Evaluation Report

Eurolyser Total Bile Acids test kit (VT0240, VT0241)

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 dogs, cats and horses and were fresh serum or li-hep plasma blood samples which have been tested with the reference method (on a Roche Cobas 8000 c701).

For all other tests dedicated controls have been used.

Sample volume: 100 µl

Equipment

- Eurolyser CUBE-VET analyser: Ca10615, Ca10910, Ca10911, Cb12910
- Eurolyser solo: Ae5050, Ae5052, Ae, 5053, Bc14783

- Test kits: VT0240, VT0241: LOT 20171013_1

- Reagents:
  - R1_90: 850 µl
  - R2 Typ E: 170 µl

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1. Introduction and Scope

Bile acids are an important parameter to evaluate liver function. Increased bile acid concentrations can occur due to various hepatopathies, portosystemic shunts or intra- or extrahepatic cholestasis. The bile acid concentration depends on several factors i.e. food ingestion, gall bladder emptying. Therefore, it is recommended to only analyse bile acids if the patient is fasting or after performance of a bile acid stimulation test.

1.1 Method comparison
Testing the correlation between the total bile acids (TBA) measurement results on Eurolyser analysers from serum/plasma and the results of the reference method on a Roche Cobas 8000 c701.
1.2 Reproducibility
Characterisation of the reproducibility of the Eurolyser TBA test at 3 levels
1.3 Stability testing
1.4 Limit of Quantification
1.5 Interferences

Principle:
Homogeneous colorimetric test.

2. Comparison Study

Eurolyser vs reference method (Roche Cobas 8000 c701)

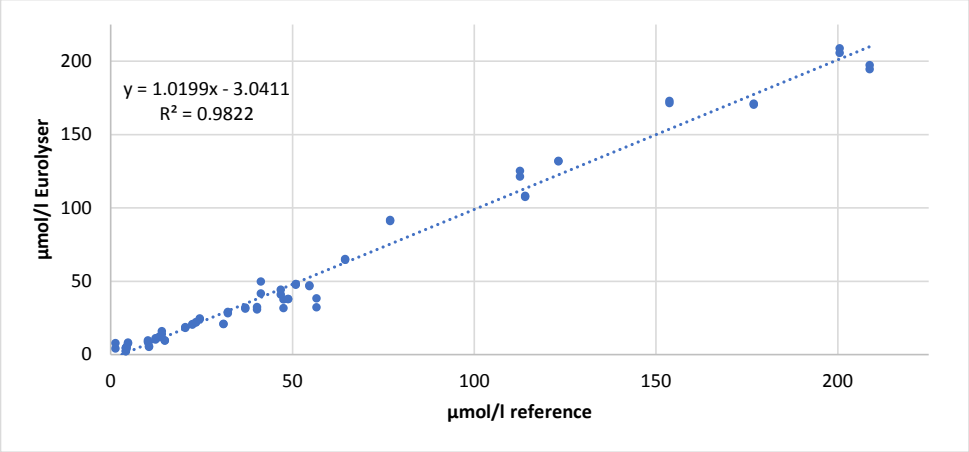
The comparison study is based on the correlation between the results of the Eurolyser TBA test and the reference method measured on a Roche Cobas 8000 c701.

72 samples have been used in the correlation test, 2 replicates of each sample have been measured. 35 canine samples, 17 feline samples and 20 equine samples have been analysed on solo and CUBE-VET analysers.

The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.90$** obtained from linear regression between the Eurolyser TBA and the reference method TBA.

