



Instructions for Use


ADMA / Arginine ELISA


Enzyme Immunoassay
for the Quantitative Determination of
Endogenous Asymmetric Dimethylarginine (ADMA)
and L-Arginine (ARG) in Serum and EDTA-Plasma

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

REF EA207/192

 2 x 96

 2 – 8 °C



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




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Symbols

	For Research Use Only		
	Contents		Expiry Date
	Lot Number		Store
	Manufactured by		Sufficient for
	Catalogue Number		Consult Instructions

Hazard Pictograms



Danger



Warning

1. Introduction and Principle of the Test

Nitric oxide (NO) which is formed in the vascular endothelium plays a crucial role in the regulation of vascular structure and function. 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 synthase (NOS) from the amino acid precursor L-arginine. NOS activity is inhibited 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 and clinical studies, including patients with hypercholesterolemia, hypertension, chronic heart failure, chronic renal failure and other internal disorders.

Elevated ADMA levels are a risk factor for future cardiovascular events and total mortality, as evidenced by prospective clinical studies comprising more than 10,000 participants. Thus, ADMA has diagnostic relevance as a novel cardiovascular risk marker.

Importantly, high ADMA levels and low L-arginine/ADMA ratio were both independent predictors of death in the community-based Framingham Offspring Study. As ADMA competes with L-arginine for binding to NO synthase, many scientists suggest that the L-arginine/ADMA ratio is a better index of NOS substrate availability and, thus, functional integrity of the NOS pathway, than L-arginine levels alone. Furthermore the measurement of both L-arginine and ADMA plasma concentrations is suitable for treatment surveillance of subjects during nutritional L-arginine supplementation.

This assay is for research use only.

The competitive ADMA-Arginine ELISA uses the microtiter plate format. Antigen is bound to the solid phase of the microtiter plate. Antigen in the samples is acylated and competes with solid phase bound antigen for a fixed number of 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 and Arginine, respectively are detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase antigen is inversely proportional to the antigen concentration of the sample.

2. Precautions

- For research use only. Not for use in diagnostic procedures. For professional use only.
- Before carrying out the test, the valid instructions for use, as included in this kit, should be read completely and the content understood.
- Material of animal origin used in the preparation of the kit have been obtained from certified healthy animals 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 any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents, as these can cause irritation and chemical burns.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be cautious with glass vials.

3. Changes to Declare

Version _7: Additions and changes are highlighted in gray.

4. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. The shelf life of the ready-to-use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to Preparation of Reagents.

Bring all reagents to room temperature before use and refrigerate immediately after use.

5. Contents of the Kit

5.1 **MT-Strips** STRIPS-ADMA 12 strips
8 wells each, break apart
precoated with ADMA; blue coloured

5.2 **MT-Strips** STRIPS-ARG 12 strips
8 wells each, break apart
precoated with L-Arginine; yellow coloured

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

Standard	1	2	3	4	5	6
ADMA $\mu\text{mol/l}$	0	0.2	0.45	0.7	1.0	3.0
Arginine $\mu\text{mol/l}$	5	15	35	70	120	300

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

5.5 **Acylation Buffer** ACYL-BUFF 1 bottle
3.5 ml, ready for use, blue coloured





Warning

5.6 **Acylation Reagent** ACYL-REAG 3 vials
lyophilised, dissolve content
in 3 ml Solvent before use; if required
combine the contents of the vials

5.7 **Antiserum ADMA** AS-ADMA 1 vial
7 ml, ready for use
Rabbit-anti-N-acyl-ADMA; blue coloured

5.8 **Antiserum Arginine** AS-ARG 1 vial
7 ml, ready for use
Rabbit-anti-N-acyl-Arginine; yellow coloured

5.9 **Enzyme Conjugate** CONJ 2 vials
13 ml, ready for use
Goat anti-rabbit-IgG-peroxidase

5.10	Wash Buffer 20 ml, concentrated Dilute content with dist. Water to 1,000 ml total volume.	WASH	2 bottles
5.11	Substrate 13 ml TMB solution, ready for use	SUB	2 vials
5.12	Stop Solution 13 ml, ready for use Contains 0.3 M sulphuric acid, not corrosive	STOP	2 vials
5.13	Reaction Plate for acylation	ACYL-PLATE	1 piece
5.14	Equalizing Reagent lyophilized, dissolve content with 21 ml dist. water, dissolve carefully to minimize foam formation	EQUA-REAG	1 vial
5.15	Solvent 10 ml, contains DMSO (please note that Solvent reacts with many plastic materials including plastic trays; Solvent does not react with normal pipette and with glass devices)	SOLVENT	1 vial
	 		
	Warning		
5.16	Self-adhesive Foil ready for use	FOIL	4 pieces

Additional materials and equipment required but not provided:

- Pipettes (10, 20, 25, 50, 100 and 200 µl)
- Multichannel pipette
- Orbital shaker
- Multipipette
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer
- Roll mixer

6. Sample Collection

EDTA-Plasma and Serum

The test can be performed with EDTA plasma and serum.

