Evaluation Report

Eurolyser SDMA test kit (VT0300, VT0301)

for solo and CUBE-VET analysers

Location: Eurolyser Diagnostica GmbH
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Report created on 30th July 2020
Report created by Michael Gruber

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 (LCMS Mass spectrometers Sciex API4000 and API4500QTRAP coupled to reversed phase chromatography).

Sample volume: 40 µl

Equipment

- Eurolyser CUBE-VET analyser: Ce19729, Ce19730, Ce19731, Ce14920
- Eurolyser solo analyser: Ae5050, Ae5052, Ae5053, Bc14783

- Test kits: LOT R+D 062020ABC

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1. Introduction and Scope

Symmetric dimethylarginine (SDMA) is a methylated arginine amino acid. SDMA is derived from intranuclear methylation of L-arginine residues and is released into the cytoplasm after proteolysis. SDMA is excreted by the kidneys.
Several studies have found that 30% of cats and 10% of dogs are likely to develop a kidney disease during their lifetime.
SDMA is correlating with glomerular filtration rate (GFR). On average, SDMA increases in chronic kidney disease (CKD) with 30 to 40% loss of kidney function. Creatinine, however, does not increase until 75% of kidney function is lost. SDMA is therefore an earlier diagnostic tool than Creatinine or Cystatin C.
SDMA is specific for kidney function. It is not impacted by other diseases such as cardiovascular disease endocrine diseases, liver disease or inflammatory disease. SDMA is not impacted by muscle mass either, which simplifies diagnosing and monitoring CKD in thin geriatric animals, especially cats and animals with other diseases that cause muscle wasting.

The Eurolyser antibody binds with acylated SDMA, with peptide bound SDMA and also with free SDMA, and, thus is considered as not being "specific" for "free SDMA" ("free SDMA" is to be understood as SDMA not being part of a polypeptide chain).

Principle:
Homogeneous immunoturbidimetric test.

2. Comparison Study

2.1 Eurolyser vs Reference method (LCMS, Sciex API4000)

The comparison study is based on the correlation between the results of the Eurolyser SDMA test and the Sciex API 4000 LCMS.

49 canine and 48 feline samples have been analysed with the Eurolyser SDMA assay.

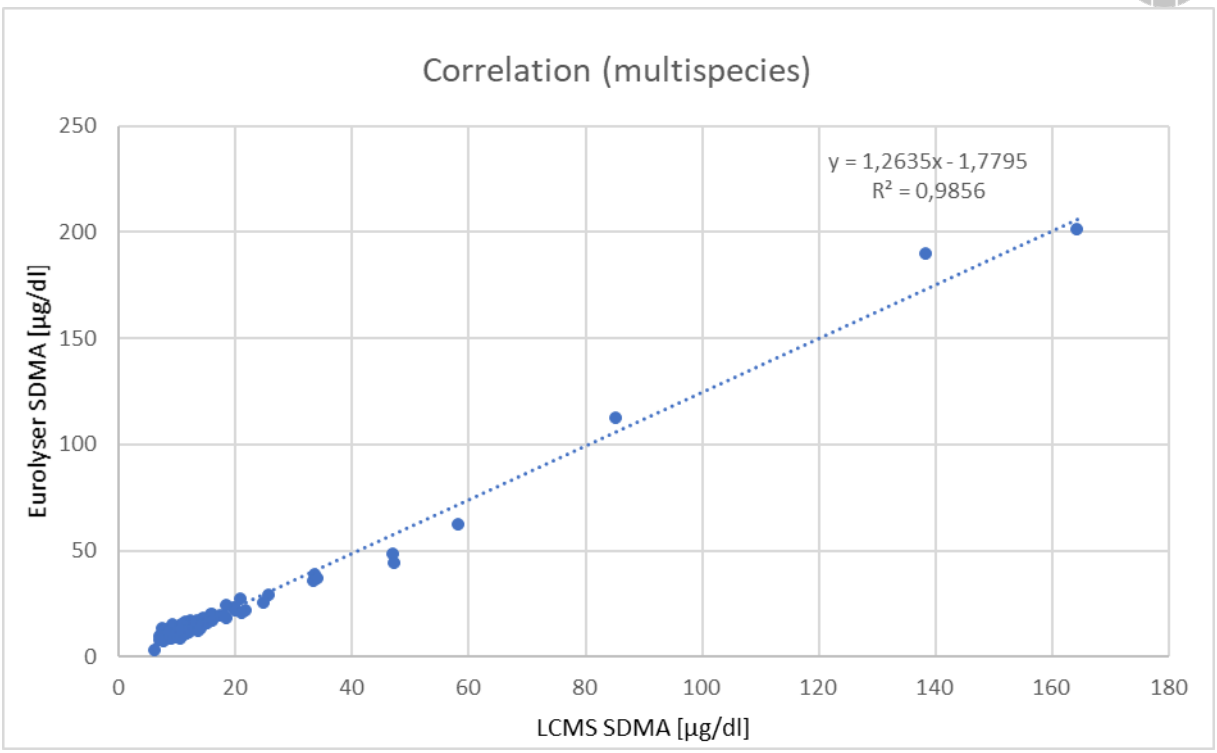
The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.95$** obtained from linear regression between the Eurolyser SDMA and the Sciex LCMS.

