DIAGNOSTIKA GMBH

Instruction for Use

SDMA vet ELISA

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

For Veterinary Diagnostic

REF EA203/96

12 x 8

2 – 8 °C

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Contents

Symbols

CONT	Contents		Expiry Date
LOT	Lot Number	+2 +8 °C	Store
	Manufactured by	Σ	Sufficient for
REF	Catalogue Number		Consult Instructions

Hazard Pictograms

Danger

Warning

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

- Disposable gloves should be used.
- 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.

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.
Do not use components beyond the expiration date shown on the labels.
Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

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

4.2 **Standards 1 - 6** **CAL 1 – 6** 6 vials
Each 4 ml, ready for use
Concentrations:

Standard	1	2	3	4	5	6
µmol/l	0	0.2	0.4	0.7	1.2	3.0
ng/ml	0	40	81	141	242	606

4.3 **Control 1 & 2** **CON 1 & 2** 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

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


Warning

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

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

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


Warning

4.8 **Wash Buffer** **WASH** 1 vial
20 ml, 50 x concentrated
Dilute contents with dist. water to 1000 ml total volume.

4.9 **Substrate** **SUB** 1 vial
13 ml TMB solution, ready for use

4.10 **Stop Solution** **STOP** 1 vial
13 ml, ready for use
Contains 0.3 M sulphuric acid, not corrosive

4.11 **Reaction Plate** **ACYL-PLATE** 1 piece
for acylation

4.12 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilised, dissolve contents with 21 ml dist. water,
dissolve carefully to minimize foam formation

4.13 **Solvent** **SOLVENT** 2 vials
5 ml, 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


Danger


Warning

4.14 **Foil** **FOIL** 2 pieces

Additional materials and equipment required but not provided:

- Pipettes (20, 50, 100 and 200 µl,)
- 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 **STRIPS**

Before opening the packet of strip wells, 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 **WASH**

Dilute the contents 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 **EQUA-REAG**

Dissolve the contents 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 **ACYL-REAG**

Dissolve the contents of one bottle in 3 ml 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 multipette, 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 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 & CON 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 all wells.

3. Pipette 200 µl reconstituted Equalizing Reagent [EQUA-REAG] into all wells.

4. Mix the reaction plate for 10 seconds.

5. Prepare Acylation Reagent freshly [ACYL-REAG + SOLVENT] and pipette 50 µl prepared Acylation Reagent each into all wells, mix immediately.
   It is recommended to use a multipette, 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

Pipette each 20 µl acylated Standards 1 to 6, 20 µl acylated controls and 20 µl acylated samples from the Reaction Plate [ACYL-PLATE] into the respective wells of the coated microtiter strips [STRIPS].

Pipette each 50 µl Antiserum [AS] into all wells.

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 Wash Buffer [WASH]. Repeat the washing procedure 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7.3 Conjugate Incubation

Pipette each 100 µl enzyme conjugate [CONJ] into all wells.
Incubate for 30 minutes at room temperature on an orbital shaker.

7.4 Washing

Repeat step 7.2.

7.5 Substrate Incubation

Pipette each 100 µl Substrate [SUB] into all wells and incubate for 25 ± 5 minutes at room temperature on an orbital shaker.

7.6 Stopping

Pipette each 100 µl Stop Solution [STOP] into 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.