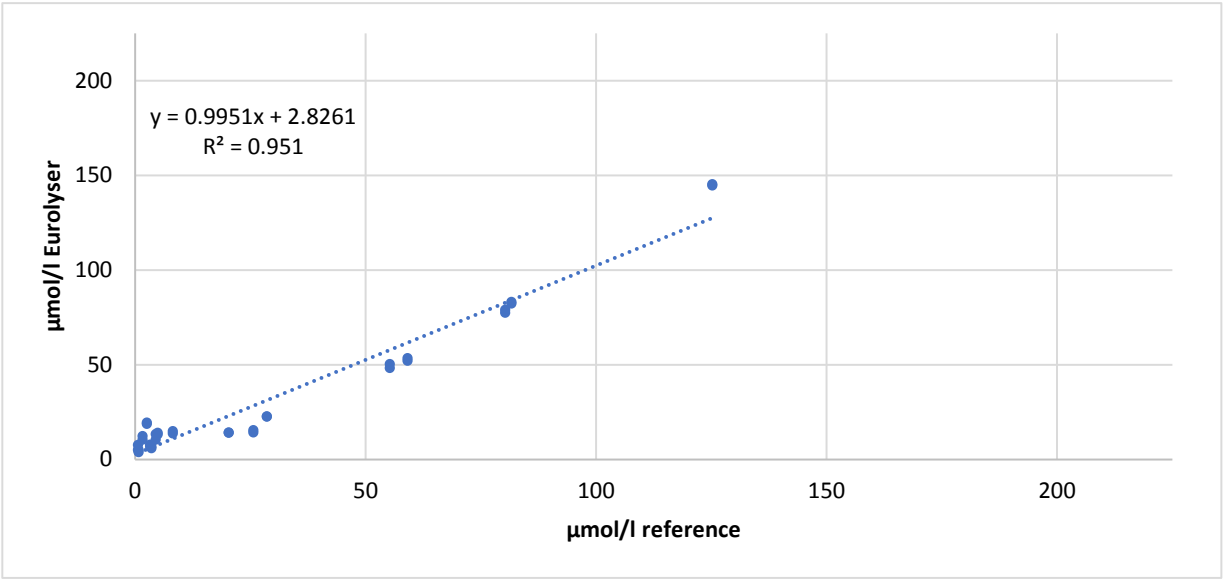
Canine correlation:

Sample N°	Reference µmol/l	Eurolyser µmol/l #1	Eurolyser µmol/l #2
1	23.54	22.0	21.8
2	20.59	18.0	18.6
3	40.28	30.6	32.3
4	208.85	197.1	194.3
5	22.53	20.6	20.2
6	24.56	24.0	24.4
7	31.06	20.8	20.7
8	32.30	28.0	29.0
9	37.10	31.2	32.0
10	41.35	41.6	49.6
11	46.85	44.1	40.7
12	47.59	31.6	37.5
13	48.88	37.9	37.7
14	176.97	170.1	170.9
15	153.73	171.3	172.7
16	64.57	64.4	65.0
17	54.72	47.0	46.6
18	50.95	47.9	47.5
19	10.31	8.4	9.5
20	10.63	5.1	5.4
21	12.39	11.0	10.1
22	13.43	11.7	12.1
23	14.14	13.8	15.8
24	14.95	9.4	9.6
25	4.20	4.5	4.3
26	4.23	2.2	2.2
27	4.42	5.3	4.1
28	1.39	4.1	7.7
29	4.79	8.2	7.5
30	56.69	32.2	38.1
31	112.62	125.1	121.2
32	76.95	91.7	90.9
33	114.07	108.1	107.4
34	123.24	131.5	131.8
35	200.60	208.6	205.5



Feline correlation:

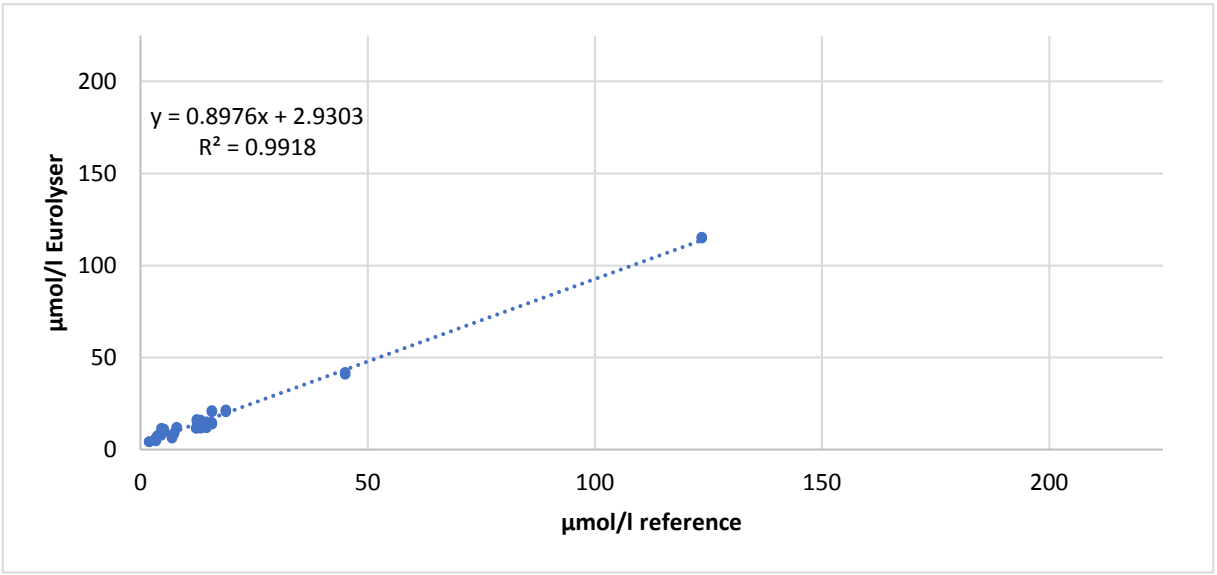
Feline Sample N°	Reference µmol/l	Eurolyser µmol/l #1	Eurolyser µmol/l #2
36	125.28	144.5	145.1
37	0.79	4.9	3.8
38	1.69	12.2	10.3
39	2.55	19.6	19.3
40	3.31	6.6	7.6
41	3.61	5.8	6.7
42	4.97	14.0	13.1
43	20.33	14.0	14.1
44	25.69	15.4	14.1
45	8.23	14.8	13.6
46	0.65	4.9	7.5
47	4.48	10.3	13.2
48	55.31	48.1	50.3
49	28.65	22.5	22.5
50	59.12	51.9	53.3
51	80.30	77.2	78.9
52	81.69	83.1	82.2



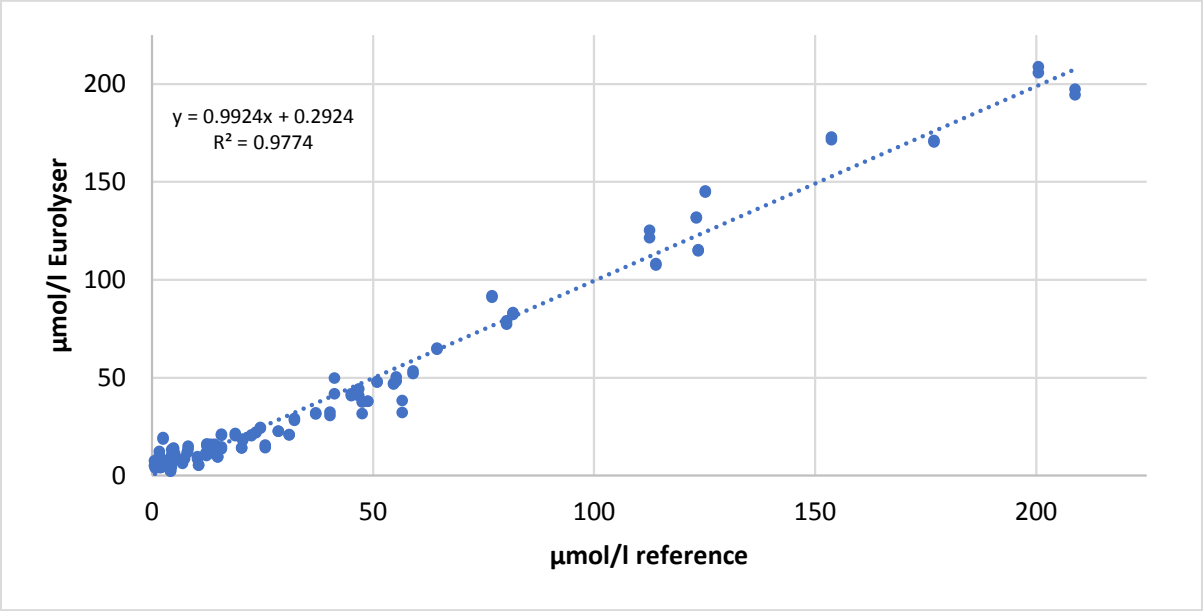
Equine correlation:

Equine Sample N°	Reference µmol/l	Eurolyser µmol/l #1	Eurolyser µmol/l #2
53	3.81	7.4	*
54	3.46	6.4	4.6
55	2.00	4.0	4.1
56	4.07	7.5	7.7
57	4.61	8.3	7.5
58	4.70	9.3	11.5
59	5.16	11.0	9.7
60	6.96	7.5	6.0
61	7.42	8.8	8.4
62	8.02	11.6	12.0
63	15.80	20.3	20.9
64	18.87	20.3	21.4
65	123.62	115.2	114.7
66	45.12	40.7	41.7
67	13.29	15.8	11.4
68	12.32	11.4	11.4
69	13.24	14.4	13.9
70	12.48	15.1	16.1
71	14.54	11.5	15.0
72	15.76	14.5	13.5

* not enough sample material for replicate



Correlation to reference method (multispecies):



Sample correlation:
The result for the correlation between the reference method and Eurolyser TBA test is the linear regression function:

y (Eurolyser) = 0.9924x (reference method) + 0.2924 and a **R^2 = 0.9774**

Based on the correlation the linearity and upper limit of measurement range is defined as 200 µmol/l.

Based on the excellent correlation data the reference ranges of the reference method will be used:
Dog: < 20 µmol/l
Cat: < 20 µmol/l
Horse: < 12 µmol/l
Nonetheless, it is recommended that each laboratory establishes its own reference ranges.

Lipaemic and haemolytic samples cause interferences with the test and shall therefore not be used.

3. Reproducibility (within-run precision)

Three controls have been measured 20 times and the CV value was calculated (tested with solo and CUBE-VET analysers). Values shown in the following table are µmol/l. The acceptance criterion for CV values shall be 5%.

Sample #	Control low µmol/l	Control mid µmol/l	Control high µmol/l
1	28.78	55.91	84.81
2	29.57	54.88	83.38
3	29.32	54.32	83.15
4	29.14	55.51	82.49
5	29.21	55.02	83.41
6	27.47	54.07	82.76
7	29.80	54.23	81.55
8	29.13	55.19	82.88
9	29.72	55.62	83.12
10	29.42	55.57	82.20
11	29.55	55.38	84.01
12	28.09	54.92	83.12
13	28.74	55.58	83.58
14	29.59	56.39	81.66
15	30.61	56.58	82.56
16	27.76	54.32	82.98
17	28.96	56.18	83.77
18	29.39	55.76	82.10
19	29.93	55.83	82.36
20	28.22	54.82	81.01
Average	**29.12**	**55.30**	**82.85**
Stdev	**0.75**	**0.71**	**0.88**
CV	**2.57%**	**1.28%**	**1.06%**

The CV values are 2.57% for the control low, 1.28% for the mid and 1.06% for the control high.

