DRG® Canine Parvo Virus Ag ELISA (EIA-2477)

Revised 23 May 2011 rm (Vers. 5.1) **For Veterinary Use Only**

Please use only the valid version of the package insert provided with the kit.

1 INTRODUCTION

For diagnosis of Canine Parvovirus (CPV) infections in dogs the demonstration of CPV antigen in feces is the most commonly used method. Possible false-negative results caused by naturally occurring variants of the virus is minimized in this assay, since two monoclonal antibodies directed against two different well conserved epitopes were used in the assay.

2 PRINCIPLE OF THE TEST

The test is based on the reaction of CPV proteins with dog antibodies. To this end CPV proteins have been coated to a 96-well microstate 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 anti-species conjugate.

The colour reaction in the wells is directly related to the concentration of CPV antibodies in the serum/plasma sample.

3 CONTENTS

- 1 x 12 **microtiter strips** coated with monoclonal anti-CPV antibody.
- 1 x strip holder
- 1 x 12 ml **Buffer**
- 1 x13 ml **HRPO-conjugated anti-Parvo antibody**
- 1 x 1 ml CPV **positive Control**
- 1 x 1 ml CPV **negative Control**
- 1 x 20 ml **Washing Solution, 200 x concentrated**. Dilute in deionized water before use!
- 1 x 8 ml **Substrate A**
- 1 x 8 ml **Substrate B**
- 1 x 8 ml **Stop Solution**
- 1 x plastic cover seal

Products needed that are not provided:

- Phosphate buffered saline (PBS)

4 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 increase non-specific reactivity.

5 WASH PROTOCOL

In ELISAs, 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 essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. 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 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 with a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are 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.

6 TEST PROTOCOL

1. Open the packet of strips. Take out the strips to be used, cover the remaining strips with a part of the seal provided; store at +4°C and use within 10 days.
   Wash according to the washing protocol. Dilute washing solution 200 x before use!
   Reconstitute freeze dried controls with 1 ml Aqua bidest.; not in case of wet controls, they are ready to use
2. Dillute the faeces samples at least 1+1 in a clean tube.
   Take a small sample of faeces/diarrhoea and add the same amount/volume of PBS (0.01 M) or aqua bidest (not provided) to the tube, mix well.
   Let cloths of faeces sink or spin down for 4 minutes at 4000 g.
   Use only the supernatant.
   Divide positive and negative controls into aliquots, and store immediately at -20°C until use.
3. Add 100 µl positive control to the first well.
   Add 100 µl negative control to the second well.
   Add 50 µl Buffer to all other needed wells and thereafter 50 µl supernatant of each centrifuged sample
4. Incubate 60 minutes at 37°C.
5. Wash as pointed out in wash protocol.
6. Add 100 µl HRPO-conjugated anti-Parvo antibodies to each well.
7. Incubate 60 min. at 37°C.
8. Wash as pointed out in wash protocol.

9. Mix equal parts of buffer A and B with gentle shaking. Prepare immediately before use!
   Dispense 100 µl substrate mixture to each well.
   Incubate for 10-15 minutes at room temperature (21°C).
10. After incubation the reaction is stopped by adding 50 µl stopping solution to each well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm. Use as a reference wavelength 620 nm.

7 PRECAUTIONS

- Handle all biological material as though capable of transmitting CPV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, by contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the 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 is from damage and dirt.

8 VALIDATION OF THE TEST

To standardize the Canine Parvo virus ELISA positive and negative controls have to be tested.

The CPV positive control should give an OD (450 nm) ≥ 0.500.

The OD (450 nm) of the negative control must be lower than 0.150.

9 INTERPRETATION OF TEST RESULTS

The test samples are considered CPV positive if the absorbency is above 3 times the absorbency of the negative control.

These animals will shed the Parvo Virus and will be infectious to other animals.

When a sample is **negative > sample < 3 x negative**,
it should be tested again within 5 days.

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.

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