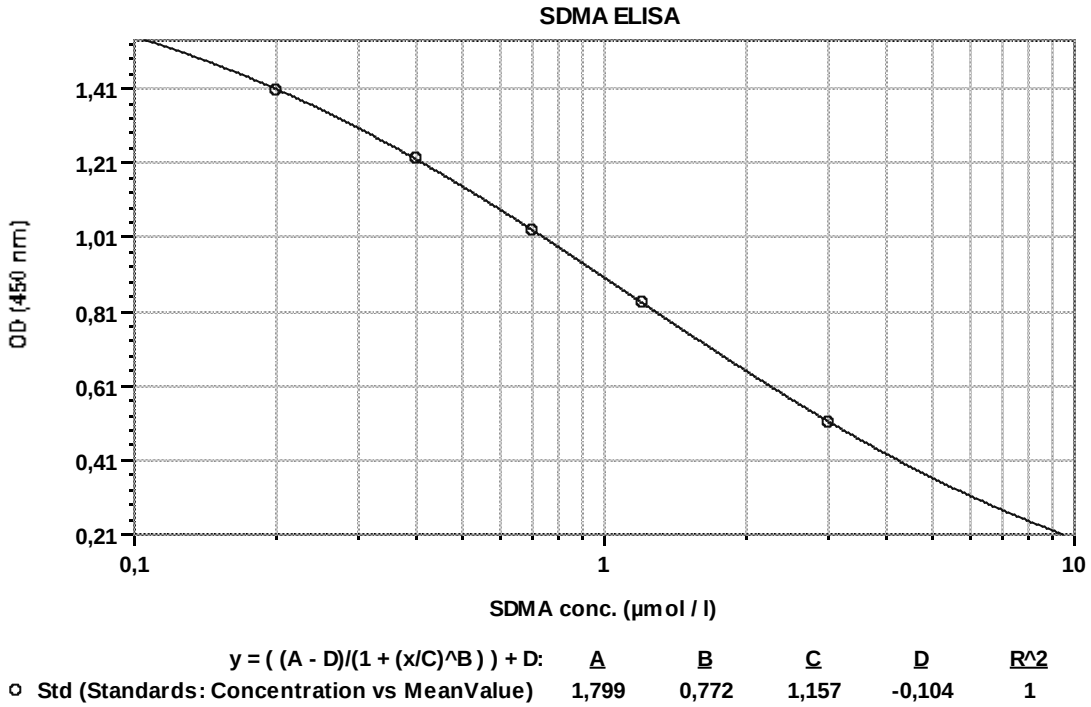
8. Calculation of the Results

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:



9. Assay Characteristics

9.1 Expected Values (Serum, EDTA-Plasma)

Dogs: 0.30 – 0.65 µmol/l (6.0 – 13 µg/dl)

Cats: 0.30 – 0.75 µmol/l (6.0 – 15 µg/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 µmol/l (0.6 µg/dl)

9.3 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	measured	expected	% 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**

concentrations in µmol/l

9.3 **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	**measured**	**expected**	**% 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**

concentrations in µmol/l

9.4 **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	recalculated value	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**

concentrations in µmol/l

9.5 **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	recalculated value	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**

concentrations in µmol/l

9.6 **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 (µmol/l)**	**Cross Reactivity (%)**
SDMA	1.39	100
ADMA	84	1.2
NMMA	182	0.76
Homoarginine	2807	0.05
Arginine	8574	0.016

9.7 **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 µmol/l):

