






Instructions for Use

SDMA vet ELISA

Enzymimmunoassay for the
Quantitative Determination of
Endogenous Symmetric Dimethylarginine (SDMA)
in Serum or Plasma of Dogs and Cats

For Veterinary Diagnostic

| | |
|---|----------|
|  REF | EA203/96 |
|  | 12 x 8 |
|  | 2 – 8 °C |


 DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
Adlerhorst 15 • 22459 Hamburg • Telefon: 040/ 555 87 10 • Fax: 040/ 555 87 111
Internet: <http://www.dld-diagnostika.de> • E-Mail: contact@dld-diagnostika.de

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Symbols

| | | | |
|---|------------------|---|-----------------------------------|
|  | Content |  | Expiry Date |
|  | Lot Number |  | Store at |
|  | Manufactured by |  | Sufficient for ... determinations |
|  | Catalogue Number |  | Consult Instructions for Use |

Hazard Pictograms



Warning



Danger

1 Introduction and Principle of the Test

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 1 in 3 cats and 1 in 10 dogs are likely to develop a kidney disease during their lifetime.

SDMA is an early biomarker of kidney function. It correlates well 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 will enable veterinarians to diagnose chronic kidney disease (CKD) much earlier than Creatinine or Cystatin C tests.

SDMA is specific for kidney function. It is not impacted by other diseases such as liver disease, cardiovascular disease, inflammatory disease and endocrine diseases. Another exciting feature of SDMA is that it 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 competitive SDMA-ELISA uses the microtiter plate format. SDMA is bound to the solid phase of the microtiter plate. SDMA in the samples is acylated and competes with solid phase bound SDMA for a fixed number of rabbit anti-SDMA antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase SDMA is detected by anti-rabbit / peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase SDMA is inversely proportional to the SDMA concentration of the sample.

2 Precautions

- Only for professional use.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- When handling the reagents, controls and samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- Some of the components of this test kit contain hazardous substances. These components bear the appropriate hazard symbol on their label. Further information can be found in 4. Contents of the kit and on the relevant safety data sheets.
- Avoid contact with individual reagents, which can cause irritation and chemical burns.
- Dispose of waste according to state and local environmental protection regulations.

3 Storage and Stability

On arrival, store the kit at 2 – 8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

4 Contents of the Kit


MT-Strips **STRIPS** 12 strips
8 wells each, break apart, precoated with SDMA



Standards (1 - 6) **CAL 1 - 6** 6 vials
each 4 ml, ready for use

| Standard | 1 | 2 | 3 | 4 | 5 | 6 |
|----------|---|-----|-----|-----|-----|-----|
| µmol/l | 0 | 0.2 | 0.4 | 0.7 | 1.2 | 3 |
| ng/ml | 0 | 40 | 81 | 141 | 242 | 606 |

Control 1 & 2 **CON 1 & 2** 2 vials
each 4 ml ready for use, Range: see QC certificate

Acylation Reagent **ACYL-REAG** 3 vials
lyoph., dissolve contents in 3 ml Solvent before use

Acylation Buffer **ACYL-BUFF** 1 vial
3.5 ml, ready for use, blue coloured  Warning

Solvent **SOLVENT** 2 vials
5 ml ready for use,
contains DMSO,
Please note that Solvent reacts with many plastic
materials including plastic trays; Solvent does not react
with normal pipette tips and with glass devices 
 Warning
Danger

Antiserum **AS** 1 vial
7 ml, ready for use, Rabbit-anti-N-acyl-SDMA, yellow coloured

Enzyme Conjugate **CONJ** 1 vial
13 ml, ready for use,
Goat-anti-rabbit-IgG-peroxidase  Warning