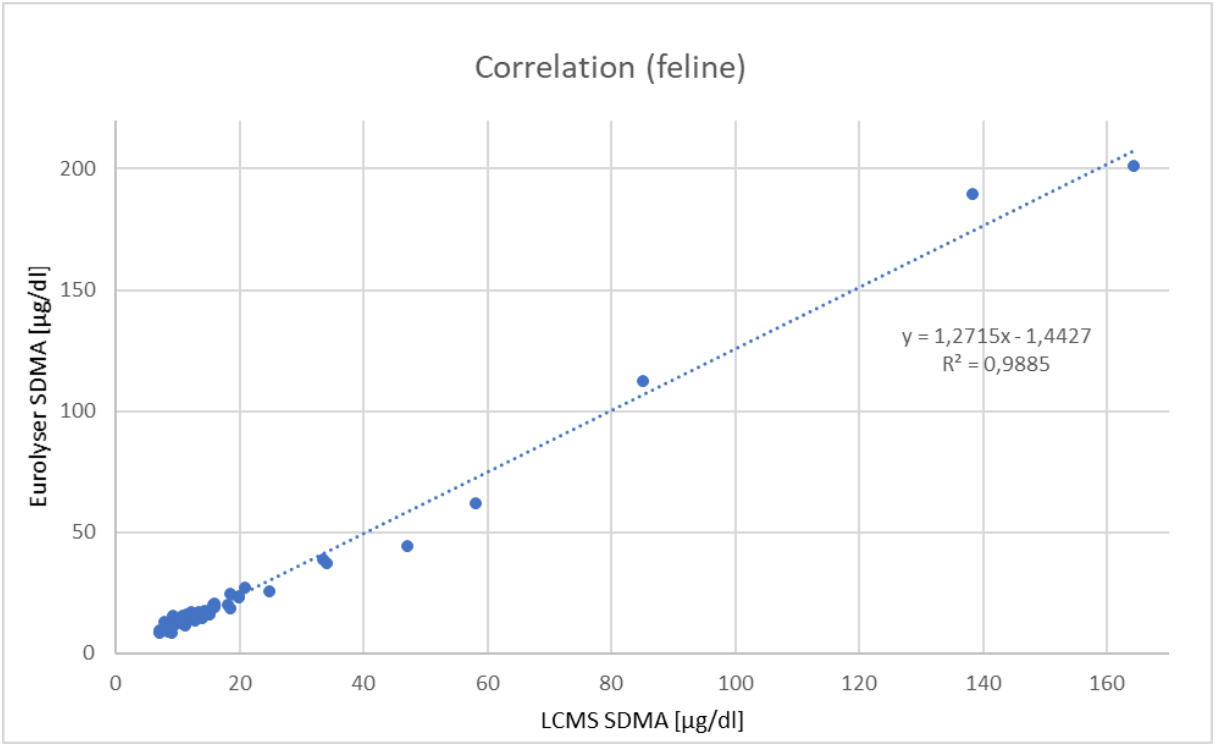
Further, slope has to be within 0.7 and 1.3, and an intercept between -2 and +2 is acceptable.
This relatively wide acceptable range is chosen because at the time of the correlation study, the calibrator set used was not assigned to any so-called reference and was not assigned species specific.
Slope and intercept can be adjusted easily by re-assigning the Eurolyser SDMA calibrators. As every lab should establish their normal ranges individually, a re-assign of the calibrators is not preferred.

