

Evaluation Report

Eurolyser T4 test kit (VT0140, VT0141) for solo and CUBE-VET analysers

Location: Eurolyser Diagnostica GmbH
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Report created on 14th November 2023
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Specimens

The specimens for sample correlation were taken from a reference lab/veterinary university from dogs, cats and horses and were fresh serum and li-hep plasma. Samples were aliquoted and tested with the reference method (Siemens Immulite 2000 xpi)

Sample volume: 20 µl

Equipment

- Eurolyser CUBE-VET analyser: Ce19729, Ce19730, Ce19731, Ch24675, Ch24676
- Eurolyser solo analyser: Ae5050, Ae5052, Ae5053, Bc14783
- Test kits: LOT_T4_20221212

1. Introduction and Scope

A thyroid problem can exist in the form of hypo- and hyperthyroidism. Hypothyroidism happens due to inadequate thyroid hormone production and is found in many species, among them dogs, cats, and horses. Hyperthyroidism, on the other hand, occurs when there is excessive thyroid hormone synthesis or release. Older cats (about 12 years old) are particularly prone to develop this condition. Symptoms of both hypo- and hyperthyroidism are diffuse and nonspecific.

The Eurolyser T4 test measures the total T4 concentration, including bound T4. 8-anilino-1-naphthalenesulfonic acid (ANS) is used to release T4 from its binding proteins. The total thyroxine then competes at the antibody binding site with the T4-analogue which is linked to Glucose-6-phosphat dehydrogenase (G6PDH) enzyme. Once, the G6PDH + T4 analogue is released from the antibody the enzyme starts to convert NAD⁺ to NADH. The change of absorption caused by the production of NADH can be measured at 340nm.

Principle:

Homogeneous enzymatic immunoturbidimetric test.

2. Comparison Study

Eurolyser vs Reference method (Immulin 2000 xpi; Siemens)

The comparison study is based on the correlation between the results of the Eurolyser T4 test and the Immulin 2000 xpi (Siemens).

47 canine and 21 feline samples have been analysed with the Eurolyser T4 assay.

The acceptance criterion for this comparison study is a coefficient of determination $R^2 > 0.90$ obtained from linear regression between the Eurolyser T4 and the Immulin 2000 xpi.

Further, slope has to be within 0.7 and 1.3, and an intercept between -1 and +1 is acceptable.

