DIAGNOSTIKA GMBH

Instruction for use

ADMA High Sensitive ELISA

Enzyme Immunoassay
for the Quantitative Determination of
Asymmetric Dimethylarginine (ADMA)
in Serum or Plasma of Mice, Rats and in Cell Culture Media

REF EA209/96

12 x 8

2 – 8 °C

DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
Adlerhorst 15 • 22459 Hamburg • Germany
Tel +49-40-555 87 10 • Fax +49-40-555 87 111
Internet: http://www.dld-diagnostika.de • E-Mail: contact@dld-diagnostika.de

Contents

1. Introduction and Principle of the Test

The vascular endothelium plays a central role in the regulation of vascular structure and function, mainly due to the formation of endothelium-derived nitric oxide (NO). NO has been named an “endogenous anti-atherogenic molecule” due to its diverse regulatory functions in vascular homeostasis.

NO is formed by the enzyme NO synthetase (NOS) from the amino acid precursor L-arginine. NOS activity can be downregulated by asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS.

The effects of ADMA on NO synthesis and NO-mediated pathophysiological processes have been described in numerous experimental studies. Moreover, elevated ADMA levels in plasma have been found in clinical studies including patients with hyper-cholesterolemia, hypertension, chronic heart failure, chronic renal failure and other internal disorders.

Recent prospective and cross-sectional studies indicated that elevated ADMA levels are a risk factor for future cardiovascular events and total mortality. ADMA may have diagnostic relevance as a novel cardiovascular risk marker.

The new competitive ADMA - high sensitive - ELISA uses the microtiter plate format. ADMA is bound to the solid phase of the microtiter plate. ADMA in the samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit anti-ADMA 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 ADMA 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 ADMA is inversely proportional to the ADMA 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
coated with ADMA

4.2 **Standards 1 - 7** **CAL 1 – 7** 7 vials
each 4 ml, ready for use
Concentrations:

Standard	1	2	3	4	5	6	7
µmol/l	0	0.1	0.3	0.6	1.0	2.0	5.0

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** 3 vials
lyophilised, dissolve content
in 2.8 ml Solvent before use; if required
combine the contents of both vials (see also 6.)

4.6 **Antiserum** **AS** 1 vial
5.5 ml, colour coded blue, ready for use
Rabbit-anti-N-Acyl-ADMA

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

4.8 **Wash Buffer** **WASH** 1 bottle
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
lyophilized, dissolve content with 20.5 ml dist. water, dissolve carefully to minimize foam formation

4.13 **Solvent** **SOLVENT** 2 vials
each 6 ml, contains acetone/ 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 200 µl)
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer
- Roll mixer

5. Sample Collection

The test can be performed with serum, EDTA or heparin plasma and cell culture media.
Culture media containing high concentrations of Arginine may influence the steepness of the standard curve and the sensitivity of the test. Therefore, it is recommended to use media with no or low level Arginine.

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 (at least 12 months) the samples must be kept frozen at -20 °C

Repeated freezing and thawing should be avoided.

6. Preparation of Reagents

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.

Wash Buffer **WASH**

Dilute the content with dist. water to a total volume of 500 ml.
The diluted wash buffer has to be stored at 2 - 8 °C.

Equalizing Reagent **EQUA-REAG**

Dissolve the content with 20.5 ml dist. water, mix shortly and leave on a roll mixer or orbital shaker 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.

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 three bottles allow three separate runs of the test. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of two vials of Acylation Reagent.

Please note that Solvent reacts with many plastic materials including plastic trays. 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.

7. Test Procedure for Serum and Plasma Samples

7.1. Preparation of Serum and Plasma 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 - 7, each 20 µl control 1 & 2 and each 20 µl 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 just before use 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 with a yellow tip (or similar device), fill the syringe directly from the vial 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 the wells or the plate; leave the plate open on the shaker.

Take each 25 µl for the ELISA.

7.2. ELISA for Serum and Plasma Samples

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

Sample Incubation
Pipette each 25 µl prepared Standards 1 to 7, 25 µl prepared controls and 25 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 briefly on an orbital shaker.

Cover the plate with adhesive foil and incubate Microtiter Strips for 15 –20 hours (overnight) at 2 – 8 °C.