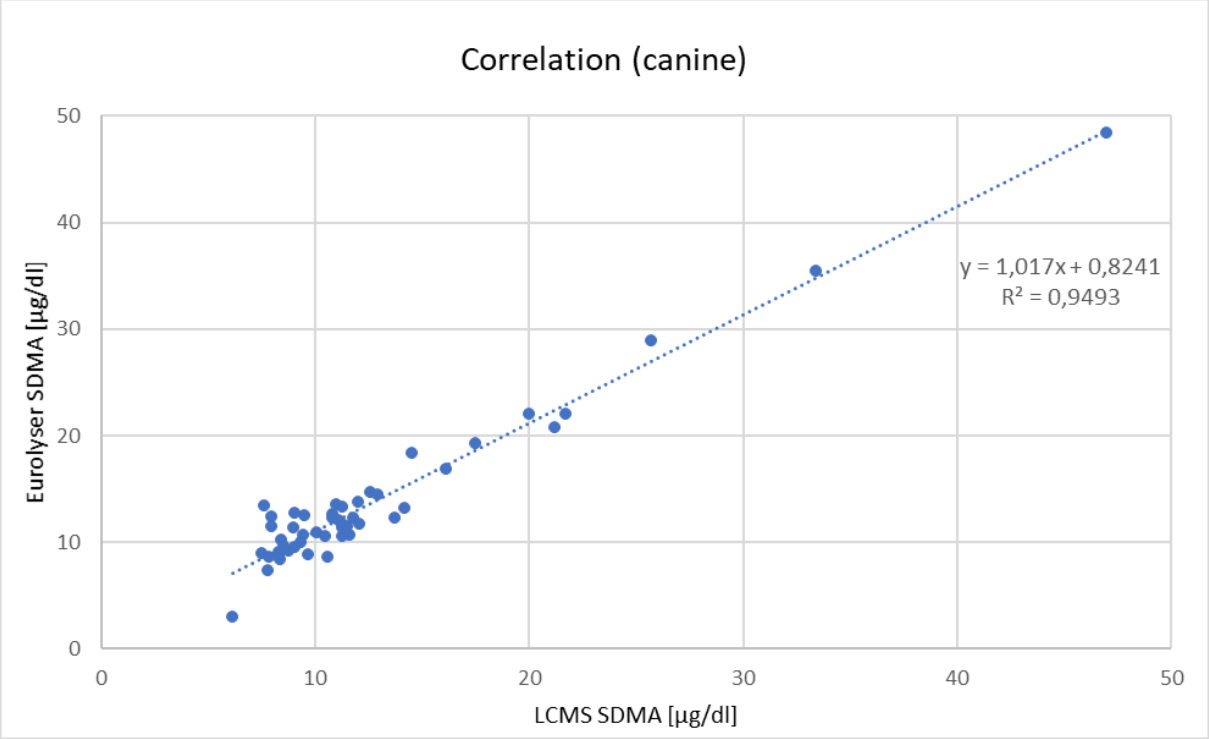
Correlation:

Species	Sample N°	haemolytic	lipaemic	SDMA LCMS (µg/dl)	SDMA Eurolyser (µg/dl)
canine	1	-	-	9.0	11.3
canine	2	-	-	9.5	10.6
canine	3	-	-	9.5	12.5
canine	4	-	-	11.3	11.7
canine	5	-	-	33.4	35.4
canine	6	-	-	10.1	10.9
canine	7	-	-	7.6	13.4
canine	8	-	-	14.2	13.2
canine	9	-	-	10.8	12.6
canine	10	-	-	11.0	13.5
canine	11	-	+	21.7	22.0
canine	12	-	+	7.5	8.9
canine	13	-	+	10.5	10.5
canine	14	-	-	8.0	12.4
canine	15	-	-	11.3	13.3
canine	16	-	+	12.6	14.7
canine	17	-	-	14.5	18.3
canine	18	-	+	8.3	9.0
canine	19	-	-	25.7	28.9
canine	20	-	-	12.0	13.8
canine	21	-	-	12.9	14.4
canine	22	-	-	17.5	19.2
canine	23	+	-	47.0	48.4
canine	24	-	-	11.8	12.3
canine	25	-	-	11.1	12.0
canine	26	-	+	8.0	11.5
feline	27	-	-	18.6	24.3
feline	28	-	-	11.3	12.8
feline	29	-	-	13.4	16.6
feline	30	-	-	9.3	13.0
feline	31	-	-	12.3	16.8
feline	32	-	-	16.0	20.1
feline	33	-	-	15.8	19.9
feline	34	-	-	14.4	17.3
feline	35	-	-	15.9	18.7
feline	36	+	-	11.4	16.0
feline	37	-	-	11.3	14.3
feline	38	-	-	10.3	13.9
feline	39	-	-	10.5	14.3
feline	40	-	-	13.2	15.6
feline	41	-	-	11.5	14.8
feline	42	-	-	9.3	15.2
feline	43	-	-	10.9	15.1
feline	44	-	-	10.7	13.9
feline	45	-	-	19.9	23.1
feline	46	-	-	19.9	22.7
feline	47	-	-	10.9	13.0
feline	48	-	-	8.0	12.7
feline	49	-	-	85.2	112.4
feline	50	-	-	33.6	38.4
feline	51	-	-	138.4	189.6
canine	52	-	+	20.0	22.0
feline	53	+	-	34.1	37.1
feline	54	-	-	47.2	44.0
canine	55	-	-	11.3	10.5