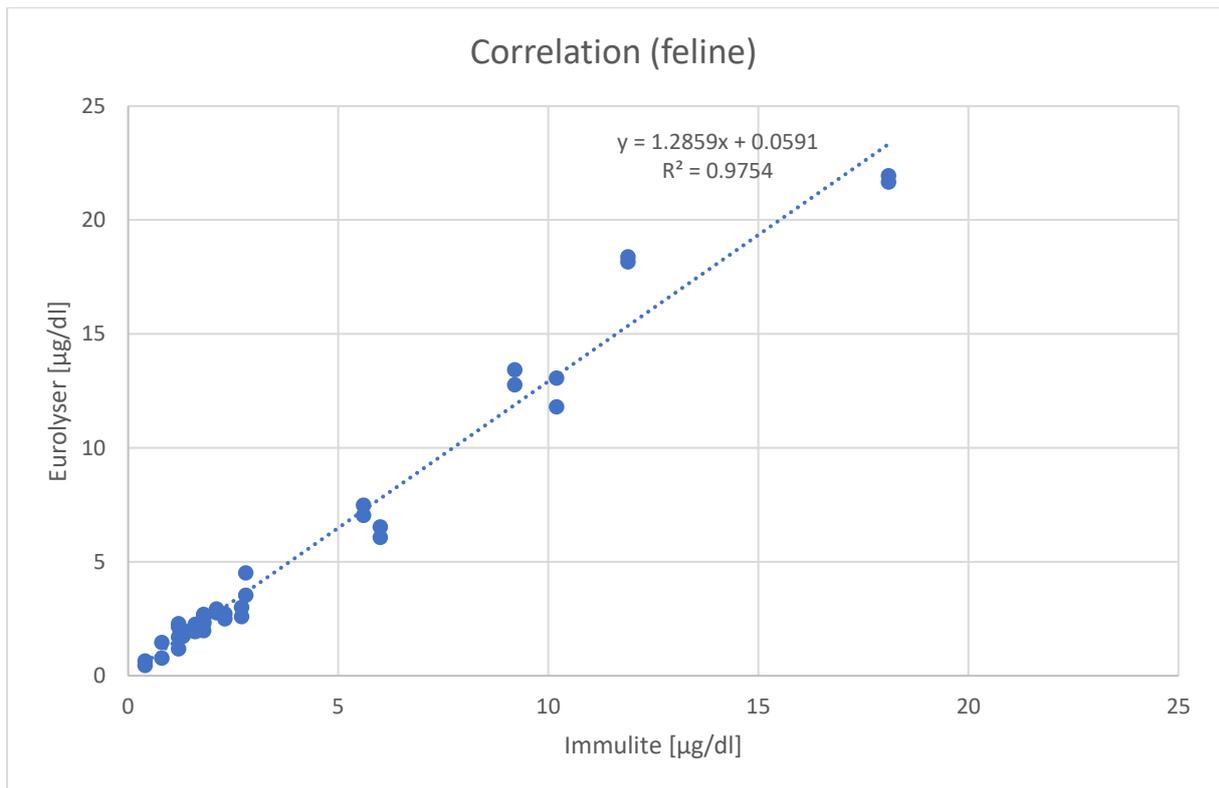
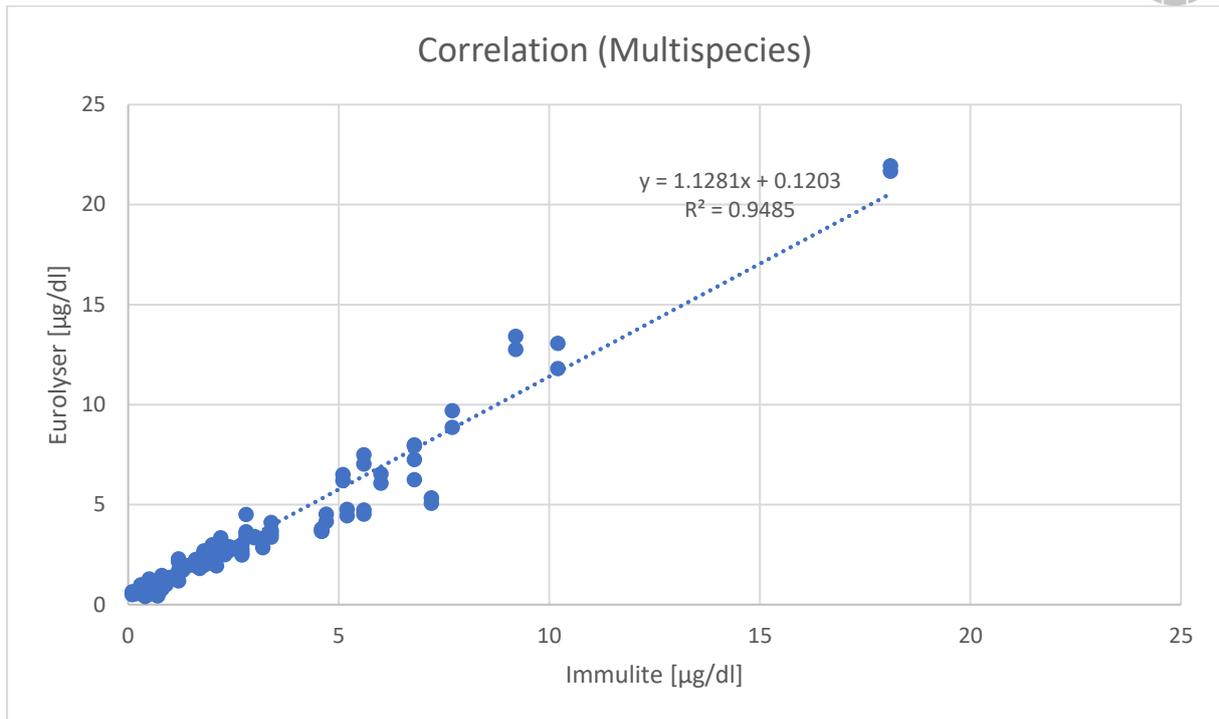
It is recommended that every laboratory establishes its own reference range.