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.

Conjugate Incubation
Pipette each 100 µl enzyme conjugate into all wells.
Incubate for 60 minutes at room temperature on an orbital shaker.

Washing
Repeat washing as described above.

Substrate Incubation
Pipette each 100 µl Substrate into all wells and incubate for 25 to 35 minutes at room temperature on an orbital shaker.

Stopping
Pipette each 100 µl Stop Solution into all wells.

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

8. Test Procedure for Cell Culture Samples

8.1. Preparation of Cell Culture 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 - 7, each 20 µl control 1 & 2 and each 20 µl cell culture sample into the respective wells of the Reaction Plate.

2. Pipette 20 µl standard 1 (zero standard) into all wells containing cell culture samples.

3. Pipette 20 µl cell culture medium into all wells containing standards 1 – 7 and controls 1 & 2 (to balance matrix differences).

4. Pipette 25 µl Acylation Buffer into all wells.

5. Pipette 200 µl Equalizing Reagent into all wells.

6. Mix the reaction plate for 10 seconds.

7. Prepare Acylation Reagent just before use 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 with a yellow tip (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

8. Incubate for 90 minutes at room temperature (approx. 20 °C) on an orbital shaker. Do not cover the wells or the plate; leave the plate open on the shaker.

Take each 25 µl for the ELISA.

8.2. ELISA for Cell Culture Samples

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

Sample Incubation
Pipette each 25 µl prepared Standards 1 to 7, 25 µl prepared controls and 25 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 briefly on an orbital shaker.

Cover the plate with adhesive foil and incubate Microtiter Strips for 15 –20 hours (overnight) at 2 – 8 °C.

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.

Conjugate Incubation
Pipette each 100 µl enzyme conjugate into all wells.
Incubate for 60 minutes at room temperature on an orbital shaker.

Washing
Repeat washing as described above.

Substrate Incubation
Pipette each 100 µl Substrate into all wells and incubate for 25 to 35 minutes at room temperature on an orbital shaker.

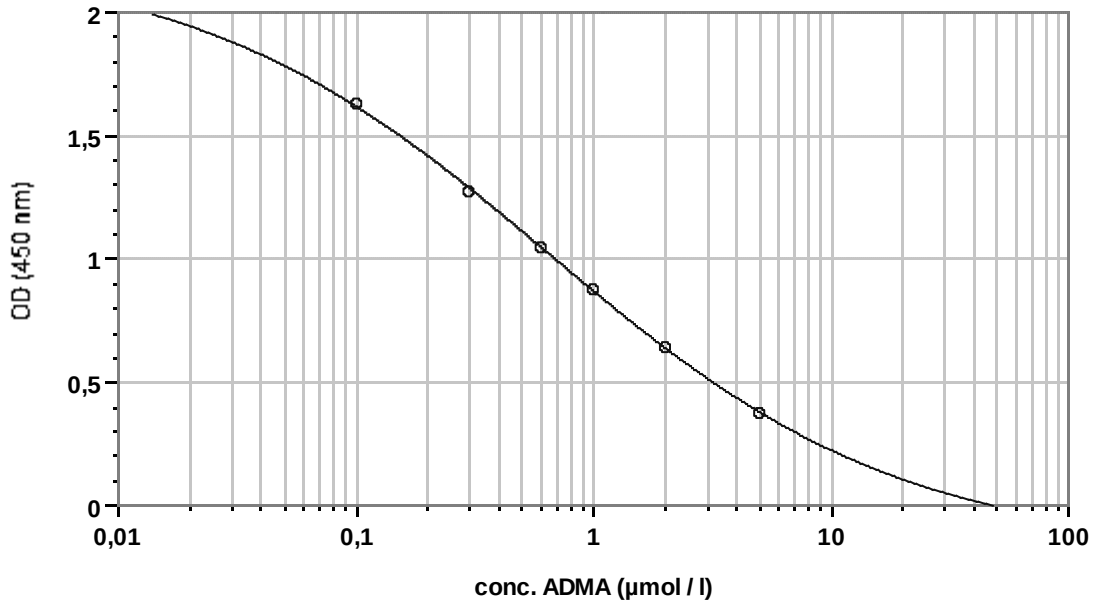
Stopping
Pipette each 100 µl Stop Solution into all wells.