Haemolytic 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.

7. Preparation of Reagents and Samples

Microtiter strips

STRIPS-ADMA**STRIPS-ARG**

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 of one bottle with dist. water to a total volume of 1,000 ml. The diluted wash buffer has to be stored at 2 - 8 °C for a maximum of 4 weeks. If the kit is to be divided into several runs, prepare for each run only the required amount of wash buffer.

Equalizing Reagent

EQUA-REAG

Dissolve the content with 21 ml dist. water, mix shortly and leave on a roll mixer or orbital shaker for 20 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until expiry date of the kit.

Acylation Reagent

ACYL-REAG

Dissolve the content of one bottle in 3 ml Solvent and shake for 10 minutes on a orbital shaker. The Acylation Reagent has always to be prepared immediately before use and is stable for 3 hours. The second and third bottles allow a second and third run, respectively, 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. After use the reagent has to be discarded.

Please note that Solvent reacts with many plastic materials including plastic trays. Solvent does not react with normal pipette tips and with glass devices. It is recommended to use an Eppendorf multipipette, 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.

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 μ l standard 1 - 6, each 20 μ l control 1 & 2 and each 20 μ l patient sample into the respective wells of the Reaction Plate.
2. Pipette 20 μ l Acylation Buffer into all wells.
3. Pipette 200 μ l Equalizing Reagent into all wells.
Mix the reaction plate for 10 seconds.
4. Prepare Acylation Reagent just before use and pipette 50 μ l prepared Acylation Reagent each into all wells, mix **immediately**. Colour changes to violet.
It is recommended to use an Eppendorf multipipette with a yellow tip (or similar device). Fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.
5. Incubate for 20 minutes at room temperature (approx. 20 °C) on an orbital shaker with medium speed.

Take each 25 μ l for the ADMA-ELISA.

Take each 10 μ l for the Arginine-ELISA.

8. Test Procedure ELISA

8.1. Preparation of Reagents

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

8.2 **ADMA-ELISA**

1. **Sample Incubation**

Pipette each 25 µl prepared Standards 1 to 6, 25 µl prepared controls and 25 µl prepared samples into the respective wells of the coated microtiter strips (blue coloured; duplicates are recommended).

Pipette each 50 µl ADMA-Antiserum **AS-ADMA** into all wells.

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

2. **Washing**

Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl prepared 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.

3. **Conjugate Incubation**

Pipette each 100 µl enzyme conjugate into all wells.

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

4. **Washing**

Repeat step 2

5. **Substrate Incubation**

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

6. **Stopping**

Pipette each 100 µl Stop Solution into all wells.

7. **Reading**

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

8.3 Arginine-ELISA

1. Sample Incubation

Pipette each 10 µl prepared Standards 1 to 6, 10 µl prepared controls and 10 µl prepared samples into the respective wells of the coated microtiter strips (yellow coloured; duplicates are recommended).

Pipette each 50 µl Arginine-Antiserum **AS-ARG** into all.

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

2. Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl prepared 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.

3. Conjugate Incubation

Pipette each 100 µl enzyme conjugate into all wells.

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

4. Washing

Repeat step 2

5. Substrate Incubation

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

6. Stopping

Pipette each 100 µl Stop Solution into all wells.