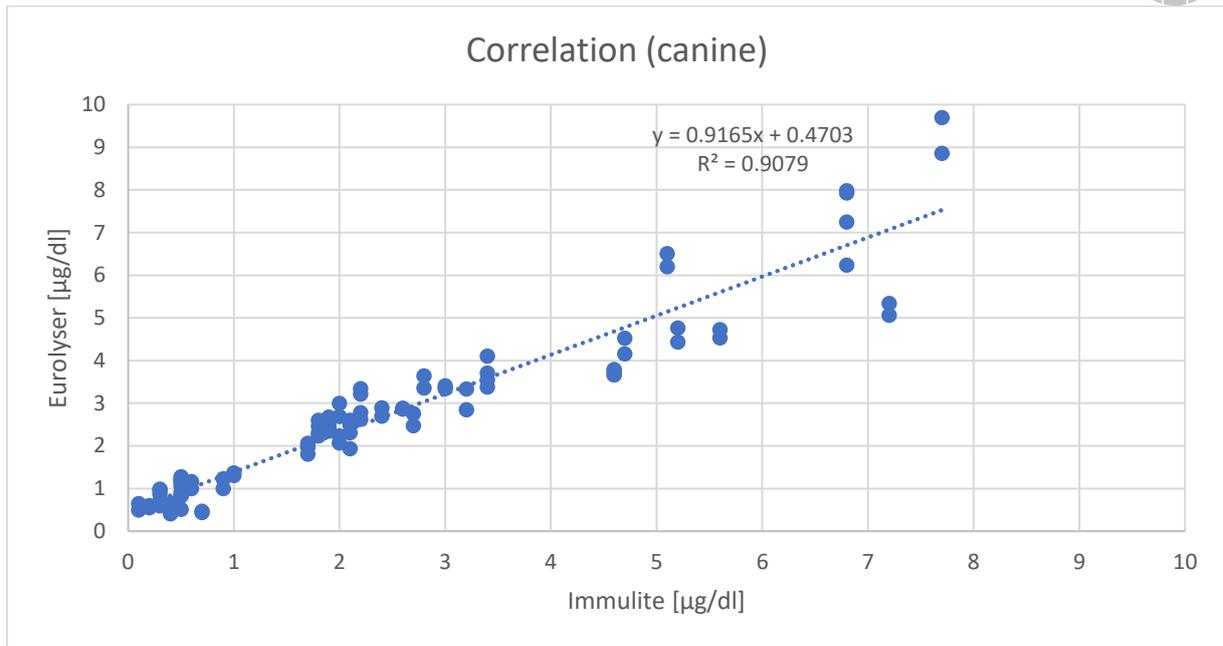
In addition, the multispecies correlation between the currently in use two sample volume setup (20µl/5µl) and the in-future use one sample volume setup (20µl) is shown.

Correlation:

Species	Sample N°	Immulite [µg/dl]	Eurolyser #1 [µg/dl]	Eurolyser #2 [µg/dl]
canine	C1	0.5	1.08	1.16
canine	C2	1	1.30	1.36
canine	C3	0.9	1.23	1.00
canine	C4	6.8	7.98	7.93
canine	C5	4.7	4.53	4.16
canine	C6	1.7	1.81	2.03
canine	C7	2.6	2.88	2.86
canine	C8	2	2.24	2.07
canine	C9	3.4	3.38	3.71
canine	C10	2.1	2.30	2.49
canine	C11	1.7	1.97	2.06
canine	C12	1.8	2.31	2.46
canine	C13	2.2	2.62	3.22
canine	C14	3.4	3.55	4.11
canine	C15	3	3.34	3.40
canine	C16	2.8	3.64	3.36
canine	C17	2	2.69	3.00
canine	C18	1.9	2.35	2.47
canine	C19	1.8	2.60	2.23
canine	C20	2.2	2.78	3.34
canine	C21	2.4	2.70	2.89
canine	C22	2.7	2.76	2.47
canine	C23	3.2	3.33	2.85
canine	C24	1.9	2.44	2.67
canine	C25	2.1	1.93	2.60
canine	C26	0.5	1.17	1.23
canine	C27	7.7	8.86	9.69
canine	C28	6.8	7.25	6.24
canine	C29	0.5	1.06	0.83
canine	C30	7.2	5.06	5.34
canine	C31	5.1	6.20	6.51
canine	C32	0.3	0.70	0.62
canine	C33	0.1	0.50	0.65
canine	C34	0.2	0.55	0.60
canine	C35	0.3	0.59	0.88
canine	C36	4.6	3.66	3.77
canine	C37	5.2	4.76	4.43
canine	C38	5.6	4.72	4.53
canine	C39	4.6	3.67	3.79
canine	C40	0.5	0.51	0.83
canine	C41	0.4	0.41	0.65
canine	C42	0.4	0.43	0.47