Species	Sample N°	haemolytic	lipaemic	SDMA LCMS (µg/dl)	SDMA Eurolyser (µg/dl)
feline	56	-	-	24.9	25.3
feline	57	-	-	58.2	61.9
feline	58	-	-	164.4	201.1
feline	59	-	-	18.5	18.3
feline	60	-	-	11.3	11.0
canine	61	-	-	11.6	10.6
feline	62	-	-	11.5	13.1
feline	63	-	-	14.0	14.2
feline	64	-	-	12.7	15.2
canine	65	-	+	8.4	8.4
canine	66	-	+	8.3	8.8
canine	67	-	-	10.6	8.6
feline	68	-	-	11.5	12.5
canine	69	-	-	13.7	12.2
canine	70	-	+	7.8	8.6
feline	71	-	-	20.9	27.0
canine	72	+	-	8.8	9.2
feline	73	-	-	18.1	19.6
canine	74	-	-	9.7	8.8
feline	75	-	-	12.9	13.4
canine	76	-	-	10.8	12.2
feline	77	-	-	9.7	11.6
canine	78	-	-	8.4	10.2
canine	79	-	-	9.0	9.5
canine	80	-	-	9.0	12.7
feline	81	-	-	15.2	15.8
feline	82	-	-	7.1	9.4
canine	83	-	-	9.3	10.0
feline	84	-	-	8.6	8.5
canine	85	-	-	21.2	20.8
canine	86	-	-	7.8	7.3
canine	87	-	-	8.5	9.6
canine	88	+	-	12.1	11.7
canine	89	-	-	16.1	16.9
feline	90	-	-	11.0	11.8
feline	91	-	+	9.1	8.4
feline	92	-	-	8.5	10.8
canine	93	-	-	11.3	11.3
canine	94	-	-	11.5	11.5
canine	95	-	+	6.2	3.0
feline	96	-	+	7.2	8.1
feline	97	-	-	10.5	12.6


Correlation (multispecies)
y = 1,2635x - 1,7795
R² = 0,9856
Eurolyser SDMA [µg/dl]
LCMS SDMA [µg/dl]
0
50
100
150
200
250
0
20
40
60
80
100
120
140
160
180


Correlation (feline)
y = 1,2715x - 1,4427
R² = 0,9885
Eurolyser SDMA [µg/dl]
LCMS SDMA [µg/dl]
0
50
100
150
200
0
20
40
60
80
100
120
140
160



Sample correlation:
The result for the correlation between the Eurolyser SDMA test and the LCMS test is the linear regression function:

Multispecies: y (Eurolyser) = 1.2635x (LCMS) - 1.7795; R^2 = 0.9856

Feline: y (Eurolyser) = 1.2715x (LCMS) - 1.4427; R^2 = 0.9885

Canine: y (Eurolyser) = 1.0170x (LCMS) + 0.8241; R^2 = 0.9493

2.2 Eurolyser SDMA versus IDEXX SDMA

Methods	Canine Correlation				Feline Correlation			
	N	R-Value	Bias %	Bias Abs.	N	R-Value	Bias %	Bias Abs.
Idexx v. Eurolyser	76	0.9840	-5.2%	-0.9	73	0.9779	-0.3%	-0.1