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

9. 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:



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
o	Std (Standards: Concentration vs MeanValue)	2,221	0,586	0,645	-0,178	1

10. Assay Characteristics

Sensitivity

The lower limit of detection was determined by taking the 2fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

lower limit of detection: **0.01 µmol/l**

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 (NMMA) und SDMA.

Substance	ED-50-Value (µmol/l)	Cross Reactivity (%)
ADMA	0.745	100
Arginin	3,993	< 0.020
SDMA	2,990	0.025
NMMA	51	1.460

Reproducibility

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

Intra-Assay Variation

sample	n =	mean value	sd	cv (%)
Rat Plasma	40	0.40	0.033	8.3
Rat Serum	40	0.99	0.075	7.6

Recovery

Increasing amounts of ADMA were added to a sample. Each spiked sample was assayed. The analytical recovery of ADMA was calculated at different concentrations by using the theoretically expected and the actually measured values. Shown concentrations are in µmol/l.

Rat Heparin Plasma

added	measured	expected	% recovery
0	0.58		
0.10	0.71	0.68	104
0.19	0.80	0.77	104
0.28	0.89	0.86	103
0.37	0.95	0.95	100
0.45	0.98	1.03	95
0.61	1.21	1.19	102
0.85	1.43	1.43	100
1.11	1.97	1.69	116
1.36	2.14	1.94	110
1.61	2.34	2.19	107
1.92	2.64	2.50	106
		mean	**104**

Rat EDTA Plasma

added	measured	expected	% recovery
0	0.51		
0.10	0.59	0.61	97
0.19	0.75	0.70	107
0.28	0.82	0.79	104
0.37	0.98	0.88	111
0.45	0.86	0.96	90
0.61	1.07	1.12	96
0.85	1.34	1.36	99
1.11	1.68	1.62	104
1.36	1.94	1.87	104
1.61	2.04	2.12	96
1.92	2.36	2.43	97
		mean	**100**

Rat Serum

added	measured	expected	% recovery
0	1.05		
0.10	1.16	1.15	101
0.19	1.22	1.24	98
0.28	1.31	1.32	99
0.37	1.27	1.42	89
0.45	1.26	1.50	84
0.61	1.51	1.66	91
0.85	2.23	1.90	117
1.11	2.42	2.16	112
1.36	2.25	2.41	93
1.61	2.63	2.66	99
1.92	2.74	2.97	92
		mean	**98**

Mouse Serum

added	measured	expected	% recovery
0	0.23		
0.19	0.47	0.42	112
0.28	0.55	0.51	108
0.37	0.60	0.60	100
0.45	0.69	0.68	101
0.61	0.88	0.84	105
0.85	1.14	1.08	106
1.11	1.22	1.34	91
1.36	1.52	1.59	96
1.61	1.90	1.84	103
1.92	1.96	2.15	91
		mean	**101**

RPMI Cell Culture Medium

added	measured	expected	% recovery
0	0.25		
0.10	0.38	0.35	109
0.19	0.44	0.44	100
0.28	0.52	0.53	98
0.37	0.55	0.62	89
0.45	0.68	0.70	97
0.67	1.05	0.92	114
0.85	1.11	1.10	101
1.11	1.26	1.36	93
1.36	1.46	1.61	91
1.74	1.83	1.99	92
2.11	2.07	2.36	88
2.56	2.52	2.81	90
		mean	**97**

DMEM Cell Culture Medium

added	measured	expected	% recovery
0	0.23		
0.10	0.33	0.33	100
0.19	0.38	0.42	90
0.28	0.44	0.51	86
0.37	0.56	0.60	93
0.45	0.65	0.68	96
0.67	1.09	0.90	121
0.85	1.09	1.08	101
1.11	1.19	1.34	89
1.36	1.34	1.59	84
1.74	1.62	1.97	82
2.11	1.87	2.34	80
2.56	2.41	2.79	86
		mean	**92**

Linearity

The linearity of the ELISA method was investigated using different dilutions of a sample. Shown concentrations are in µmol/l.

