DRG® Feline Infectious Peritonitis Ab ELISA (EIA-2468)

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1 INTRODUCTION

Feline infectious peritonitis (FIP) is an important and complex disease of both wild and domestic cats induced by corona virus.
The great majority of cats that become infected recover completely and develop immunity to Corona virus. Some of the recovered cats become carriers of the virus and can infect other cats. A few infected cats do not build up immunity to corona virus and the disease progress to a fatal form. The fatal, disseminated form of FIP is a chronic, progressive disease characterized by antibiotic-resistant fever, weakness, loss of appetite, lethargy and often anemia. Inflammation is present in the linings of the chest and abdominal cavities often accompanied by accumulation of fluid. In addition, there is marked variation in the organs that may be involved and in the extent of the involvement.
Important in the diagnosis of FIP are:
- clinical history
- clinical signs
- eye examination
- examination of abdominal or chest fluid, if present
- laboratory findings

This test measures corona virus antibodies which are present in the blood or ascetic fluid. Most antibody positive cats (especially those with high titers) are possible virus carriers and may shed FIP.

2 INTENDED USE OF THE TEST KIT

The FIP ELISA test kit is designed to detect antibodies against FIP proteins (mostly glycoproteins).
FIP proteins are attached to the solid phase. After washing the strips are incubated with the cat sera to be tested. The strips are washed after incubation to remove unbound materials. A HRP-labeled anti-species conjugate is added to detect bound cat antibodies to FIP proteins. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE FIPV TEST

The test is based on the reaction of FIP proteins (mostly glycoproteins) with polyclonal cat antibodies.
To this end FIP proteins have been coated to a 96-well microtiter plate.
The diluted cat serum/ascetic fluid sample is added to the wells of the coated plate.
After washing the bound cat antibodies are detected by a HRP-conjugated antispecies conjugate.
The color reaction in the wells is directly related to the concentration of FIP antibodies in the serum/ascetic sample.

4 CONTENTS

- 12 x 8-well **microtiter strips** coated with FIP proteins
- 1 x stripholder
- 2 x 6 ml HRP-**conjugated (anti-species) antibody**
- 1 x 1 ml FIP inactivated **positive control** serum (freeze-dried)
- 1 x 1 ml FIP inactivated **negative control** serum (freeze-dried)
- 1 x 60 ml **wash solution 200 x concentrated**, which must be diluted in deionized water before use!
- 3 x 5 ml **ELISA buffer**
- 1 x 8 ml **substrate buffer A**
- 1 x 8 ml **substrate buffer B**

- 1 x 8 ml **stop solution**
- 1 x plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS.

The kit should be stored at +4°C. An open packet should be used within 10 days.

Samples may be used fresh or may be kept frozen below -20°C before use.

Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date. Avoid repeated freezing and thawing as this increases non-specific reactivity.

6 WASH PROTOCOL

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is advised to carefully follow the washing procedures outlined below. Both manual washing and washing with automatic equipment can be performed. (Automatic washing equipment usually gives better results).

Manual washing

1. Empty each well by turning the microtitre plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times.
4. Turn the plate upside down and empty the wells by a firm short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

When using automatic plate washing equipment, check that all wells can be aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 TEST PROTOCOL

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C and use them within 10 days.
   Wash the microtiter strip(s) with washing solution, according to washing protocol.
   The washing solution provided must be diluted 10 x in deionized water!

   Reconstitute directly before use the positive and negative control in 1 ml deionized water divide into aliquots and store immediately at -20°C until use.
2. Make 3-step dilution of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate.
   Make also a 3 step dilution of the positive and negative control.
3. Transfer 100 µl of this dilution to the FIP coated microtiter strips.
   Seal and incubate for 60 min. at 37°C.

4. Wash as in 1.
5. Dispense 100 µl conjugated anti-species antibody to all wells.
6. Seal and incubate 60 min. at 37°C.
7. Wash as in 1.
8. Mix equal parts of buffer A and buffer B with gentle shaking. Prepare immediately before use!
   Dispense 100 µl substrate solution to each well.
   Incubate 15-25 min. at room temperature (21°C).
9. Add 50 µl stop solution to each well; mix well.
10. Read the absorbency values immediately (within 10 min.!) at 450 nm.

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting FIP.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin and when swallowed; observe care when handling the substrate.
- Do not use components past their expiry date and do not mix components from different serial lots together.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the positive control should give an extinction > 0.500 OD units and an end point titer > 270.
The negative control should give an OD < 0.250 and an end point titer < 30.

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

a. qualitatively: positive or negative
A sample is scored positive if
the OD of the sample is above the value of the negative control + 0.150 OD units.

b. quantitatively: end point titer
The end point titer of the sample is the dilution which gives an extinction just above 0.300 OD units (450 nm) Antibody titers of 270 and higher in diseased animals showing signs suggestive of FIP are considered positive and the cat will be suspected of shedding FIP.

A rise in antibody titer in a cat with FIP represents an exaggerated, ineffective immune response and is indicative of a worsening of the disease process.

Fulminating FIP, characterized by very rapid accumulation of fluid may on occasion be associated with lower titers (1:90/1:270).

In summary:
< 30 = no antibodies found
90-270 = antibodies found, retest in 3 months
> 810 = high titer of antibodies found in
- diseased animal: suggestive for FIP
- healthy animal : retest in 3 months.

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