

SDMA ELISA

Cat. No.: REA203/96



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

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 sought, 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 A-F** **CAL 1 – 6** 6 vials
- Each 4 ml, ready for use
Concentrations:

Standard	A	B	C	D	E	F
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 bottle
- 3.5 ml, ready for use

4.5 Acylation Reagent lyophilised, dissolve content in 1.5 ml dimethylformamide (DMF), if necessary combine the contents of both vials (see 6.4. also)	ACYL-REAG	2 vials
4.6 Antiserum 5.5 ml, ready for use Rabbit-anti-N-acyl-SDMA	AS	1 vials
4.7 Enzyme Conjugate 12 ml, ready for use goat anti-rabbit-IgG-peroxidase	CONJ	1 vial
4.8 Wash Buffer 50 ml, concentrated Dilute content with dist. water to 500 ml total volume.	WASH	1 bottle
4.9 Substrate 12 ml TMB solution, ready for use	SUB	1 vial
4.10 Stop Solution 12 ml, ready for use Contains 0.3 M sulphuric acid, not corrosive	STOP	1 vial
4.11 Reaction Plate for acylation	ACYL-PLATE	1 piece
4.12 Equalizing Reagent lyophilized, dissolve content with 5 ml dist. water, dissolve carefully to minimize foam formation	EQUA-REAG	1 vial
4.13 Solvent) 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)	SOLVENT	1 vial

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. Repeal 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 ml dist. water, mix shortly and leave on a roll mixer for 30 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable for a minimum 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.

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 A - F, 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**.
6. Incubate for 90 minutes at room temperature (approx. 20 °C) on an orbital 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 A to F, 50 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°C.

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

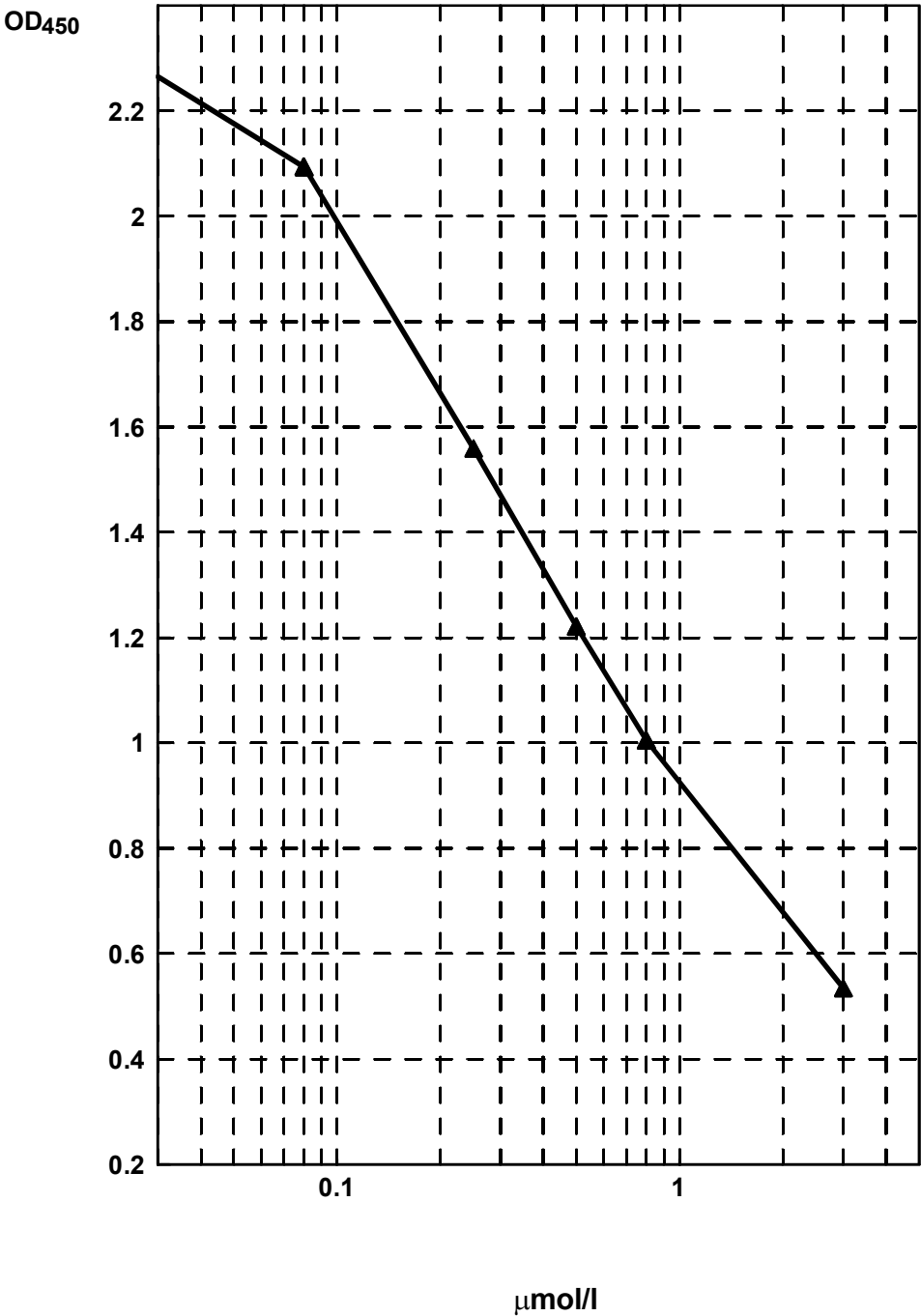
Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer.

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).

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 $\mu\text{mol/l}$ (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 $\mu\text{mol/l}$

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 $\mu\text{mol/l}$

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% (94 - 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

Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against ADMA used in the ELISA method. The tested compounds were Arginine, Monomethylarginine (NMMAI) and 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 determining the intra- und inter-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

10. 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

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

10 seconds shaking

freshly prepared Acylation Reagent	μl	50	50	50
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90 minutes incubation at room temperature on an orbital shaker

Pipetting Scheme ELISA

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

Shortly shaking on an orbital shaker

12 – 20 hours (overnight) incubation at 2 - 8 °C

4 x washing with each 250 µl Wash Buffer

Enzyme Conjugat	µl	100	100	100
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60 minutes shaking at room temperature

4 x washing with each 250 µl Wash Buffer

Substrate	µl	100	100	100
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20 - 30 minutes shaking at room temperature

Stop Solution	µl	100	100	100
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Reading of absorbance at 450 nm

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