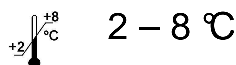
Instruction for Use

SDMA - ELISA

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

REF EA203/96

12 x 8

 2 – 8 °C

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August 2011

Contents

1. Introduction and Principle of the Test Page 4
2. Precautions Page 5
3. Storage and Stability Page 5
4. Contents of the Kit Page 5
5. Sample Collection Page 7
6. Preparation of Reagents and Samples Page 7
7. Test Procedure (ELISA) Page 9
8. Calculation of the Results Page 10
9. Assay Characteristics Page 11
10. Literature Page 14

Pipetting Scheme Sample Preparation Page 15
Pipetting Scheme ELISA Page 16

1. Introduction and Principle of the Test

Dosing of most drugs must be adapted in renal insufficiency, making accurate assessment of renal function an essential component of diagnostics in clinical medicine. Furthermore, even modest impairment of renal function has been recognized as a cardiovascular risk factor. As the most commonly used marker of renal excretory function, serum creatinine concentration, does not adequately respond to mild to moderate impairment of renal function, more sensitive markers for renal excretory function are urgently seeked, especially in mild stages of renal impairment. SDMA is a methylated derivative of the amino acid L-arginine (symmetric dimethylarginine). SDMA is eliminated from the body exclusively by renal excretion; therefore SDMA plasma concentration is tightly related to renal function. Thus, quantification of plasma SDMA is an adequate means to assess renal function, as could be demonstrated in a series of recent clinical trials: In 18 clinical studies involving more than 2,100 patients systemic SDMA concentrations were highly correlated with inulin clearance as well as with various clearance estimates and better corresponded to mild renal function impairment than serum creatinine.

Thus, SDMA exhibits properties of a reliable marker of renal function. Furthermore, there is evidence showing that elevated SDMA levels, as they may occur in renal function impairment, may prospectively indicate future risk of cardiovascular disease and mortality independently of the level of renal impairment.

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

- For in vitro use only.
- 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	0.08	0.25	0.5	0.8	3.0

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

4.5 **Acylation Reagent** — ACYL-REAG — 2 vials
lyophilised, dissolve content
in 2.8 ml Solvent, if necessary
combine the contents of both vials (see 6.4. also)

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

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

4.8 **Wash Buffer** WASH 1 vial
20 ml, concentrated
Dilute content with dist. water to 500 ml total volume.

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

4.10 **Stop Solution** STOP 1 vial
12 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
lyophilzed, dissolve content with 20.5 ml dist. water,
dissolve carefully to minimize foam formation

4.13 **Solvent** SOLVENT 1 vial
6 ml Aceton/ 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)

Additional materials and equipment required but not provided:

- Pipettes (20, 25, 50, 100 and 250 µl,)
- 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 24 hours at 2 - 8 °C. For a longer storage (up to 24 months) the samples must be 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 content with dist. water to a total volume of 500 ml.
The diluted wash buffer must be stored at 2 - 8 °C.

6.3. Equalizing Reagent EQUA-REAG

Dissolve the content with 20.5 ml dist. water, mix shortly and leave on a roll mixer for 30 minutes. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable for a mini mum of 1 year.

6.4. Acylation Reagent ACYL-REAG

Dissolve the content of one bottle in 2.8 ml Solvent and shake for 5 minutes on a orbital shaker. After use the reagent has to be discarded. The Acylation Reagent has always to be prepared immediately before use. The second bottle allows a second 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.

Attention
Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette, or similar device, fill the syringe directly from the vial (using a yellow tip) with dissolved Acylation Reagent and add well by well.

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, each 20 µl control 1 & 2 and each 20 µl patient sample into the respective wells of the Reaction Plate.
2. Pipette 25 µl Acylation Buffer into all wells.
3. Pipette 200 µl Equalizing Reagent into all wells.
4. Mix the reaction plate for 10 seconds.
5. Prepare Acylation Reagent freshly and pipette 50 µl prepared Acylation Reagent each into all wells, mix **immediately.**

   Attention
   Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette, or similar device, fill the syringe directly from the vial (using a yellow tip) with dissolved Acylation Reagent and add well by well.
6. Incubate for 90 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 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 prepared Standards 1 to 6, 20 µl prepared controls and 20 µl prepared samples into the respective wells of the coated microtiter strips (duplicates are recommended).