4. Stability Test

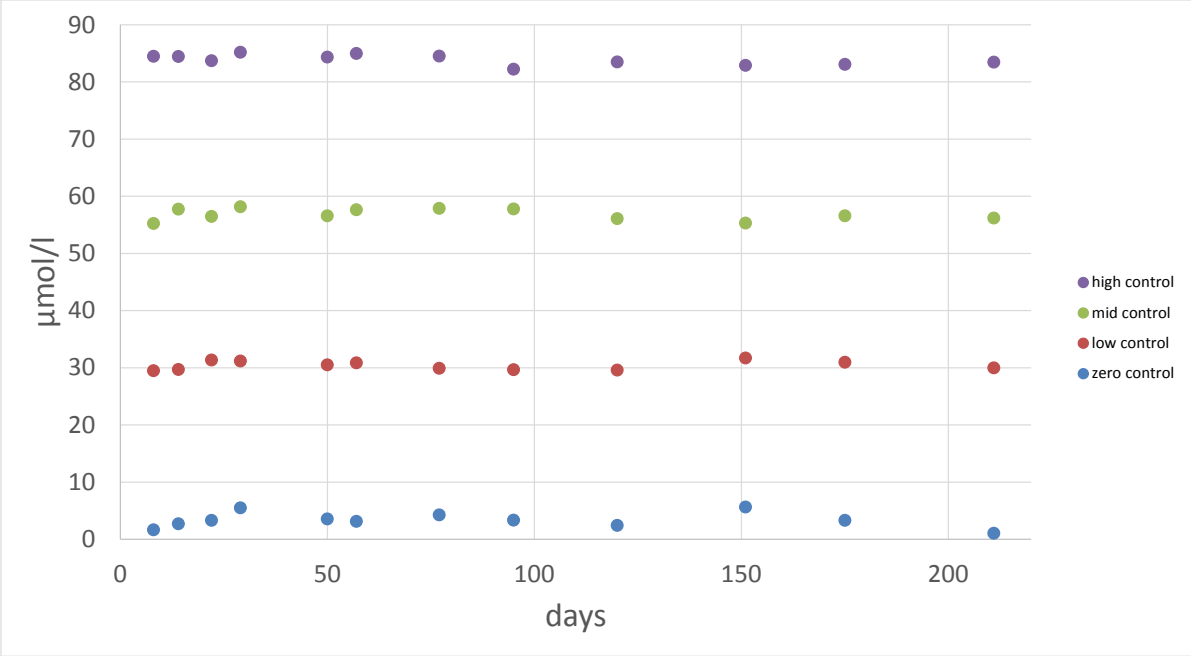
A real time stability test was performed. Reagent stability was recorded over 7 months. During this time cuvettes have been stored at +4 °C.

Cuvettes prepared on: day 0
Measurement date: day 8 – day 211

3 control levels have been used.
In addition, a 0 control was measured.

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

Reagent stability:



Recovery:

day	Control low		Control mid		Control high	
	µmol/l	% recovery	µmol/l	% recovery	µmol/l	% recovery
8	29.49	102.25%	55.22	102.62%	84.47	99.18%
14	29.68	101.57%	57.74	98.15%	84.45	99.21%
22	31.35	96.16%	56.45	100.38%	83.72	100.08%
29	31.15	96.77%	58.16	97.43%	85.19	98.35%
50	30.50	98.84%	56.57	100.17%	84.36	99.32%
57	30.86	97.70%	57.63	98.34%	84.99	98.58%
77	29.90	100.82%	57.89	97.90%	84.50	99.15%
95	29.65	101.69%	57.76	98.12%	82.23	101.89%
120	29.59	101.90%	56.09	101.03%	83.50	100.34%
151	31.70	95.10%	55.31	102.46%	82.88	101.09%
175	30.96	97.39%	56.59	100.14%	83.07	100.86%
211	29.99	100.54%	56.19	100.85%	83.45	100.40%

The reagent shows very good stability shown in the graph above and reflected in recovery rates in a 7 months real time stability test.

This data supports a 9-month stability for the TBA test kit.

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	4.46	1.26
stdev	0.45	0.33
CV (%)	10.18%	26.21%

Based on these results the LOQ is set to 3 µmol/l.

6. Interferences in Plasma

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

no interference up to:

Bilirubin	35 mg/dl
Haemoglobin	120 mg/dl
Triglyceride	1200 mg/dl

Lipaemic and haemolytic samples cause interferences with the test and shall therefore not be used.

7. Summary

The TBA test kit designed for solo and CUBE-VET analysers has a good correlation to the reference method measured on a Roche Cobas 8000 c701.

The reproducibility and stability of the test are excellent.