Species	Sample N°	Immulite [µg/dl]	Eurolyser #1 [µg/dl]	Eurolyser #2 [µg/dl]
canine	C43	0.6	1.16	1.00
canine	C44	0.3	0.98	0.98
canine	C45	0.5	0.91	0.88
canine	C46	0.5	1.28	1.22
canine	C47	0.7	0.44	0.46
feline	F1	1.2	1.70	1.18
feline	F2	1.3	1.76	1.72
feline	F3	1.2	2.29	2.14
feline	F4	1.8	2.54	2.69
feline	F5	2.3	2.50	2.72
feline	F6	1.4	1.94	1.94
feline	F7	1.8	2.29	2.32
feline	F8	2.1	2.93	2.77
feline	F9	1.6	1.94	2.25
feline	F10	1.7	2.01	2.18
feline	F11	1.8	2.37	1.97
feline	F12	2.8	3.54	4.51
feline	F13	2.7	2.59	3.00
feline	F14	9.2	12.76	13.42
feline	F15	10.2	13.06	11.80
feline	F16	18.1	21.93	21.66
feline	F17	5.6	7.03	7.49
feline	F18	11.9	18.15	18.39
feline	F19	6	6.53	6.07
feline	F20	0.8	1.46	0.77
feline	F21	0.4	0.45	0.65





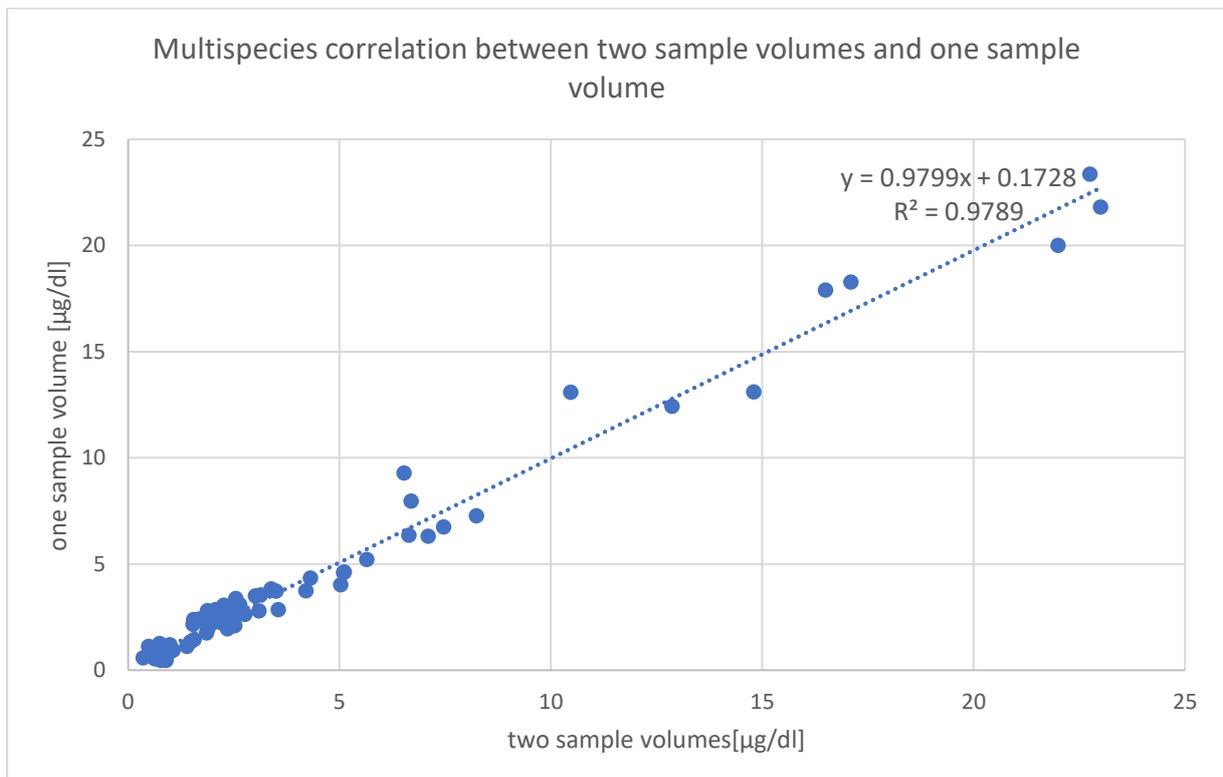
Sample correlation:

The result for the correlation between the Eurolyser T4 test and the Immulite 2000 test is the linear regression function:

Multispecies: y (Eurolyser) = 1.1281x (Immulite) + 0.1203; $R^2 = 0.9485$

Feline: y (Eurolyser) = 1.2859x (Immulite) + 0.0591; $R^2 = 0.9754$

Canine: y (Eurolyser) = 0.9165x (Immulite) + 0.4703; $R^2 = 0.9079$



The result for the correlation between the Eurolyser in-use two sample volume setup and the Eurolyser in-future-use one sample volume setup is the linear regression function:

Multispecies: y (one sample volume) = $0.9799x$ (two sample volumes) + 0.1728 ; $R^2 = 0.9789$

3. Reference Ranges

It is highly recommended that each laboratory establishes its own reference ranges.
If a lab is not able to establish its own reference range. the following ranges can be used.

Normal range cat:	1.0 – 4.0 µg/dl (13 – 52 nmol/l)
Normal range dog:	1.3 – 4.5 µg/dl (17 – 58 nmol/l)
Normal range horse:	1.3 – 4.1 µg/dl (17 – 53 nmol/l)

4. Reproducibility (within-run precision)

Five controls have been tested 16 times each and the CV values were calculated (tested with solo and CUBE-VET analysers):

Sample #	Control 1 [$\mu\text{g}/\text{dl}$]	Control 2 [$\mu\text{g}/\text{dl}$]	Control 3 [$\mu\text{g}/\text{dl}$]
1	2.07	5.66	10.02
2	1.88	5.46	9.30
3	2.01	5.25	9.21
4	2.03	5.34	8.90
5	1.95	5.67	9.31
6	2.01	5.44	9.71
7	1.92	5.28	9.85
8	1.96	5.30	9.10
9	1.94	5.73	8.88
10	1.97	5.22	8.89
11	1.92	5.52	10.22
12	1.99	5.79	9.88
13	1.89	5.50	9.13
14	1.91	5.29	9.56
15	1.92	5.60	9.86
16	1.89	5.80	9.83
Average	1.95	5.49	9.48
Stdev	0.06	0.19	0.43
CV	2.83%	3.51%	4.49%

The CV values for the tested controls are:

- 2.83% (1.95 $\mu\text{g}/\text{dl}$)
- 3.51% (5.49 $\mu\text{g}/\text{dl}$)
- 4.49% (9.48 $\mu\text{g}/\text{dl}$)

5. Day-to-day Imprecision

In the study two controls were tested in three runs per day over five working days on three different Eurolyser CUBE analyzers. Results are listed in the following table:

day	Control low [$\mu\text{g}/\text{dl}$]			Control high [$\mu\text{g}/\text{dl}$]		
1	2.08	1.95	1.93	4.04	4.11	3.84
2	1.99	2.16	1.91	4.12	4.15	3.73
3	2.21	1.99	1.92	4.20	3.97	4.12
4	2.30	2.13	1.92	4.45	4.13	4.77
5	2.23	2.18	1.99	4.67	4.30	4.05
mean	2.06			4.18		
stdev	0.13			0.27		
CV[%]	6.22%			6.47%		

Conclusion: For two levels of T4 samples (commercial T4 controls) the day-to-day imprecision was from 6.22% to 6.47%.

6. Stability Test

A real time stability test was performed. Reagent stability was recorded over 6 months. During this time cuvettes were stored at 2 – 8 °C. The software drift compensation feature was enabled.

