitotane loading dose	> 5 µg/dl	Increase weekly dose or repeat loading dose.

4. Reproducibility (within-run precision)

Two controls have been tested 20 times each and the CV values were calculated (tested with solo and CUBE-VET analysers):

Sample #	Control 1 µg/dl	Control 2 µg/dl
1	5.5	9.5
2	5.7	9.8
3	6.1	10.4
4	6.3	9.5
5	6.0	11.9
6	5.6	10.3
7	5.2	9.3
8	5.5	9.7
9	5.7	10.7
10	5.5	10.4
11	5.6	9.8
12	5.2	9.0
13	6.2	9.1
14	5.3	9.5
15	5.7	9.4
16	5.8	9.9
17	5.9	9.9
18	4.9	9.9
19	5.3	9.1
20	6.3	9.0
Average	**5.7**	**9.8**
Stdev	**0.39**	**0.71**
CV	**6.93%**	**7.24%**

The CV values for the tested controls are 6.93% and 7.24%.

5. Stability Test

A real time stability test was performed. Reagent stability was recorded over 5 months, during this time cuvettes have been stored at 4 °C.

Cuvettes prepared on: day 0
Measurement date: day 1 – day 141

3 control levels have been used.

The recovery of the control low, mid and high has to be within 15% of the target value.

Recovery:

day	Control low		Control mid		Control high	
	µg/dl	% recovery	µg/dl	% recovery	µg/dl	% recovery
1	1.3	100.0%	3.9	100.0%	7.6	100.0%
8	1.3	97.4%	3.8	97.7%	7.1	93.8%
15	1.2	94.7%	3.5	89.9%	6.8	89.9%
22	1.4	105.3%	3.8	98.6%	8.0	105.2%
29	1.4	110.2%	3.7	95.0%	7.2	94.1%
43	1.3	98.0%	3.8	97.9%	7.1	93.6%
57	1.2	93.5%	3.8	99.0%	7.5	97.9%
71	1.2	88.3%	3.4	89.2%	6.8	89.6%
85	1.4	106.7%	3.7	95.4%	7.3	96.0%
113	1.4	103.8%	3.7	95.9%	7.1	92.8%
141	1.4	104.2%	3.8	98.0%	7.6	99.5%

The reagent shows good stability in case of storage at 4 °C over 5 months, therefore, a 9 months expiry date can be assumed and is implemented.

6. Linearity Study

Do not use diluted samples for measurement.

7. 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	2.61	0.99
stdev	0.33	0.19
CV (%)	12.62%	18.72%

Based on these results the LOQ is set to 0.8 µg/dl.

8. Interferences

The test system has been analyzed for various interferences. Criterion was the recovery within 15% of initial values. The following substances show no interferences up to:

Haemoglobin	131 mg/dl
Albumin	12 g/dl
Bilirubin, conjugated	36 mg/dl
Bilirubin, unconjugated	36 mg/dl
Cholesterol	620 mg/dl
Rheumatoid factor	540 IU/ml
Triglycerides	835 mg/dl
Uric acid	30 mg/dl

9. Summary

The Eurolyser cortisol test kit designed for solo and CUBE-VET analysers has a good correlation to the Roche Elecsys Cortisol II Kit measured on a Cobas 8000 e602.
The reproducibility and stability of the test are very good.