Pipette each 50 µl Antiserum into all wells and shake shortly on an orbital shaker.

Cover the plate with adhesive foil and incubate Microtiter Strips for 12 –20 hours (overnight) at 2-8℃.

7.2 Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer (Shake shortly on an orbital shaker). 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 into all wells.
Incubate for 60 minutes at room temperature on an orbital shaker.

7.4 Washing

Repeat step 7.2.

7.5 Substrate Incubation

Pipette each 100 µl Substrate into all wells and incubate for 20 to 30 minutes at room temperature on an orbital shaker.

7.6 Stopping

Pipette each 100 µl Stop Solution into all wells.

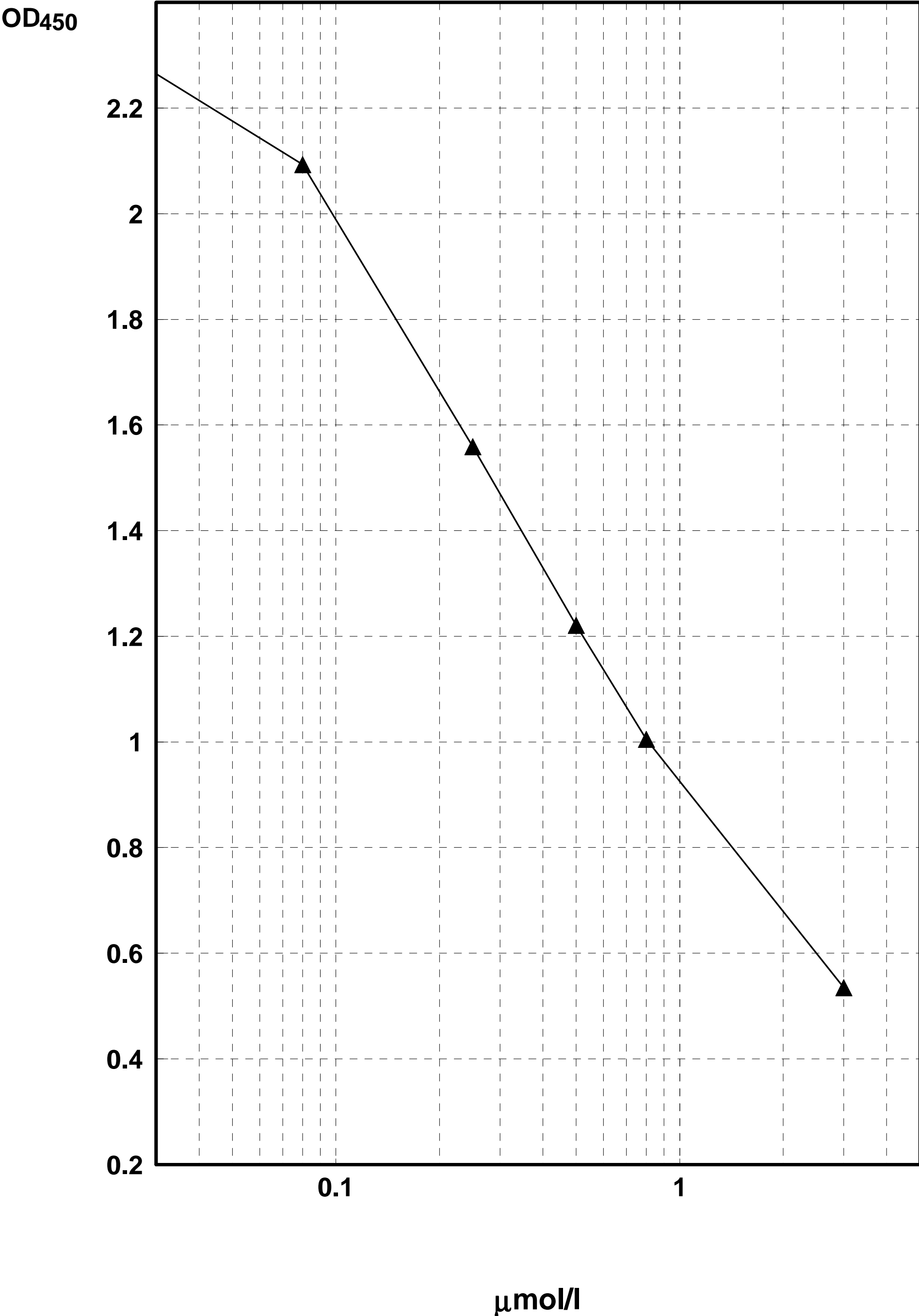
7.7 Reading

Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer.

8. Calculation of the Results

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

Expected Values

0.3 – 0.7 µmol/l (60 – 140 ng/ml)

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

Sensitivity

0.05 µmol/l

Recovery

Increasing amounts of SDMA were added to a serum sample. Each spiked sample was assayed. The analytical recovery of SDMA was estimated at 14 different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 104% (97 - 109%).

added	measured	expected	% recovery
0	0.36		
0.1	0.48	0.46	104
0.2	0.58	0.56	104
0.3	0.72	0.66	109
0.4	0.82	0.76	108
0.5	0.93	0.86	108
0.6	0.95	0.96	99
0.8	1.23	1.16	106
1.00	1.43	1.36	105
1.25	1.56	1.61	97
1.5	1.96	1.81	108
1.75	2.29	2.11	109
2.0	2.43	2.36	103
2.25	2.74	2.61	105
2.5	2.77	2.86	97

mean value **104**

concentrations in µmol/l

Linearity

The linearity of the ELISA method was investigated using nine different dilutions of a serum sample. The mean linearity from all dilutions was 98% (90 - 106%)

dilution	measured	recalculated value	recovery %
orig.	2.36		
4 + 1	1.693	2.12	90
2 + 1	1.661	2.49	106
1 + 1	1.133	2.27	96