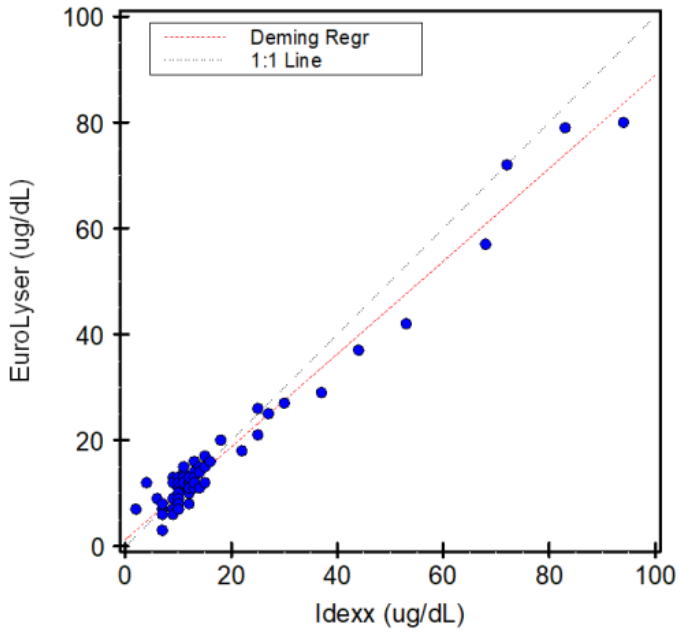


Figure 1. Canine Idexx vs. Eurolyser

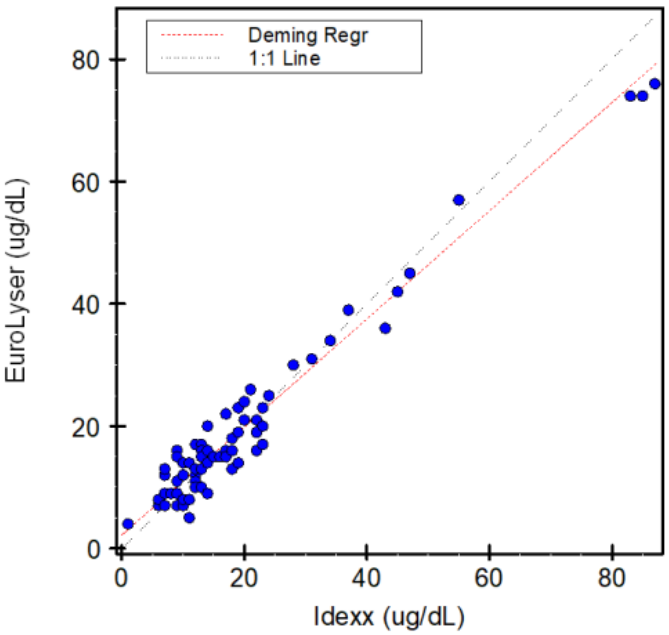
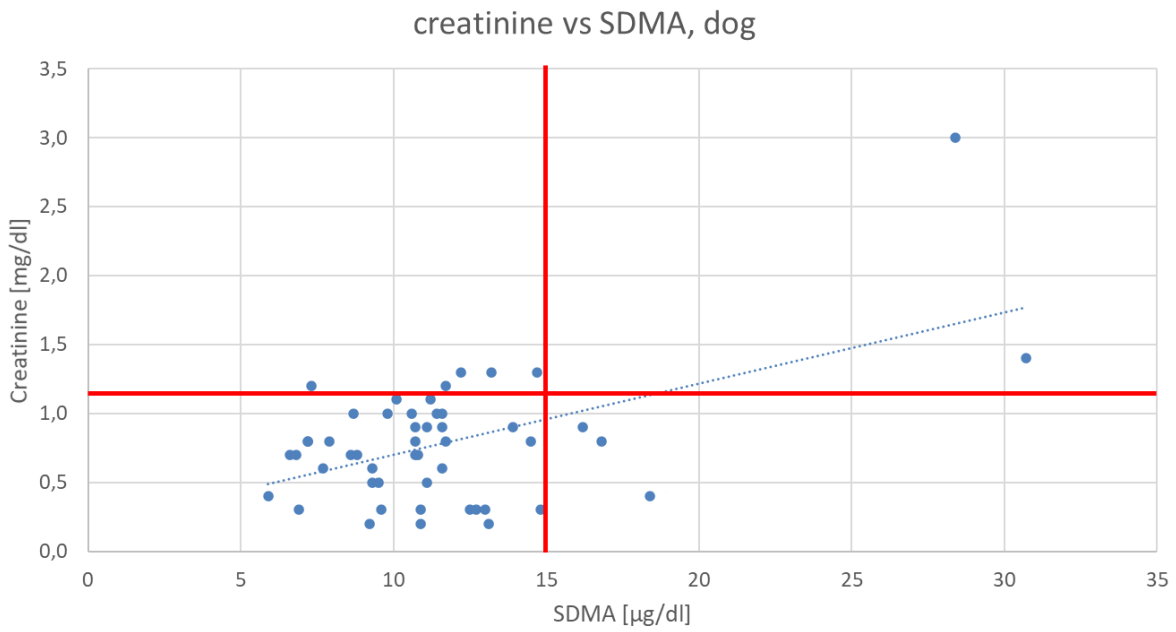