Rat Heparin Plasma

dilution	measured	recalculated value	recovery %
orig.	2.55		
3 + 1	1.86	2.48	97
2 + 1	1.61	2.42	95
1 + 1	1.26	2.52	99
1 + 2	0.82	2.46	96
1 + 3	0.60	2.40	94
1 + 5	0.41	2.46	96
1 + 9	0.27	2.70	106
1 + 15	0.18	2.88	113
1 + 20	0.11	2.31	91
		mean recovery	**99**

Rat EDTA Plasma

dilution	measured	recalculated value	recovery %
orig.	2.40		
3 + 1	1.96	2.61	109
2 + 1	1.61	2.42	101
1 + 1	1.40	2.80	117
1 + 2	0.78	2.34	98
1 + 3	0.62	2.48	103
1 + 5	0.41	2.46	103
1 + 9	0.24	2.40	100
1 + 15	0.15	2.40	100
1 + 20	0.11	2.31	96
		mean recovery	**103**

Rat Serum

dilution	measured	recalculated value	recovery %
orig.	2.72		
3 + 1	1.84	2.45	90
2 + 1	1.68	2.52	93
1 + 1	1.45	2.90	107
1 + 2	0.95	2.85	105
1 + 3	0.76	3.04	112
1 + 5	0.49	2.94	108
1 + 9	0.28	2.80	103
1 + 15	0.19	3.04	112
1 + 20	0.15	3.15	116
		mean recovery	**105**

Mouse Serum

dilution	measured	recalculated value	recovery %
orig.	3.31		
3 + 1	2.27	3.03	92
2 + 1	2.08	3.12	94
1 + 1	1.62	3.24	98
1 + 2	1.07	3.21	97
1 + 3	0.74	2.96	89
1 + 5	0.53	3.18	96
1 + 9	0.33	3.30	100
1 + 15	0.18	2.88	87
1 + 20	0.15	3.15	95
		mean recovery	**94**

RPMI Cell Culture Medium

dilution	measured	recalculated value	recovery %
orig.	2.25		
3 + 1	1.89	2.52	112
2 + 1	1.82	2.74	122
1 + 1	1.22	2.45	109
1 + 2	0.79	2.38	106
1 + 3	0.62	2.49	111
1 + 5	0.38	2.30	102
1 + 9	0.20	1.98	88
		mean recovery	**107**

DMEM Cell Culture Medium

dilution	measured	recalculated value	recovery %
orig.	1.97		
3 + 1	1.51	2.01	102
2 + 1	1.52	2.29	116
1 + 1	1.05	2.10	107
1 + 2	0.72	2.16	110
1 + 3	0.55	2.18	111
1 + 5	0.34	2.05	104
1 + 9	0.24	2.36	120
		mean recovery	**110**

11. Literature

Literature using the ADMA-ELISA from DLD Diagnostika

Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP, Böger RH.
Determination of ADMA using a novel ELISA assay.
Clin. Chem. Lab. Med. 2004; 42: 1377-1383

Krempl TK, Kähler J, Maas R, Silberhorn L, Meinertz T, Böger RH.
Elevation of asymmetric dimethylarginine (ADMA) in patients with unstable angina and recurrent cardiovascular events.
Eur. Heart J. 2005; 26: 1846-1851

Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn L, Böger RH.
Determination of a reference value for N,N-dimethyl-L-arginine in 500 subjects.
Eur. J. Clin. Invest. 2005; 35 : 622-626

Schnabel R, Blankenberg S, Lubos E, Lackner KJ, Rupprecht HJ, Espinola-Klein C, Jachmann N, Post F, Peetz D, Bickel C, Cambien F, Tiret L, Münzel T.
Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: results from the AtheroGene Study.
Circ. Res. 2005; 97: e53-59

O`Dwyer MJ, Dempsey F, Crowley V, Kelleher D, McManus R, Ryan T.
Septic shock correlates with ADMA levels which may be influenced by a polymorphism in DDAH II: a prospective observational study.
Crit. Care 2006; 10: (5): R139

Antoniades C, Tousoulis D, Marinou K, Vasiliadou C, Tentolouris C, Bouras G, Pitsavos C, Stefanidis C.
Asymmetrical dimethylarginine regulates endothelial function in methionine-induced but not in chronic homocystinemia in humans: effect of oxidative stress and proinflammatory cytokines
Am. J. Clin. Nutr. 2006; 84: 781-788