3 control levels have been used.

The recovery of the control low, mid and high has to be within 10% of the target value.

Recovery:

	day 1 [$\mu\text{g}/\text{dl}$]	day 184 [$\mu\text{g}/\text{dl}$]	Recovery
Control low	3.95	3.73	94.46%
Control mid	8.27	7.72	93.47%
Control high	19.94	19.42	97.43%

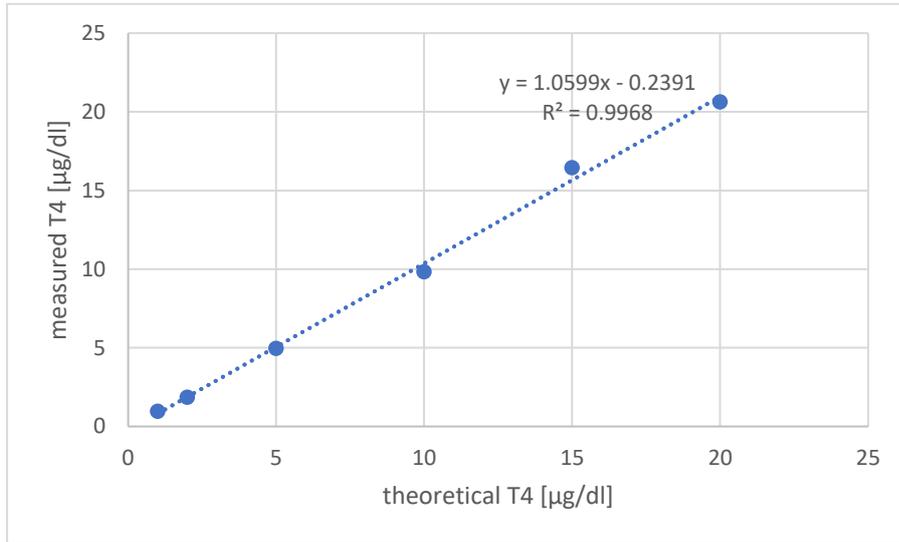
The reagent shows good stability in case of storage at 2 – 8 °C over 6 months. Therefore, a 9 months expiry date can be assumed and is implemented.

7. Linearity Study

The target for the linearity study is 90-110%.

	Control value $\mu\text{g}/\text{dl}$	$\mu\text{g}/\text{dl}$	$\mu\text{g}/\text{dl}$	$\mu\text{g}/\text{dl}$	$\mu\text{g}/\text{dl}$	$\mu\text{g}/\text{dl}$	mean [$\mu\text{g}/\text{dl}$]	stdev	CV	recovery
Con	20	22.42	19.99	20.25	19.91	18.58	20.64	1.03	5.0%	103.2%
Con dil.	15	15.87	17.96	15.46	17.19	15.81	16.46	0.95	5.8%	109.7%
Con dil.	10	9.86	9.86	9.92	10.02	9.51	9.83	0.17	1.8%	98.3%
Con dil.	5	4.90	4.79	4.93	5.43	4.82	4.98	0.23	4.7%	99.5%
Con dil.	2	1.90	1.75	1.74	1.84	2.12	1.87	0.14	7.5%	93.4%
Con dil.	1	0.84	0.97	1.04	0.98	1.01	0.97	0.07	7.1%	96.7%

Based on these results sample out of linearity can be diluted 1+1 with saline and retested (multiply result by 2).



8. Limit of Quantitation (LOQ)

LOQ is calculated based on the lowest sample run that displays a CV value < 20%.

	Control dilution	
average	0.26 µg/dl	0.42 µg/dl
stdev	0.10	0.08
CV (%)	37.28	18.15

The estimated LOQ is 0.4 µg/dl determined by regression analysis.

9. Interferences

The test system has been analysed for various interferences. Criterion was the recovery within 10% of initial values.

Bilirubin	up to 30 mg/dl
Cholesterol	up to 400 mg/dl
Hemoglobin	up to 800 mg/dl
Triglycerides	up to 1000 mg/dl

10. Summary

The Eurolyser T4 test kit designed for solo and CUBE-VET analyzers has a good correlation to Siemens Immulite 2000 xpi.

The reproducibility as well as the stability of the test are very good and no interfering substances are found.