1 + 2	0.775	2.33	99
1 + 4	0.455	2.28	97
1 + 6	0.344	2.41	102
1 + 9	0.228	2.28	97
1 + 15	0.143	2.29	97
1 + 24	0.089	2.23	94
		mean recovery	**98**

concentrations in µmol/l

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, Monomethylarginine (NMMA) und ADMA.

Substance	ED-50-Value (ng/ml)	Cross Reactivity (%)
SDMA	0,36	100
Arginin	4,500	< 0.01
NMMA	43	0.70
ADMA	81	0.44

Reproducibility

The reproducibility of the ELISA method was investigated by determing the intra-assay-coefficients of variation (cv) by repeated measurements of different serum samples with different SDMA concentrations.

Intra-Assay Variation

sample	n =	mean value	sd	cv (%)
1	40	0.524	0.030	5.7
2	40	0.752	0.046	6.1
3	40	1.723	0.081	4.7

9. Literature

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

Wanby P., Teerlink T., Brudin L., Brattström L., Nilsson I., Palmqvist P., Carlsson M.
Asymmetric dimethylarginine (ADMA) as a risk marker for stroke and TIA in a Swedish population
Atherosclerosis (2006) **185**: 271 - 277

Pipetting Scheme
Sample Preparation

		Standard	Control	Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Patient Sample	µl			20
Acylation Buffer	µl	25	25	25
Equalizing Reagent	µl	200	200	200

shake for 10 seconds

freshly prepared Acylation Reagent	µl	50	50	50

incubate for 90 minutes at room temperature on an orbital shaker
do not cover wells or plate, leave the plate open on the shaker

Pipetting Scheme ELISA

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

shake shortly on an orbital shaker

incubate 12 – 20 hours (overnight) at 2 - 8 °C cove red with foil

wash 4 x with each 250 µl Wash Buffer

Enzyme Conjugate	µl	100	100	100

shake for 60 minutes at room temperature

wash 4 x with each 250 µl Wash Buffer

Substrate	µl	100	100	100

shake for 20 - 30 minutes at room temperature

Stop Solution	µl	100	100	100

read absorbance at 450 nm