Wang TZ., Chen WJ., Cheng WC., Lin JW., Chen MF., Lee YT.
Relation of improvement in endothelium-dependent flowmediated vasodilation after Rosiglitazone to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome
Am. J. Cardiol. 2006; 9: 1057-1062

Wanby P., Nilsson I., Brudin L., Nyhammar I., Gustafsson I., Carlsson M.
Increased plasma levels of asymmetric dimethylarginine in patients with carotid stenosis: no evidence for the role of the common FABBP2 A54T gene polymorphism
Acta Neurol. Scand. 2007; 115: 90-96

Konishi H, Sydow K, Cooke JP.
Dimethylarginine dimethylaminohydrolase promotes endothelial repair after vascular injury
J. Am. Coll. Cardiol. 2007; 49: 1099-1105

Iribarren C, Husson G, Sydow K, Wang BY, Sidney S, Cooke JP.
Asymmetric dimethyl-arginine and coronary artery calcification in young adults entering middle age: the CARDIA Study
Eur. J. Cardiovasc. Prev. Rehabil. 2007; 14:222-229

Melikian N, Wheatcroft SB, Ogah OS, Murphy C, Chowienczyk PJ, Wierzbicki AS, Sanders TA, Jiang B, Duncan ER, Shah AM, Kearney MT.
Asymmetric dimethylarginine and reduced nitric oxide bioavailability in young Black African men
Hypertension 2007; 49: 873-877

Horowitz JD, Heresztyn T.
An overview of plasma concentrations of asymmetric dimethylarginine (ADMA) in health and disease and in clinical studies: Methodological considerations.
J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2007; epub ahead of print

Korish AA, Arafah MM.
Catechin combined with vitamins C and E ameliorates insulin resistance (IR) and atherosclerotic changes in aged rats with chronic renal failure (CRF)
Arch. Gerontol. Geriatr. 2007; in press

Charitidou C, Farmakiotis D, Zournatzi V, Pidonia I, Pegiou T, Karamanis N, Hatzistilianou M, Katsikis I, Panidis D.
The administration of estrogens, combined with anti-androgens, has beneficial effects on the hormonal features and asymmetric dimethyl-arginine levels, in women with the polycystic ovary syndrome
Atherosclerosis 2007; in press

General Literature

Vallance P, Leone A, Calver A, Collier J, Moncada S.
Accumulation of an endogenous inhibitor of NO synthesis in chronic renal failure
Lancet 1992; 339: 572 - 575

Stühlinger M, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS.
Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor
J. Am. Med. Assoc. 2002; 287: 1420-1426

Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frölich JC, Böger RH.
Asymmetric dimethylarginine (ADMA): An endogenous inhibitor of nitric oxide synthase predicts mortality in end-stage renal disease (ESRD)
Lancet 2001; 358: 2113-2117

Nijveldt RJ, Teerlink T, Van der Hoven B, Siroen MP, Kuik DJ, Rauwerda JA, van Leeuwen PA.
Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality
Clin. Nutr. 2003; 22: 23-30

Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH.
Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia
Lancet 2003; 361: 1511-1517

Böger RH.
The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor
Cardiovasc. Res. 2003; 59: 824-833

Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC.
Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention.
Eur Heart J. 2003; 24: 1912-1919

Pipetting Scheme Sample Preparation Plasma and Serum

		Standards	Control	Sample
Standard 1 - 7	µl	20		
Control 1 & 2	µl		20	
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 Sample Preparation Cell Culture Samples

		Standards	Control	Sample
Standard 1 - 7	µl	20		
Control 1 & 2	µl		20	
Sample	µl			20
Standard 1	µl			20
Cell Culture Medium	µl	20	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 - 7	µl	25		
Control 1 & 2	µl		25	
Sample	µl			25
Antiserum	µl	50	50	50

shake shortly on an orbital shaker

incubate 15 – 20 hours (overnight) at 2 - 8 °C covered with foil

wash 4 x with each 250 µl Wash Buffer

Enzyme Conjugat	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 25 - 35 minutes at room temperature

Stop Solution	µl	100	100	100

read absorbance at 450 nm