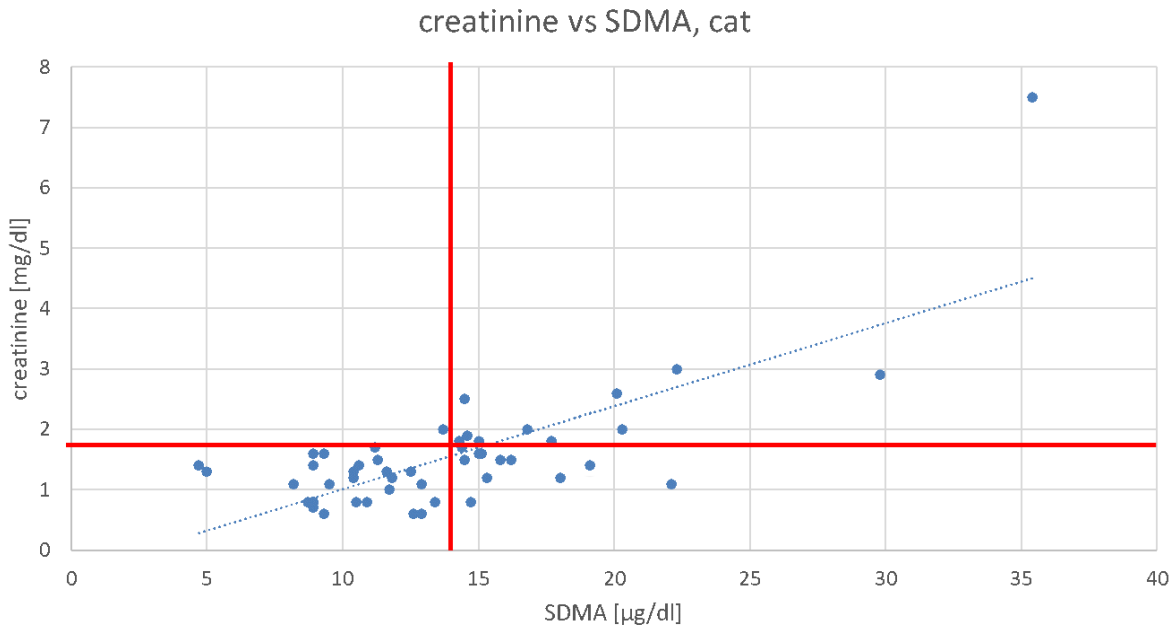
Figure 2. Feline Idexx vs. Eurolyser

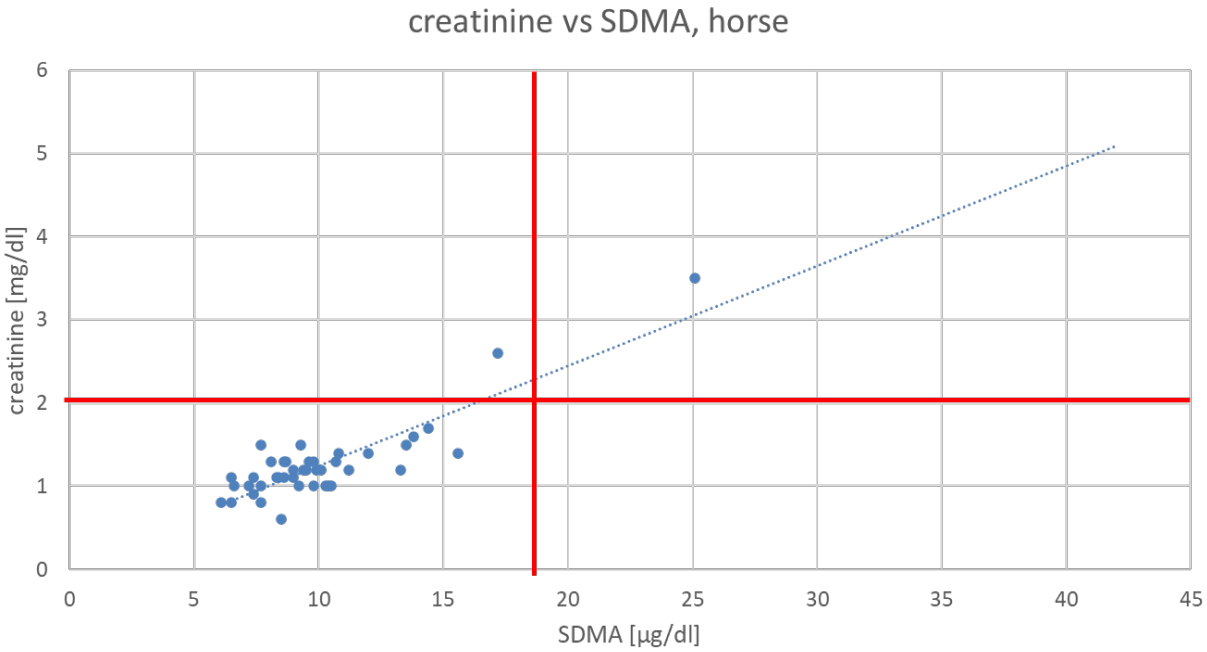
Conclusion: Eurolyser SDMA shows excellent correlation to Idexx SDMA

Relationship between SDMA and serum creatinine

50 samples from dogs and cats as well as 42 samples from horses have been analysed with serum creatinine measured on a routine chemistry analyser and compared with Eurolyser SDMA.