7. Reading

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

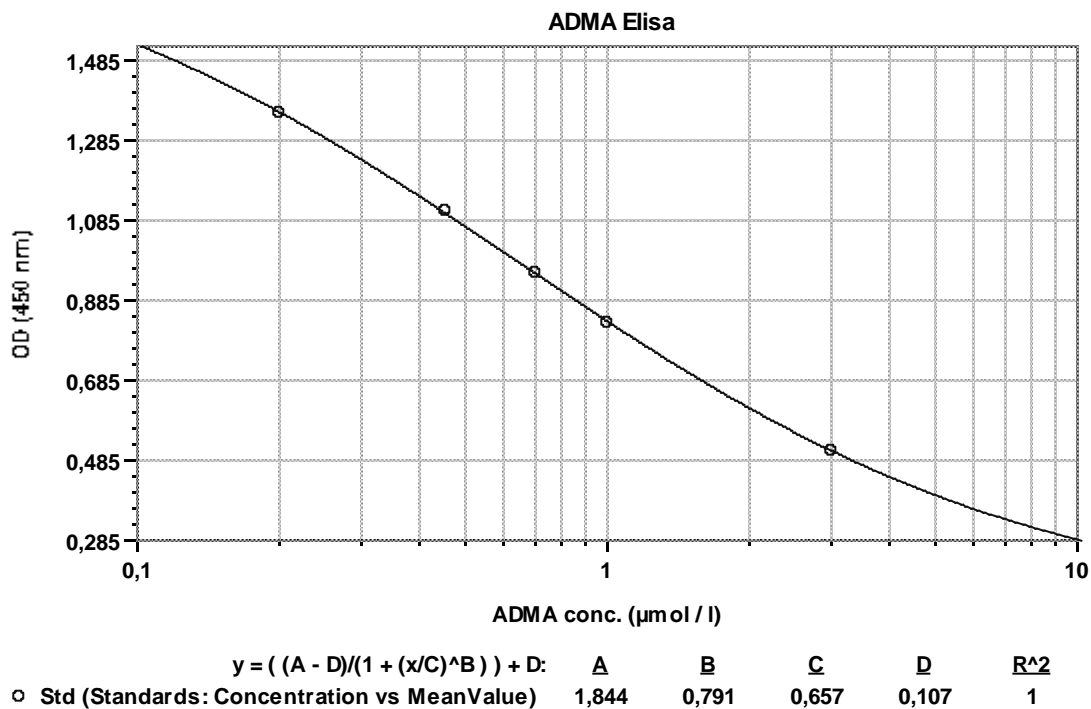
9. Calculation of the Results

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.

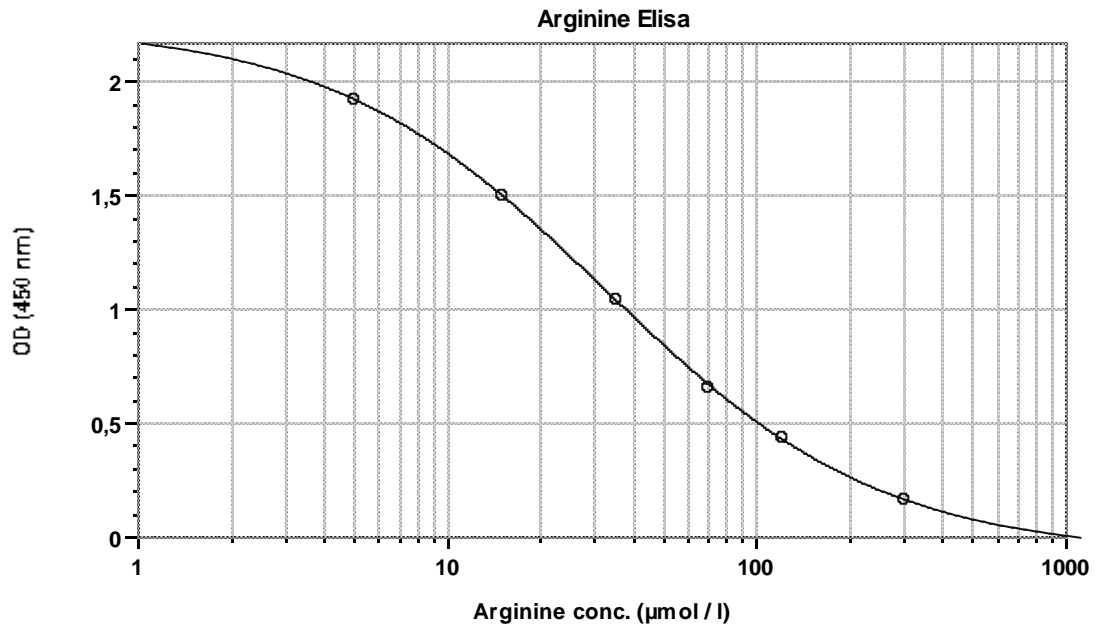
The concentration of the controls and samples can be read directly from this standard curve by using their average optical density.

Conversion: ADMA: 1 $\mu\text{mol} / \text{l} = 202 \text{ ng} / \text{ml}$
 Arginin: 1 $\mu\text{mol} / \text{l} = 174 \text{ ng} / \text{ml}$

Typical standard curve ADMA ELISA (do not use for calculation of results):



Typical standard curve Arginine ELISA (do not use for calculation of results):



$y = ((A - D)/(1 + (x/C)^B)) + D$: A B C D R²
○ Std (Standards: Concentration vs MeanValue) 2,248 0,975 32,2 -0,071 1

10. Assay Characteristics

10.1 Test Characteristics ADMA

Reference Range

This kit is for research use only, the values below are not for use in diagnostic procedures and should only be taken as a guideline. It is recommended that each laboratory should establish its own reference values.

	Reference Range
EDTA-Plasma, Serum	0.40 – 0.75 $\mu\text{mol} / \text{l}$

Sensitivity

Lower Limit of Detection	Calculation
0.03 $\mu\text{mol} / \text{l}$	$\text{OD}_{\text{cal1}} - 3 \times \text{SD}$

Specificity (Cross Reactions)

Substance	Cross Reactivity (%)
ADMA	100
SDMA	0.05
Monomethylarginine (NMMA)	1.93
Homoarginine	< 0.01
Arginine	0.03

Recovery

	Range ($\mu\text{mol} / \text{l}$)	Mean (%)	Range (%)
EDTA-Plasma	0.43 – 1.55	99	90 - 107
Serum	0.54 – 1.72	92	87 - 102

Linearity

	Range ($\mu\text{mol} / \text{l}$)	Highest Dil.	Mean (%)	Range (%)
EDTA-Plasma	0,23 – 1,53	1 : 6 with water	99	92 - 105

Reproducibility

	Range ($\mu\text{mol} / \text{l}$)	Intra-Assay-CV
EDTA-Plasma	0.58 – 1.04	4.9 – 5.4 %

	Range ($\mu\text{mol} / \text{l}$)	Inter-Assay-CV
EDTA-Plasma	0.57 – 1.34	4.3 – 9.6 %

Method Comparison

	Method	Correlation
Serum + Plasma	LC/MS	$Y = 0.99 \times \text{LC/MS} + 0.02$; $R = 0.983$; $N = 32$

10.2 Test Characteristics Arginine

Reference Range

This kit is for research use only, the values below are not for use in diagnostic procedures and should only be taken as a guideline. It is recommended that each laboratory should establish its own reference values.

	Reference Range
EDTA-Plasma, Serum	20 – 80 $\mu\text{mol} / \text{l}$

Sensitivity

Lower Limit of Detection	Calculation
6 $\mu\text{mol} / \text{l}$	$\text{OD}_{\text{Cal1}} - 3 \times \text{SD}$

Specificity (Cross Reactions)

Substance	Cross Reactivity (%)
Arginine	100
ADMA	< 0.37
Homoarginine	2.92
SDMA	0.88

Recovery

	Range ($\mu\text{mol} / \text{l}$)	Mean (%)	Range (%)
EDTA-Plasma	48 – 163	97	93 - 100
Serum	82 – 211	100	96 - 103

Linearity

	Range ($\mu\text{mol} / \text{l}$)	Highest Dil.	Mean (%)	Range (%)
EDTA-Plasma	28 – 193	1 : 6 with water	102	94 - 106

Reproducibility

	Range ($\mu\text{mol} / \text{l}$)	Intra-Assay-Vk
EDTA-Plasma	56 – 125	3.6 – 2.3 %

	Range ($\mu\text{mol} / \text{l}$)	Inter-Assay-Vk
EDTA-Plasma	53 – 170	3.2 – 6.3 %

Method Comparison

	Method	Correlation
Serum + Plasma	LC/MS	$Y = 0.95 \times \text{LC/MS} - 0.68$; $R = 0.991$; $N = 32$

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

Krempel 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

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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

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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

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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

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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, Nicolaidis 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
(ADMA and Arginine)**

		Standards	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
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mix immediately and incubate for 20 minutes
at room temperature on an orbital shaker

Take each 25 μl for the ADMA-ELISA.

Take each 10 μl for the Arginine-ELISA.

Pipetting Scheme ADMA-ELISA

		Standard	Control	Patient-Sample
Standard 1 - 6	μl	25		
Control 1 & 2	μl		25	
Patient Sample	μl			25
Antiserum	μl	50	50	50

cover plate with foil and incubate 90 minutes at room temperature

wash 4 x with 300 μl Wash Buffer per well

Enzyme Conjugat	μl	100	100	100
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shake for 30 minutes at room temperature

wash 4 x with 300 μl Wash Buffer per well

Substrate	μl	100	100	100
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shake for 25 ± 5 minutes at room temperature

Stop Solution	μl	100	100	100
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read absorbance at 450 nm (ref. 570 – 650 nm)

Pipetting Scheme Arginine-ELISA

		Standard	Control	Patient-Sample
Standard 1 - 6	μl	10		
Control 1 & 2	μl		10	
Patient Sample	μl			10
Antiserum	μl	50	50	50

cover plate with foil and incubate 90 minutes at room temperature

wash 4 x with 300 μl Wash Buffer per well

Enzyme Conjugat	μl	100	100	100
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shake for 30 minutes at room temperature

wash 4 x with 300 μl Wash Buffer per well

Substrate	μl	100	100	100
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shake for 25 ± 5 minutes at room temperature

Stop Solution	μl	100	100	100
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read absorbance at 450 nm (ref. 570 – 650 nm)