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

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

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

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

9.8 Correlation ELISA to LC-MS/MS

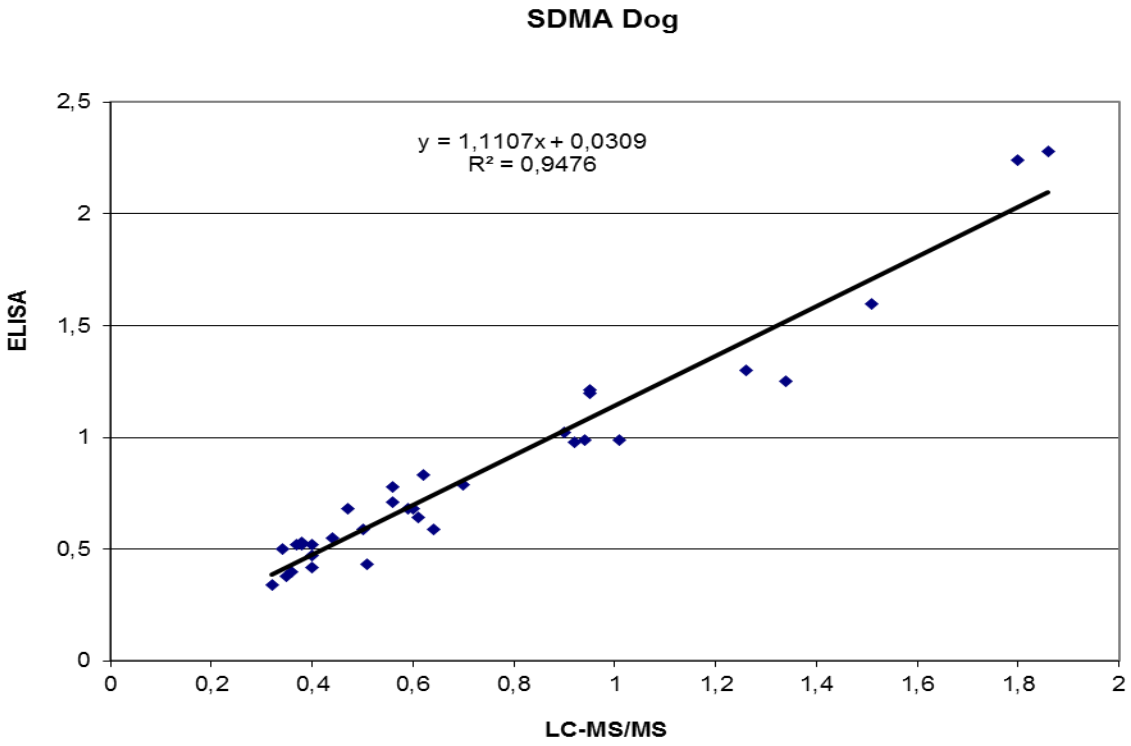


Figure 1

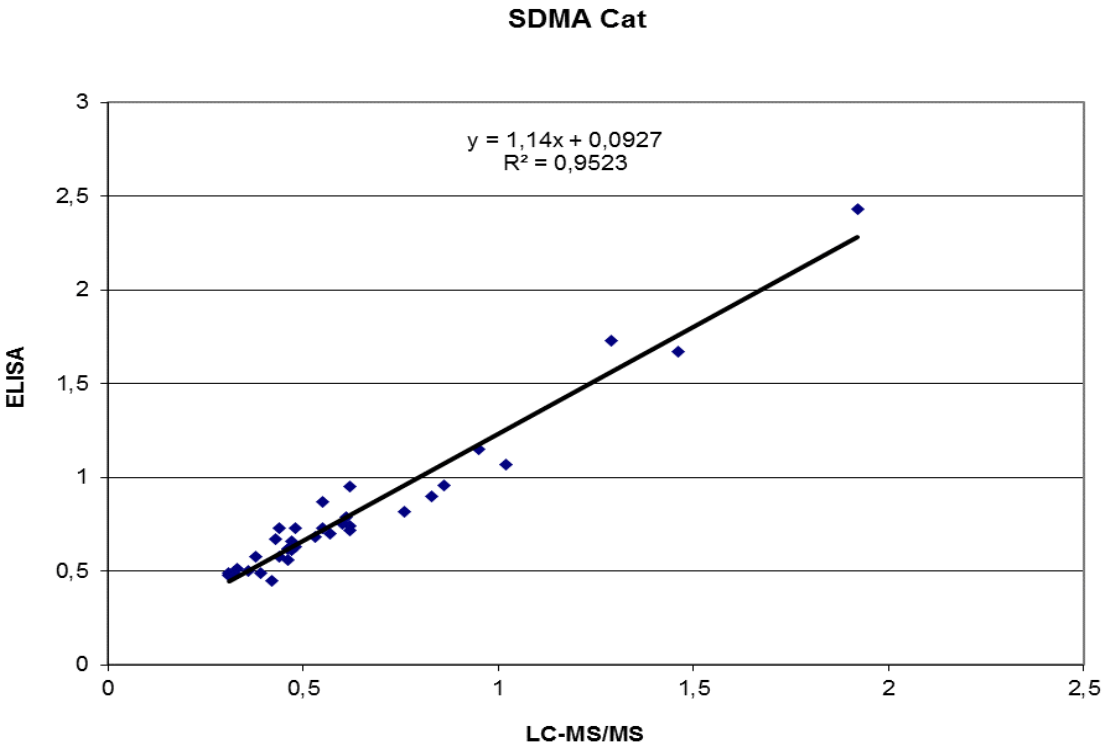


Figure 2

The figures 1 & 2 shows the 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

Pipetting Scheme
Sample Preparation

		Standard	Control	Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Patient Sample	µl			20
Acylation Buffer	µl	20	20	20
Equalizing Reagent	µl	200	200	200

shake for 10 seconds

freshly prepared Acylation Reagent	µl	50	50	50

incubate for 20 minutes at room temperature on an orbital shaker

take 20 µl each for ELISA

Pipetting Scheme ELISA

	Standard	Control	Sample
Acylated Standard 1 - 6 µl	20		
Acylated Control 1 & 2 µl		20	
Acylated Patient Sample µl			20
Antiserum µl	50	50	50

cover frame with foil and incubate on an orbital shaker
for 90 minutes at room temperature

wash 4 x with each 300 µl Wash Buffer

Enzyme Conjugate µl	100	100	100

incubate for 30 minutes at room temperature on an orbital shaker

wash 4 x with each 300 µl Wash Buffer

Substrate µl	100	100	100

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

Stop Solution µl	100	100	100

within 15 minutes, read absorbance at 450 nm