Instruction for use

ADMA – Arginine – Plasma ELISA

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

CE IVD

REF EA207/192

Σ  2 x 96

2 – 8 °C

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April 2011
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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.

The new competitive ADMA-Arginin-Plasma 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 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-ADMA**  
12 strips  
8 wells each, break apart  
precoated with ADMA; blue coloured

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

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

<table>
<thead>
<tr>
<th>Standard</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA µmol/l</td>
<td>0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.6</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Arginine µmol/l</td>
<td>0</td>
<td>6</td>
<td>20</td>
<td>60</td>
<td>200</td>
<td>600</td>
</tr>
</tbody>
</table>

4.4 **Control 1 & 2**  
**CON 1 & 2**  
2 vials  
Each 4 ml, ready for use  
Range: see q.c. certificate
4.5 **Acylation Buffer** *(ACYL-BUFF)*
3.5 ml, ready for use

4.6 **Acylation Reagent** *(ACYL-REAG)*
Lyophilised, dissolve content in 2.8 ml Solvent before use; if required combine the contents of both vials

4.7 **Antiserum ADMA** *(AS-ADMA)*
5.5 ml, ready for use
Rabbit-anti-N-acyl-ADMA; blue coloured

4.8 **Antiserum Arginine** *(AS-ARG)*
5.5 ml, ready for use
Rabbit-anti-N-acyl-Arginine; yellow coloured

4.9 **Enzyme Conjugate** *(CONJ)*
21 ml, ready for use
goat anti-rabbit-IgG-peroxidase

4.10 **Wash Buffer** *(WASH)*
20 ml, concentrated
Dilute content with dist. water to 500 ml total volume.

4.11 **Substrate** *(SUB)*
21 ml TMB solution, ready for use

4.12 **Stop Solution** *(STOP)*
21 ml, ready for use
Contains 0.3 M sulphuric acid, not corrosive

4.13 **Reaction Plate** *(ACYL-PLATE)*
1 piece
for acylation

4.14 **Equalizing Reagent** *(EQUA-REAG)*
Lyophilized, dissolve content with 20.5 ml dist. water, dissolve carefully to minimize foam formation

4.15 **Solvent** *(SOLVENT)*
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)
4.16 **Self-adhesive Foil**

**FOIL**

| 2 pieces |

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**

5.1. **EDTA-Plasma**

The test can be performed with EDTA plasma only. 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**

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**

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.
6.3. 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.

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. 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 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 ADMA-ELISA.**

**Take each 10 µl for the Arginine-ELISA.**
7. **Test Procedure ELISA**

7.1. **Preparation of reagents**

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

7.2 **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 Antiserum into all wells and shake shortly on an orbital shaker.
   Cover the plate with adhesive foil and incubate Microtiter Strips for 15 –20 hours (overnight) at 2 – 8 °C.

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.

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

4. **Washing**
   Repeat step 7.2.

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.

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.
7.3 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 Antiserum into all wells and shake shortly on an orbital shaker.
   Cover the plate with adhesive foil and incubate Microtiter Strips for 15 –20 hours (overnight) at 18 – 24 °C.

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.

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

4. **Washing**
   Repeat step 7.2.

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.

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.
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.
9. Assay Characteristics

Expected Values

<table>
<thead>
<tr>
<th>ADMA</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 – 0.75 µmol/l</td>
<td>20 - 80 µmol/l</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>ADMA</th>
<th>Arginin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 µmol/l</td>
<td>3.0 µmol/l</td>
</tr>
</tbody>
</table>

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 ADMA, SDMA and Arginine.

<table>
<thead>
<tr>
<th>Substance</th>
<th>ADMA (%)</th>
<th>Arginine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>100</td>
<td>0.01</td>
</tr>
<tr>
<td>SDMA</td>
<td>1.34</td>
<td>0.68</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.01</td>
<td>100</td>
</tr>
</tbody>
</table>

Recovery

Increasing amounts of ADMA were added to a plasma sample. Each spiked sample was assayed. The analytical recovery of ADMA was estimated at twelve different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 97%.
Increasing amounts of Arginine were added to a plasma sample. Each spiked sample was assayed. The analytical recovery of Arginine was estimated at eleven different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 102%.

<table>
<thead>
<tr>
<th>added</th>
<th>measured</th>
<th>expected</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.55</td>
<td>0.53</td>
<td>104</td>
</tr>
<tr>
<td>0.19</td>
<td>0.61</td>
<td>0.62</td>
<td>98</td>
</tr>
<tr>
<td>0.28</td>
<td>0.67</td>
<td>0.71</td>
<td>94</td>
</tr>
<tr>
<td>0.37</td>
<td>0.79</td>
<td>0.80</td>
<td>99</td>
</tr>
<tr>
<td>0.45</td>
<td>0.83</td>
<td>0.88</td>
<td>97</td>
</tr>
<tr>
<td>0.73</td>
<td>1.08</td>
<td>1.16</td>
<td>93</td>
</tr>
<tr>
<td>0.97</td>
<td>1.33</td>
<td>1.40</td>
<td>95</td>
</tr>
<tr>
<td>1.20</td>
<td>1.60</td>
<td>1.63</td>
<td>98</td>
</tr>
<tr>
<td>1.42</td>
<td>1.67</td>
<td>1.85</td>
<td>90</td>
</tr>
<tr>
<td>1.92</td>
<td>2.24</td>
<td>2.35</td>
<td>95</td>
</tr>
<tr>
<td>2.33</td>
<td>2.76</td>
<td>2.76</td>
<td>100</td>
</tr>
<tr>
<td>2.80</td>
<td>3.32</td>
<td>3.23</td>
<td>103</td>
</tr>
</tbody>
</table>

mean value 97

concentrations in µmol/l
**Linearity**

The linearity of the ELISA method was investigated using nine different dilutions of a plasma sample with ADMA. The mean linearity from all dilutions was 97%.

<table>
<thead>
<tr>
<th>dilution measured</th>
<th>recalculate value</th>
<th>recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>orig. 2.819</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 + 1 2.090</td>
<td>2.787</td>
<td>99</td>
</tr>
<tr>
<td>2 + 1 1.745</td>
<td>2.618</td>
<td>93</td>
</tr>
<tr>
<td>1 + 1 1.236</td>
<td>2.472</td>
<td>88</td>
</tr>
<tr>
<td>1 + 2 0.860</td>
<td>2.580</td>
<td>91</td>
</tr>
<tr>
<td>1 + 4 0.535</td>
<td>2.675</td>
<td>95</td>
</tr>
<tr>
<td>1 + 7 0.361</td>
<td>2.888</td>
<td>102</td>
</tr>
<tr>
<td>1 + 10 0.286</td>
<td>3.146</td>
<td>112</td>
</tr>
<tr>
<td>1 + 15 0.178</td>
<td>2.848</td>
<td>101</td>
</tr>
<tr>
<td>1 + 25 0.104</td>
<td>2.704</td>
<td>96</td>
</tr>
</tbody>
</table>

mean recovery 97

concentrations in µmol/l

The linearity of the ELISA method was investigated using nine different dilutions of a plasma sample with Arginine. The mean linearity from all dilutions was 98%.

<table>
<thead>
<tr>
<th>Dilution measured</th>
<th>recalculate value</th>
<th>recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>orig. 443</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 + 1 370</td>
<td>463</td>
<td>105</td>
</tr>
<tr>
<td>2 + 1 319</td>
<td>479</td>
<td>108</td>
</tr>
<tr>
<td>1 + 1 232</td>
<td>465</td>
<td>105</td>
</tr>
<tr>
<td>1 + 2 148</td>
<td>444</td>
<td>100</td>
</tr>
<tr>
<td>1 + 4 87</td>
<td>435</td>
<td>98</td>
</tr>
<tr>
<td>1 + 6 60</td>
<td>420</td>
<td>95</td>
</tr>
<tr>
<td>1 + 9 42</td>
<td>420</td>
<td>95</td>
</tr>
<tr>
<td>1 + 12 30</td>
<td>390</td>
<td>188</td>
</tr>
<tr>
<td>1 + 20 19</td>
<td>390</td>
<td>90</td>
</tr>
</tbody>
</table>

mean recovery 98

concentrations in µmol/l
Reproducibility

The reproducibility of the ELISA method was investigated determining the intra- and inter-assay-coefficients of variation (cv) by repeated measurements of different plasma samples with different ADMA and Arginine concentrations.

Intra-Assay Variation ADMA

<table>
<thead>
<tr>
<th>sample</th>
<th>n =</th>
<th>mean value</th>
<th>sd</th>
<th>cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>0.66</td>
<td>0.037</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>1.01</td>
<td>0.066</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Intra-Assay Variation Arginine

<table>
<thead>
<tr>
<th>sample</th>
<th>n =</th>
<th>mean value</th>
<th>sd</th>
<th>cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>39.3</td>
<td>1.43</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>72.2</td>
<td>2.59</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Inter-Assay Variation

<table>
<thead>
<tr>
<th>sample</th>
<th>n =</th>
<th>mean value</th>
<th>sd</th>
<th>cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>0.63</td>
<td>0.07</td>
<td>10.3</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>1.01</td>
<td>0.10</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>1.38</td>
<td>0.13</td>
<td>9.4</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>2.26</td>
<td>0.19</td>
<td>8.3</td>
</tr>
</tbody>
</table>
10. Literature

Literature using the ADMA-ELISA from DLD Diagnostika


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


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, Hereszty n T.  
**An overview of plasma concentrations of asymmetric dimethylarginine (ADMA) in health and disease and in clinical studies: Methodological considerations.**

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)**

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

**Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor**

**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**

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**

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)

<table>
<thead>
<tr>
<th></th>
<th>Standards</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 - 6 µl</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1 &amp; 2 µl</td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Patient Sample µl</td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Acylation Buffer µl</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Equalizing Reagent µl</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

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

**Take each 25 µl for the ADMA-ELISA.**

**Take each 10 µl for the Arginine-ELISA.**
# Pipetting Scheme ADMA-ELISA

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Control</th>
<th>Patient-Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 - 6</td>
<td>µl</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Control 1 &amp; 2</td>
<td>µl</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Patient Sample</td>
<td>µl</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Antiserum</td>
<td>µl</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

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 20 - 30 minutes at room temperature

| Stop Solution    | µl | 100 | 100 | 100 |

read absorbance at 450 nm
## Pipetting Scheme Arginine-ELISA

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Control</th>
<th>Patient-Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 - 6</td>
<td>µl</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Control 1 &amp; 2</td>
<td>µl</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Patient Sample</td>
<td>µl</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Antiserum</td>
<td>µl</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

shake shortly on an orbital shaker

incubate 15 – 20 hours (overnight) at **18 - 24 °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 20 - 30 minutes at room temperature

| Stop Solution   | µl | 100 | 100 | 100 |

read absorbance at 450 nm