Cat α-1-Acid Glycoprotein (α-1-AGP) ELISA

INTRODUCTION

α-1-AGP is an acute phase protein that is elevated in cat serum as a result of injury, infection or disease. Kajikawa, et al. reported a 19-fold increase of α-1-AGP in serum from hospitalised cats and a 5.7 fold increase after injection of normal cats with lipopolysaccharide. Of the positive acute phase proteins investigated (SAA, α-1-AGP, haptoglobin and CRP), they reported that α-1-AGP was the most responsive.1 More recently, Paltrinieri et al., reported α-1-AGP to be a powerful discriminating biomarker for diagnosis of feline infectious peritonitis.2

PRINCIPLE OF THE TEST

The cat α-1-AGP ELISA uses affinity purified anti-cat α-1-AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-cat α-1-AGP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in α-1-AGP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of α-1-AGP is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-cat α-1-AGP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (lyophilized)
- 10x Diluent, 25 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18- 25°C) before use.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The cat α-1-AGP standard is provided as a lyophilized stock. Add the volume of distilled or deionized water indicated on the vial label and mix gently until dissolved (the reconstituted standard remains stable for at least 7 days at 2-8°C but should be aliquoted and frozen at -20°C after reconstitution if use beyond this time is intended).

2. Label 5 polypropylene or glass tubes as 50, 25, 12.5, 6.25 and 3.13 ng/ml.

3. In the tube labeled 50 ng/ml, prepare the 50 ng/ml working standard as described on the label of the reconstituted reference standard vial.

4. Dispense 250 μl of diluent into the remaining tubes.

5. Prepare the 25 ng/ml standard by diluting and mixing 250 μl of the 50 ng/ml standard with 250 μl of diluent in the tube labeled 25 ng/ml.

6. Similarly prepare the 12.5, 6.25, and 3.13 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: We observed α-1-AGP concentrations of ~0.15 mg/ml in serum of healthy American short haired cats. However, in serum from diseased cats concentrations can reach 5 mg/ml or greater.1 4 In order to obtain values within the range of the standard curve we suggest that samples initially be diluted 10,000 fold using the following procedure for each sample to be tested:

1. Dispense 197.5 μl and 492 μl of 1x diluent into separate polypropylene or glass tubes.

2. Pipette and mix 2.5 μl of the serum/plasma sample into the tube containing 197.5 μl of diluent. This provides an 80 fold dilution of the sample.

3. Mix 8 μl of the 80 fold diluted sample with the 492 μl of diluent in the second tube. This provides a 10,000 fold dilution of the sample.

4. Repeat this procedure for each sample to be tested.

Caution: We found that α-1-AGP concentrations were lower than expected at dilutions less than 4000 (i.e., 2000 fold). Serum and plasma should therefore be diluted at least 4000 fold.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 μl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in 4 and 5 above.
9. Dispense 100 μl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
11. Stop the reaction by adding 100 μl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS
1. Calculate the average absorbance values (A450) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of α-1-AGP in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of α-1-AGP in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE
A typical standard curve with optical density readings at 450nm on the Y-axis against α-1-AGP concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>α-1-AGP (ng/ml)</th>
<th>Absorbance (450 nm)</th>
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<tbody>
<tr>
<td>50.00</td>
<td>2.940</td>
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<td>25.00</td>
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<td>12.50</td>
<td>0.991</td>
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<td>6.25</td>
<td>0.540</td>
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<td>3.13</td>
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ASSAY CHARACTERISTICS
Typical results obtained with five American short-haired cat serum samples are shown in the table below. Samples were diluted as indicated and concentrations (ng/ml) determined. Serum concentrations (μg/ml) were calculated by multiplying the concentrations of the samples by their respective dilution factors. Average values, standard deviation (SD) and coefficient of variation (CV) were then calculated.

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<tr>
<th>Sample</th>
<th>Dilution</th>
<th>A450</th>
<th>ng/ml</th>
<th>ug/ml</th>
<th>Average ug/ml</th>
<th>SD (μg/ml)</th>
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REFERENCES

Rev 041015NC
For technical assistance please email us at techsupport@lifediagnostics.com