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:	< 14 µg/dl
Normal range dog:	< 15 µg/dl
Normal range puppy dog:	< 17 µg/dl

4. Reproducibility (within-run precision)

Five controls have been tested 15 times each and the CV values were calculated (tested with solo and CUBE-VET analysers):

Sample #	Ctrl 1 (µg/dl)	Ctrl 2 (µg/dl)	Ctrl 3 (µg/dl)	Ctrl 4 (µg/dl)	Ctrl 5 (µg/dl)
1	93.7	51.0	31.2	14.3	10.0
2	94.9	53.3	33.9	18.6	6.3
3	99.3	51.1	30.0	14.4	8.1
4	78.8	48.3	30.7	14.0	9.4
5	89.2	51.5	30.1	16.3	7.6
6	99.8	49.9	27.9	14.2	7.9
7	96.9	52.3	27.4	15.4	8.6
8	88.7	49.9	32.9	17.0	9.0
9	92.8	50.0	31.6	15.0	8.0
10	85.5	54.2	30.8	17.1	9.0
11	93.4	47.2	31.6	18.2	8.3
12	92.3	53.0	29.4	16.3	6.9
13	97.1	57.3	34.7	19.0	8.0
14	95.7	51.9	35.3	16.3	8.0
15	92.9	51.8	33.2	16.6	8.2
Average	**92.7**	**51.5**	**31.4**	**16.2**	**8.2**
Stdev	**5.47**	**2.46**	**2.61**	**1.62**	**0.92**
CV	**5.90%**	**4.77%**	**7.35%**	**9.98%**	**11.19%**

The CV values for the tested controls are:

5.90% (92.7 µg/dl)
4.77% (51.5 µg/dl)
7.35% (31.4 µg/dl)
9.98% (16.2 µg/dl)
11.19% (8.2 µg/dl)

5. Stability Test

A real time stability test was performed. Reagent stability was recorded over 5 months. During this time cuvettes were stored at 2 – 8 °C. The software drift compensation feature was enabled.

4 control levels have been used.

The recovery of the controls from 15 – 100 µg/dl has to be within 15% of the target value.

Recovery:

	day 1 (µg/dl)	day 150 (µg/dl)	Recovery (%)
Control 1	91.0	89.2	98.0%
Control 2	51.1	48.9	95.7%
Control 3	30.1	29.8	99.0%
Control 4	15.4	14.7	95.4%

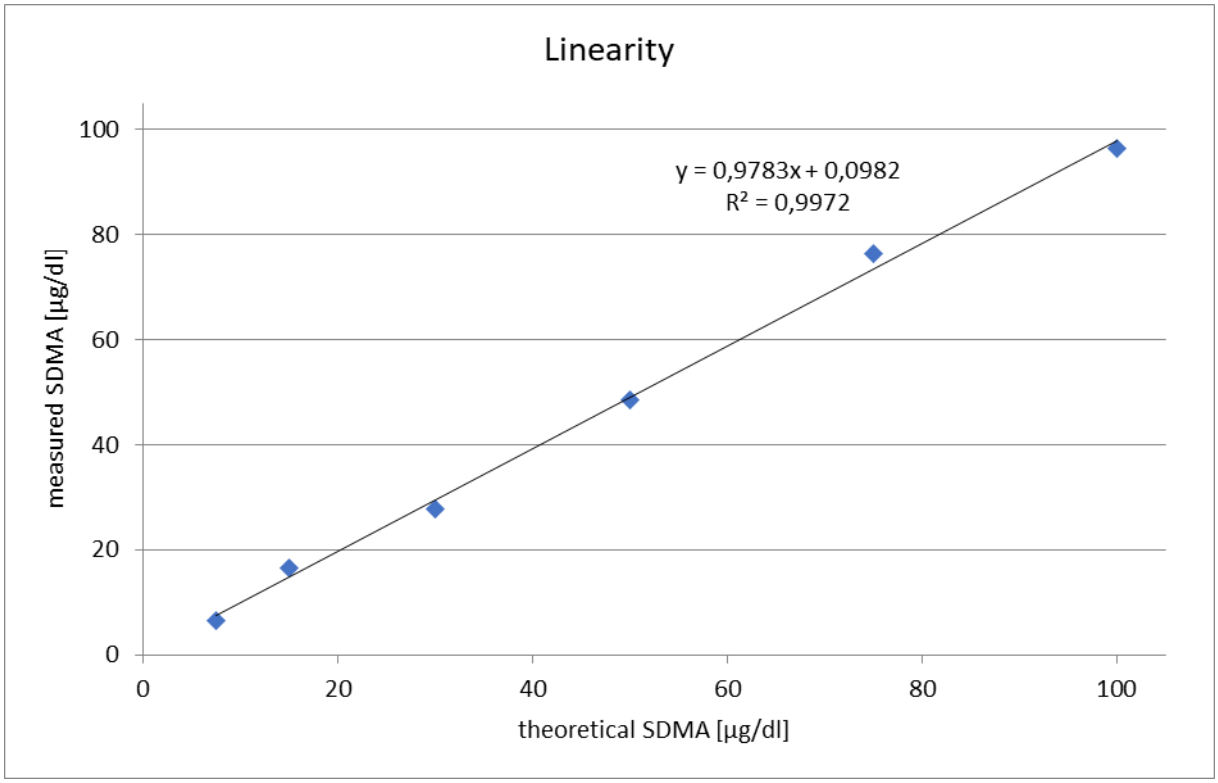
The reagent shows good stability in case of storage at 2 – 8 °C over 5 months. Therefore, a 9 months expiry date can be assumed and is implemented.

