DRG® Canine Corona Virus IgM ELISA (EIA-2483)

REVISED 24 APRIL 2005 **RUO IN THE USA**

INTRODUCTION

Canine Corona Virus (CCV) is an important and complex disease of both wild and domestic dogs. The great majority of dogs that become infected recover completely and develop immunity to CCV. Some of the recovered dogs become carriers of the virus and can infect other dogs. A few infected dogs do not build up immunity to CCV and the disease progresses to a fatal form. The fatal, disseminated form of CCV is a chronic, progressive disease characterized by diarrhea, intestinal disease, weakness, loss of appetite.

Important in the diagnosis of CCV are:
- clinical history
- clinical signs
- laboratory findings: - antigen detection
- antibody detection

This test measures coronavirus antibodies which are present in the blood or plasma. Most antibody positive dogs (especially those with intermediary titers) are possible virus carriers and may shed CCV.

INTENDED USE OF THE TEST KIT

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

PRINCIPLE OF THE TEST KIT

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

CONTENTS

- 12 x 8-well **microtiter strips** coated with CCV proteins
- 1 x stripholder
- 2 x 6 ml HRPO-**conjugated (IgM) (anti-species) antibody,** (ready to use)
- 1 x 1 ml CCV weak **positive control** serum, (ready to use)
- 1 x 1 ml CCV **negative control** serum, (freeze-dried)
- 1 x 60 ml **wash solution 200 x concentrated**, which must be diluted in deionized water before use!
- 2 x 6 ml **ELISA buffer,** (ready to use)
- 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

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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 expire date.
Avoid repeated freezing and thawing as this increases non-specific reactivity

WASHING PROTOCOL

In ELISA's, between each immunological incubation step un-complexed components have to be removed efficiently. 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 microtiter plate upside down, followed by a firm vertical movement.
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 wash 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.

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 200 x in deionized water!
   Reconstitute directly for use the negative control in 1 ml deionized water, the positive control is ready to use. Store immediately at -20°C until use.
2. **Qualitative:**
   Make a dilution 1:150 of each sample in ELISA buffer in an round bottomed titer plate.
   Make a dilution 1:50 of the (weak) positive and negative control.

   Quantitative:
   Make 3-step dilutions 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 (for the positive control begin with 1:10) and negative control.
3. Transfer 100 µl of this dilutions to the CCV coated microtiter strips.

4. Seal and incubate for 60 min. at 37°C.
5. Wash as in 1.
6. Dispense 100 µl conjugated anti-species antibody to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash as in 1.
9. 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).
10. Add 50 µl stop solution to each well; mix well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm. Use as a reference a wavelength of 620 nm.

PRECAUTIONS

1. Handle all biological material as though capable of transmitting CCV.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
4. TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
5. Do not use components past the expire date and do not mix components from different serial lots together.
6. 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.
7. Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect is from damage and dirt.

VALIDATION OF THE TEST

- To standardize the CCV ELISA a weak positive control and negative control have to be tested.
- In order to confirm appropriate test conditions, the weak positive control should give an extinction > 0.500 OD units and an end point titer > 90.
- The negative control should given an OD < 0.250 and an end point titer < 30.

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 is higher than 2,5 x OD of the negative control.

- **b. quantitatively:** end point titer

The end point titer of the sample is the dilution which gives an extinction just above 0.250 OD units (450 nm).
Antibody titers of 90 and higher in diseased animals showing signs suggestive of CCV are considered positive and the dog will be suspected of shedding CCV.
A rise in antibody titer in a dog with CCV represents an exaggerated, effective immune response.

In summary:

< 30 = no antibodies found
90-270 = antibodies found, probably shedding CCV, retest in 3 months
810 = high titer of antibodies found in recovered diseased animals

The entire risk as to the performance of these products is assumed by the purchaser. DRG shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.