Wash Buffer **WASH** 1 vial
20 ml, conc. (50x), Dilute with dist. Water to 1000 ml total volume

| | | |
|---|-------------------|----------|
| Substrate 13 ml TMB Solution, ready for use | SUB | 1 vial |
| Stop Solution 13 ml, ready for use, contains 0.3M sulphuric acid, not corrosive | STOP | 1 vial |
| Reaction Plate For acylation | ACYL-PLATE | 1 piece |
| Equalizing Reagent lyoph., dissolve contents with 21 ml dist. water, dissolve carefully to minimize foam formation | EQUA-REAG | 1 vial |
| Foil Ready for use | FOIL | 2 pieces |

Additional materials and equipment required but not provided:

- Pipettes (20, 50, 100 and 200 μ l,)
- Multipette
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer, roll mixer

5 Sample Collection

5.1 Serum and Plasma

The test can be performed with serum as well as with EDTA plasma.

Hemolytic and lipemic samples should not be used.

The samples can be stored up to 6 hours at 2 – 8 °C. For a longer storage (up to 18 months) the samples must be kept frozen at -20 °C

Repeated freezing and thawing should be avoided.

6 Preparation of Reagents and Samples

6.1 Microtiter strips

Before opening the packet of strip wells **STRIPS**, allow it to stand at room temperature for at least 10 minutes. After opening, keep any unused wells in the original foil packet with the desiccant provided. Reseal carefully and store at 2 – 8 °C.

6.2 Wash Buffer

Dilute the contents of **WASH** with dist. water to a total volume of 1000 ml, mix shortly. The diluted wash buffer must be stored at 2 – 8 °C and is stable for 4 weeks. For longer storage the diluted wash buffer has to be stored frozen at -20 °C.

6.3 Equalizing Reagent

Dissolve the contents of **EQUA-REAG** with 21 ml dist. water, mix shortly and leave on a roll mixer for 20 minutes. Avoid excess formation of foam. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until expiry date.

6.4 Acylation Reagent

Dissolve the contents of one bottle **ACYL-REAG** with 3 ml Solvent **SOLVENT** and shake for 10 minutes on an orbital shaker. After use the reagent has to be discarded. The Acylation Reagent has always to be prepared immediately before use and is stable for minimum 3 hours. The two other bottles allow a

second and third run of the test. If the whole kit is to be used in one run, it is recommended to pool the dissolved contents of the two vials of Acylation Reagent.

Please note that Solvent reacts with many plastic materials including plastic trays which are used as reservoir for multichannel pipettes. Solvent does not react with normal pipette tips and with glass devices. It is recommended to use a multipipette, fill it directly from the vial and add the Acylation Reagent to the wells.

All other reagents are ready for use.

6.5 Preparation of Samples (Acylation)

The wells of the reaction plate **ACYL-PLATE** for the acylation can be used only once. Please mark the respective wells before use to avoid repeated use.

1. Pipette each 20 µl standard 1 - 6 **CAL 1 – 6**, each 20 µl control 1 & 2 **CON 1 & 2** and each 20 µl patient sample into the respective wells of the Reaction Plate **ACYL-PLATE** (duplicates are recommended).
2. Pipette 20 µl Acylation Buffer **ACYL-BUFF** into each well.
3. Pipette 200 µl reconstituted Equalizing Reagent **EQUA-REAG** (see 6.3) into each well.
4. Mix the reaction plate for 10 seconds.
5. Pipette 50 µl of freshly prepared Acylation Reagent **ACYL-REAG** (see 6.4) into each well, mix immediately.
It is recommended to use a multipipette, fill it directly from the vial and add the Acylation Reagent to the wells.
Colour changes to violet.
6. Incubate for 20 minutes at room temperature (approx. 20 °C) on an orbital shaker. Do not cover wells or plate, leave the plate open on the shaker.

Take each 20 µl of the acylated samples for the SDMA-ELISA.

7 Test Procedure ELISA

Bring all reagents to room temperature and mix them carefully, avoid development of foam.

7.1 Sample Incubation

Transfer each 20 µl acylated Standards 1 to 6, 20 µl acylated controls and 20 µl acylated samples from the Reaction Plate into the respective wells of the coated microtiter strips **STRIPS**.

Pipette 50 µl Antiserum **AS** into each well.

Cover the plate with adhesive foil **FOIL** and incubate Microtiter Strips for 90 minutes at room temperature (20 – 25 °C) on an orbital shaker.

7.2 Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl diluted Wash Buffer **WASH** (see 6.2). Repeat the washing procedure 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7.3 Conjugate Incubation

Pipette 100 µl enzyme conjugate **CONJ** into each well.

Incubate for 30 minutes at room temperature on an orbital shaker.

7.4 Washing

Repeat step 7.2.

7.5 Substrate Incubation

Pipette 100 µl Substrate **SUB** into each well and incubate for 25 ± 5 minutes at room temperature on an orbital shaker.

7.6 Stopping

Pipette 100 µl Stop Solution **STOP** into each well and mix on an orbital shaker for approx. 30 seconds.

7.7 Reading

Within 15 minutes, read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer.

8 Calculation of the Results

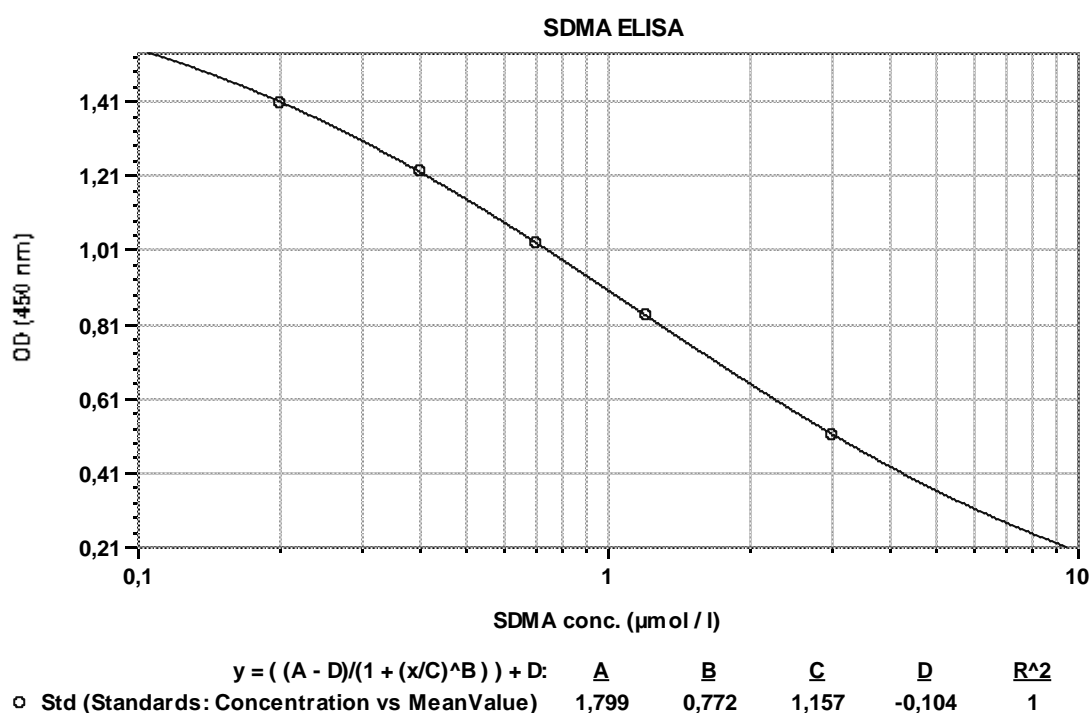
| | | | | | | |
|----------|---|-----|-----|-----|-----|-----|
| Standard | 1 | 2 | 3 | 4 | 5 | 6 |
| µmol/l | 0 | 0.2 | 0.4 | 0.7 | 1.2 | 3 |
| ng/ml | 0 | 40 | 81 | 141 | 242 | 606 |

Conversion factor: 1 µmol/l = 202 ng/ml = 20.2 µg/dl

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Cubic spline, 4 parameter or similar iteration procedures are recommended for evaluation of the standard curve.

The concentration of the controls and samples can be read directly from this standard curve by using their average optical density.

Typical standard curve:



Quality Control: Test results are valid only if the kit controls are within the ranges specified on the QC Certificate. Otherwise, the test should be repeated.

9 Assay Characteristics

9.1 Expected Values (Serum, EDTA-Plasma)

Dogs: 0.30 – 0.65 $\mu\text{mol/l}$ (6.0 – 13 $\mu\text{g/dl}$)

Cats: 0.30 – 0.75 $\mu\text{mol/l}$ (6.0 – 15 $\mu\text{g/dl}$)

The reference ranges given above should only be taken as a guideline. It is recommended that each laboratory should establish its own reference values.

9.2 Sensitivity

0.03 $\mu\text{mol/l}$ (0.6 $\mu\text{g/dl}$)

9.3 Recovery

9.3.1 Recovery Cat

Increasing amounts of SDMA were added to a cat serum sample. Each spiked sample was assayed. The analytical recovery of SDMA was estimated at 10 different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 97% (90 - 104 %).

| added [$\mu\text{mol/l}$] | measured [$\mu\text{mol/l}$] | expected [$\mu\text{mol/l}$] | % recovery |
|--------------------------------|-----------------------------------|-----------------------------------|------------|
| 0.00 | 0.58 | | |
| 0.12 | 0.68 | 0.70 | 97 |
| 0.24 | 0.77 | 0.82 | 91 |
| 0.35 | 0.86 | 0.93 | 92 |
| 0.45 | 0.91 | 1.03 | 88 |
| 0.55 | 0.99 | 1.13 | 88 |
| 0.65 | 1.24 | 1.23 | 101 |
| 0.77 | 1.71 | 1.35 | 127 |
| 1.04 | 1.90 | 1.62 | 117 |
| 1.35 | 1.95 | 1.93 | 101 |
| 1.65 | 2.29 | 2.23 | 103 |

mean value

97

9.3.2 Recovery Dog

Increasing amounts of SDMA were added to a dog serum sample. Each spiked sample was assayed. The analytical recovery of SDMA was estimated at 10 different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 104 %.

| added [$\mu\text{mol/l}$] | measured [$\mu\text{mol/l}$] | expected [$\mu\text{mol/l}$] | % recovery |
|---|--|--|-------------------|
| 0.00 | 0.54 | | |
| 0.12 | 0.74 | 0.66 | 112 |
| 0.24 | 0.74 | 0.78 | 95 |
| 0.35 | 0.86 | 0.89 | 97 |
| 0.45 | 0.94 | 0.99 | 95 |
| 0.55 | 1.01 | 1.09 | 93 |
| 0.65 | 1.19 | 1.18 | 101 |
| 0.77 | 1.51 | 1.31 | 115 |
| 1.04 | 1.73 | 1.58 | 109 |
| 1.35 | 2.19 | 1.89 | 116 |
| 1.65 | 2.23 | 2.19 | 102 |

mean value

104

9.4 Linearity

9.4.1 Linearity Cat

The linearity of the ELISA method was investigated using seven different dilutions of a cat serum sample. The mean linearity from all dilutions was 96%.

| dilution | measured [$\mu\text{mol/l}$] | recalculated value [$\mu\text{mol/l}$] | recovery % |
|----------|-----------------------------------|---|------------|
| orig. | 2.09 | | |
| 4 + 1 | 1.73 | 2.16 | 103 |
| 2 + 1 | 1.30 | 1.95 | 93 |
| 1 + 1 | 0.95 | 1.90 | 91 |
| 1 + 2 | 0.59 | 1.77 | 85 |
| 1 + 3 | 0.52 | 2.08 | 100 |
| 1 + 5 | 0.33 | 1.98 | 95 |
| 1 + 7 | 0.28 | 2.24 | 107 |

mean recovery 96

9.4.2 Linearity Dog

The linearity of the ELISA method was investigated using seven different dilutions of a dog serum sample. The mean linearity from all dilutions was 92%.

| dilution | measured [$\mu\text{mol/l}$] | recalculated value [$\mu\text{mol/l}$] | recovery % |
|----------|-----------------------------------|---|------------|
| orig. | 1.88 | | |
| 4 + 1 | 1.30 | 1.63 | 87 |
| 2 + 1 | 1.21 | 1.82 | 97 |
| 1 + 1 | 0.92 | 1.84 | 98 |
| 1 + 2 | 0.59 | 1.77 | 94 |
| 1 + 3 | 0.43 | 1.72 | 91 |
| 1 + 5 | 0.28 | 1.68 | 89 |
| 1 + 7 | 0.20 | 1.60 | 85 |

mean recovery 92

9.5 Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against SDMA used in the ELISA method. The tested compounds were Arginine, Homoarginine, Monomethylarginine (NMMA) and ADMA.

| Substance | ED-50-Value ($\mu\text{mol/l}$) | Cross Reactivity (%) |
|--------------|-----------------------------------|----------------------|
| SDMA | 1.39 | 100 |
| ADMA | 84 | 1.2 |
| NMMA | 182 | 0.76 |
| Homoarginine | 2807 | 0.05 |
| Arginine | 8574 | 0.016 |

9.6 Reproducibility

The reproducibility of the ELISA method was investigated by determining the intra- and inter-assay-coefficient of variation (cv) by repeated measurements of different serum samples with different SDMA concentrations (concentrations in $\mu\text{mol/l}$):

9.6.1 Intra-Assay Cat

| sample | n = | mean value | sd | cv (%) |
|--------|-----|------------|-------|--------|
| K1 | 40 | 0.837 | 0.064 | 7.6 |
| K2 | 40 | 0.862 | 0.049 | 5.7 |
| K3 | 40 | 0.770 | 0.058 | 7.7 |

9.6.2 Intra-Assay Dog

| sample | n = | mean value | sd | cv (%) |
|--------|-----|------------|-------|--------|
| H1 | 40 | 0.531 | 0.061 | 11.5 |
| H2 | 40 | 0.804 | 0.068 | 8.5 |
| H3 | 40 | 0.776 | 0.046 | 5.9 |

9.6.3 Inter-Assay Cat

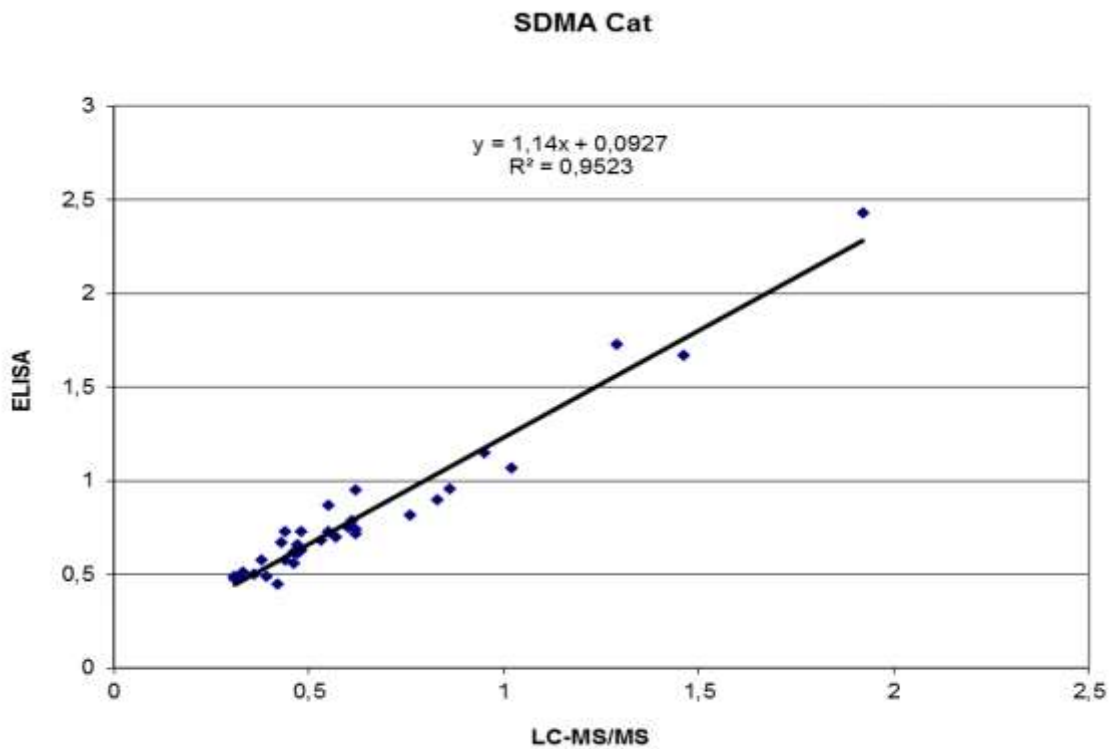
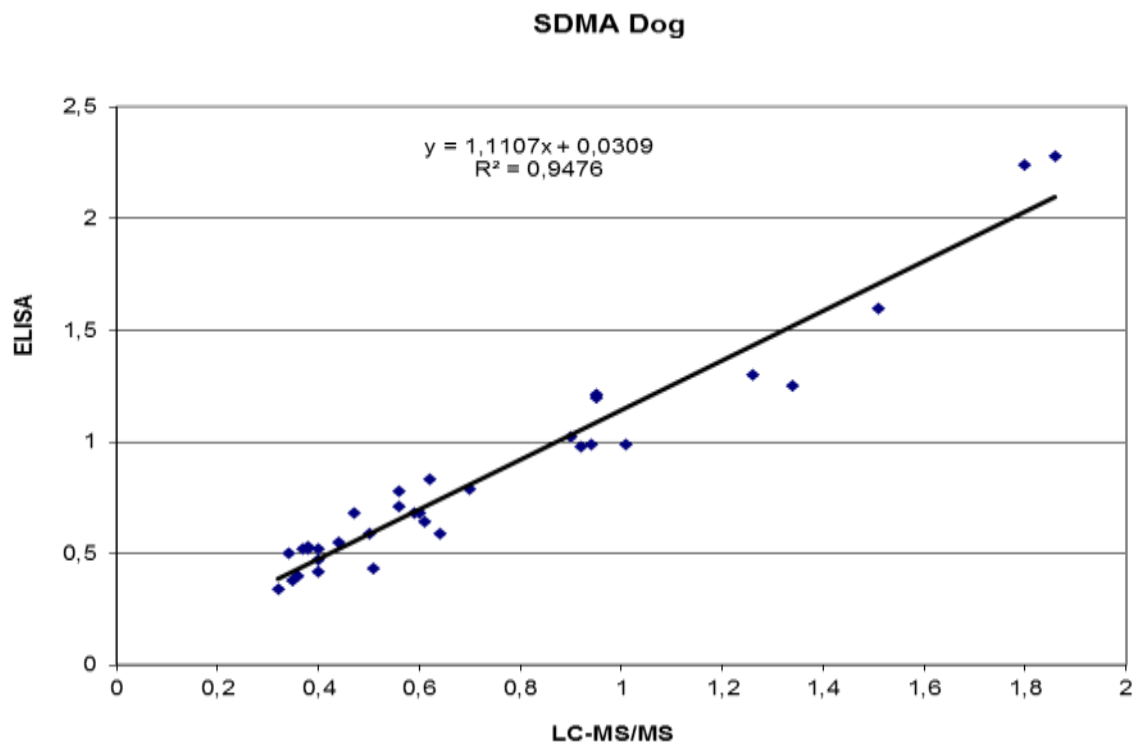
| sample | n = | mean value | sd | cv (%) |
|---------------|------------|-------------------|-----------|---------------|
| K1 | 32 | 0.49 | 0.037 | 7.5 |
| K2 | 32 | 0.59 | 0.043 | 7.3 |
| K3 | 32 | 0.72 | 0.079 | 11.0 |
| K4 | 32 | 0.74 | 0.058 | 7.8 |
| K5 | 32 | 0.84 | 0.068 | 8.1 |
| K6 | 32 | 0.84 | 0.982 | 9.7 |

9.6.4 Inter-Assay Dog

| sample | n = | mean value | sd | cv (%) |
|---------------|------------|-------------------|-----------|---------------|
| H1 | 32 | 0.49 | 0.042 | 8.5 |
| H2 | 32 | 0.66 | 0.046 | 7.0 |
| H3 | 32 | 0.76 | 0.063 | 8.2 |
| H4 | 32 | 0.93 | 0.069 | 7.4 |
| H5 | 32 | 1.21 | 0.128 | 10.6 |
| H6 | 32 | 1.35 | 0.152 | 11.3 |

9.7 Correlation ELISA to LC-MS/MS

Correlation ELISA to the LC-MS/MS method



10 Literature

- Josipa Kuleš, Petra Bilić, Blanka Beer Ljubić, Jelena Gotić, Martina Crnogaj, Mirna Brkljačić, Vladimir Mrljak
Glomerular and tubular kidney damage markers in canine babesiosis caused by *Babesia canis*
Ticks and Tick-borne Diseases (2018) **9** 1508 - 1517
- Bode-Böger S.M., Scalera F., Kielstein J.T., Martens-Lobenhoffer J., Breithardt G., Fobker M., Reinecke H.
Symmetrical Dimethylarginine: A new combined parameter for renal function and extent of coronary artery disease
J. Am. Soc. Nephrol. (2006) **17**: 1128-1134
- Kielstein J.T., Salpeter S.R.; Bode-Böger S.M., Cooke J.P., Fliser D.
Symmetric dimethylarginine (SDMA) as endogenous marker of renal function – a meta-analysis
Nephrol. Dial. Transplant (2006) **21**: 2446 – 2451

11 Changes to declare

Version _11: IFU has been re-formatted. Precautions and 8 Calculation of the Results have been complemented (highlighted in grey). Component names as printed on labels were included in pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols.

Pipetting Scheme - Sample Preparation

| | | Standards | Controls | Samples |
|------------------|----|-----------|----------|---------|
| ACYL-PLATE: | | | | |
| CAL 1 - 6 | µl | 20 | | |
| CON 1 & 2 | µl | | 20 | |
| Sample | µl | | | 20 |
| ACYL-BUFF | µl | 20 | 20 | 20 |
| recon. EQUA-REAG | µl | 200 | 200 | 200 |

Shake for 10 seconds

| | | | | |
|-----------------------------|----|----|----|----|
| recon. ACYL-REAG (fresh) | µl | 50 | 50 | 50 |
|-----------------------------|----|----|----|----|

Incubate for 20 minutes at room temperature on an orbital shaker
Take 20 µl each for ELISA

Pipetting Scheme - ELISA

| | | Acyl. Standards | Acyl. Controls | Acyl. Samples |
|---|----|--------------------|-------------------|------------------|
| STRIPS: | | | | |
| Transfer from ACYL-PLATE into STRIPS | µl | 20 | 20 | 20 |
| AS | µl | 50 | 50 | 50 |

Cover frame with FOIL and incubate on an orbital shaker
for 90 minutes at room temperature
Wash 4 x with 300 µl WASH per well

| | | | | |
|------|----|-----|-----|-----|
| CONJ | µl | 100 | 100 | 100 |
|------|----|-----|-----|-----|

Incubate for 30 minutes at room temperature on an orbital shaker
Wash 4 x with 300 µl WASH per well

| | | | | |
|-----|----|-----|-----|-----|
| SUB | µl | 100 | 100 | 100 |
|-----|----|-----|-----|-----|

Incubate for 25 ± 5 minutes at room temperature on an orbital shaker

| | | | | |
|------|----|-----|-----|-----|
| STOP | µl | 100 | 100 | 100 |
|------|----|-----|-----|-----|

Within 15 minutes, read absorbance at 450 nm