6. Linearity Study

The target for the linearity study is 90 – 110% above 15 µg/dl.

	Control value µg/dl	Ae 5052 µg/dl	Ce19731 µg/dl	Ce14920 µg/dl	mean	stdev	CV	recovery
Con	100	98.4	99.1	91.5	96.3	4.2	4.4%	96%
Con dil.	75	70.1	77.3	81.5	76.3	5.8	7.6%	102%
Con dil.	50	45.5	48.9	51.2	48.5	2.9	5.9%	97%
Con dil.	30	26.1	29.9	27.5	27.8	1.9	6.9%	93%
Con dil.	15	17.5	17.1	15.0	16.5	1.3	8.1%	110%
Con dil.	7.5	5.8	6.7	7.1	6.5	0.7	10.2%	87%

Sample out of linearity should be diluted 1+1 with saline and retested (multiply result by 2).



7. Limit of Quantitation (LOQ)

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

	Control dilution
average	5.9
stdev	1.18
CV (%)	19.85%

Based on these results the LOQ is set to 6.0 µg/dl.

8. Interferences

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

Haemoglobin	100 mg/dl
Albumin	11 g/dl
Bilirubin. conjugated	35 mg/dl
Bilirubin. unconjugated	35 mg/dl
Cholesterol	600 mg/dl
Rheumatoid factor	500 IU/ml
Triglycerides	800 mg/dl
Uric acid	30 mg/dl

9. Cross-reactivity

Potential cross-reactants (ADMA. MMA. L-arginine. N-acyl SDMA) were spiked into Eurolyser synthetic matrix at 50 or 100 µg/dl with or without SDMA (19 µg/dl). The LC-MS results and Eurolyser results are shown in the tables below.

LC-MS cross-reactivity:

Cross-reactant		Spiked SDMA µg/dl	Recovery SDMA µg/dl	Recovery SDMA %
ADMA	100 µg/dl	0	0.9	n.a.
L-Arg	100 µg/dl	0	0.6	n.a.
MMA	100 µg/dl	0	0.0	n.a.
N-acyl SDMA	100 µg/dl	0	0.5	n.a.
ADMA	50 µg/dl	19	25.4	133.7
L-Arg	50 µg/dl	19	25.8	135.8
MMA	50 µg/dl	19	26.9	141.6
N-acyl SDMA	50 µg/dl	19	25.7	135.3

Eurolyser cross-reactivity:

Cross-reactant		Spiked SDMA µg/dl	Recovery SDMA µg/dl	Recovery SDMA %
ADMA	100 µg/dl	0	0.0	n.a.
L-Arg	100 µg/dl	0	0.0	n.a.
MMA	100 µg/dl	0	3.3	n.a.
N-acyl SDMA	100 µg/dl	0	1.9	n.a.
ADMA	50 µg/dl	19	18.0	94.7
L-Arg	50 µg/dl	19	17.5	92.1
MMA	50 µg/dl	19	19.8	104.2
N-acyl SDMA	50 µg/dl	19	18.7	98.4

The Eurolyser SDMA tests had no cross-reactivity to ADMA and L-arginine and had low (< 5%) cross-reactivity to MMA or N-Acyl SDMA.
LC-MS did not show significant cross-reactivity to any compound tested (< 1%).

When the cross-reactants were co-spiked with SDMA, LC-MS showed a positive bias in recovery, whereas the Eurolyser assay did show excellent recovery.

10. Summary

The Eurolyser SDMA test kit designed for solo and CUBE-VET analysers has a good correlation to LCMS.
The reproducibility as well as the stability of the test are very good and cross-reactivity is low.

11. Literature

1. Relationship between serum iohexol clearance, serum SDMA concentration, and serum creatinine concentration in nonazotemic dogs.
   Myles McKenna1 | Ludovic Pelligand1 | Jonathan Elliott2 | Daniel Cotter1 | Rosanne Jepson1
   1Department of Clinical Science and Services, Royal Veterinary College, London, UK
   2Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK