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

SDMA ELISA

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INTRODUCTION

Chronic kidney disease is prevalent, progressive and insidious. It is recognized that kidney disease affects nearly one out of three cats and one out of ten dogs. Although the glomerular filtration rate is the gold standard measurement for assessing kidney function in pets, this analysis is impractical and rarely used.

The SDMA (symmetric dimethylarginine) is a tool for the early diagnosis of chronic kidney disease. The SDMA is a protein methylation degradation product, and it is excreted more than 90% by the kidneys. Urine specific density will decrease when 67% of renal function is lost, and the creatinine value will increase when 70% of renal function is lost. The advantage of the SDMA is that the value will increase when only 40% of the renal function is lost, thus allowing for early detection of altered renal function. The SDMA can also be used to monitor the progression of renal disease.

In tubulointerstitial disease, the leading cause of chronic kidney disease in cats and dogs, patients may exhibit normal or slightly increased urinary protein/creatinine ratio. The SDMA will therefore be a better indicator for the early detection of chronic kidney disease. It is imperative to rule out renal and post-renal causes of proteinuria before measuring the urinary protein/creatinine ratio. Dehydration can increase the SDMA. It is therefore important to measure urine specific gravity when the SDMA is increased. Preliminary studies have shown that puppies, kittens and greyhounds may exhibit a slightly higher SDMA level.

PRINCIPLE OF THE TEST

The Petcheck® SDMA ELISA is a competitive enzyme immunoassay for the quantitative determination of endogenous SDMA in serum and plasma. The modification of SDMA takes place during the sample preparation. SDMA is quantitatively converted into N-acyl-SDMA by the acylation reagent. 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 antibodies binding sites. When the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibodies bound to the immobilized SDMA is detected using goat anti-rabbit IgG conjugated to peroxidase. The reaction between the substrate and the enzymatic conjugate 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.

The Petcheck® SDMA ELISA offers the advantages of a specific and sensitive method for routine use. The detection range of the Petcheck® SDMA ELISA is 0.2 to 3.0 µmol/L (4.0 to 60 µg/dL). The reference ranges (serum/plasma) for dogs and cats are the following:

- Dogs: < 0.7 µmol/L (< 14.0 µg/dL)
- Cats: < 0.84 µmol/L (< 17.0 µg/dL)

Conversion factor: 1 µmol/L = 20.2 µg/dL

Persistent high levels point to a diminished renal elimination and thus to a possible restricted renal function.

The range given should be considered as a guideline. Each laboratory should establish its own reference ranges.

SDMA values should always be assessed together with the creatinine values and other urine parameters, especially the urine specific gravity.

MATERIAL

COMPONENTS SUPPLIED IN THE KIT

Component	Description						Qty	Vol
Strips coated with SDMA	Strips of 8 wells each, breakable, precoated with SDMA (96 wells in total)						12	
Standards 1 to 6	Ready for use. Concentration						6 vials	6 x 4 ml
	1	2	3	4	5	6		
µmol/L	0	0.2	0.4	0.7	1.2	3.0		
ng/ml	0	40	81	141	242	606		
Controls 1 and 2	Ready for use. Concentrations: see Q.C. certificate						2 vials	4 ml
Acylation Buffer	Ready for use, blue colored						1 vial	3.5 ml
Acylation reagent	Lyophilized, dissolve contents in 3 ml Solvent before use.						3 vials	
Antiserum	Ready for use, yellow colored, rabbit-anti-N-acyl-SDMA						1 vial	7 ml
Enzyme Conjugate	Ready for use goat anti-rabbit-IgG-peroxidase						1 vial	13 ml
wash buffer	50 x concentrated, dilute contents with distilled water to 1000 ml total volume						1 vial	20 ml
Substrate	TMB solution, ready for use						1 vial	13 ml
Stop Solution	Ready for use (contains 0.3 M sulphuric acid)						1 vial	13 ml
Acylation plate	Reaction Plate for acylation						1 piece	
Equalizing Reagent	Lyophilized, dissolve contents with 21 ml distilled. water, dissolve carefully to minimize foam formation						1 vial	
Solvent	Contains DMSO.						2 vials	5 ml
Foil							2	

STORAGE AND STABILITY

On arrival, store the kit at 2-7 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Micropipettes (20, 50, 100 and 200 µl) and tips
- Multipette
- Orbital shaker (400 rpm)
- Microplate washing device (optional)
- Microplate reader (450 nm)
- Vortex mixer, roll mixer
- Distilled water

PRECAUTIONS

- For *in vitro* veterinary use only.
- **Solvent (DMSO) reacts with many plastic materials including plastic trays but does not react with normal pipette tips or with glass devices**
- Do not use components beyond the expiration date
- Do not mix various lots of any kit component
- Disposable gloves should be used.
- Material of animal origin used in the preparation of the kit has been obtained from healthy animals but these materials should be handled as potentially infectious.

EXECUTION

Sample Collection

- The test is to be performed with serum or plasma (EDTA).
- Hemolytic and lipemic samples should not be used.
- Samples can be stored up to 6 hours at 2 - 7 °C. For a longer storage (up to 18 months) the samples must be kept frozen at -20 °C
- Repeated freezing and thawing of samples should be avoided.

Preparation of Reagents

1. **Microtiter strips**
   Prior 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 2-7 °C.
2. **Wash Buffer**
   Dilute the contents with distilled water to a total volume of 1000 ml, mix shortly. The diluted wash buffer must be stored at 2 - 7 °C and is stable for 4 weeks. For longer storage the diluted wash buffer has to be stored frozen at -20°C.
3. **Equalizing Reagent**
   Dissolve the contents with 21 ml distilled water, mix shortly and leave on a roll mixer for 20 minutes. Avoid excess formation of foam. The reconstituted Equalizing Reagent must be stored frozen at -20 °C and is stable until expiry date.
4. **Acylation Reagent**
   Dissolve the contents of one bottle in 3 ml Solvent and shake for 10 minutes on roll mixer. **The Acylation Reagent has to be prepared immediately before use but at least 10 minutes prior to use it. Prepared Acylation Reagent is stable for maximum 3 hours at room temperature.**
   The two other bottles allow a second and third 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.
5. **Solvent**
   It is recommended to use a multipette, fill it directly from the vial and add the Acylation Reagent to the wells.
6. **Reaction Plate**
   The wells of the reaction plate for the acylation can be used only once.

Preparation of Samples (Acylation)

Sample acylation is critical. Every step has to be done rapidly.

1. Add 20 µl of each six standards to be used to the appropriate wells.
2. Add 20 µl of each control 1 & 2 to be used to the appropriate wells.
3. Add 20 µl of each sample to be tested into the respective wells of the Reaction Plate.
4. Immediately add 20 µl Acylation Buffer with the multipette into every used well. Take care not to contaminate the multipette tip with the samples.
5. Add 200 µl reconstituted Equalizing Reagent into every used well.
6. Mix the reaction plate for 10 seconds by gently shaking it manually
7. Add 50 µl of the prepared Acylation Reagent each into every used well and **MIX IMMEDIATELY**. Colour changes to violet.
   It is strongly recommended to use a multipette, fill it directly from the Acylation Buffer and the Acylation Reagent vial and add the reagent as fast as possible to the wells. Take care not to contaminate the multipette tip with the samples.
8. Incubate for 20 minutes at room temperature (23 ± 2°C) on an orbital shaker (400 rpm). Do not cover wells or plate, leave the plate open on the shaker.
9. Take each 20 µl of the acylated samples for the SDMA-ELISA.

Test Procedure ELISA

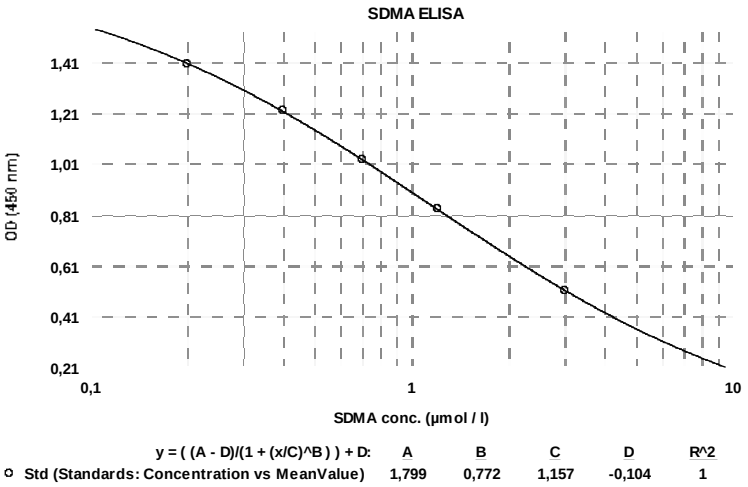
Bring all reagents to room temperature (sort them from the refrigerator at least 1 hour prior to be used and mix them carefully while avoiding development of foam).

1. **Sample Incubation**
   a. Pipette each 20 µl prepared Standards 1 to 6 (duplicates are recommended), 20 µl prepared controls (duplicates are recommended), and 20 µl prepared samples into the respective wells of the coated microtiter strips.
   b. Pipette each 50 µl Antiserum into all wells.
   c. Cover the plate with adhesive foil and incubate Microtiter Strips for 90 minutes at room temperature (23 ± 2°C) on an orbital shaker (400 rpm).
2. **Washings**
   Washings are critical. Plates have to be washed thoroughly.
   Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl Wash Buffer.
   Repeat the washing procedure 4 more times.
   Remove residual liquid by tapping the inverted plate onto clean absorbent paper after each washing not only at the end of the washing step.
3. **Conjugate Incubation**
   Add 100 µl of the Enzyme Conjugate to every used well. Incubate for 30 minutes at room temperature (23 ± 2°C) on an orbital shaker (400 rpm).
4. **Washings**
   Repeat step 2.
5. **Substrate Incubation**
   Add 100 µl of the Substrate to every used well and incubate for 25 to 30 minutes at room temperature (23 ± 2°C) on an orbital shaker (400 rpm).
6. **Stopping**
   Add 100 µl of the Stop Solution to every used well.
7. **Reading**
   Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate reader. The reading should be done no later than 15 minutes after the addition of the Stop Solution.

Calculation of the Results

- The concentration of the standards (x-axis, logarithmic) can be plotted against their corresponding optical density (y-axis, linear) on a semi-logarithmic graph paper. The concentration of the controls and samples can be read directly from this standard curve by using their average optical density.
- It is recommended to use 5-parameter logistic regression (available for free at www.elisaanalysis.com) or similar iteration procedures to evaluate the standard curve and calculate the sample SDMA concentration.

Typical standard curve:



- R^2 of the standard curve : 0.99
- OD of standard #1 (0 µmol/L): 1.85