DRG® CDV IgM (EIA-2479)

Revised 24 April 2007 **For Veterinary Use Only**

INTRODUCTION

For diagnosis of Canine Distemper Virus (CDV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. Antibodies induced through infection or vaccination are caught by the virus which is attached to the solid phase by use of monoclonal antibodies. After reaching peak values within one or two weeks, antibody titers fall back to a threshold level. Re-exposure results in an anomnestic response.

PRINCIPLE OF THE TEST

The CDV test kit is based on monoclonal antibodies against a common epitope of CDV, which are coated to the solid phase. The distemper virus is attached to the solid phase by the monoclonal antibody. After the attachment of the antigen (distemper virus) sera containing antibodies are able to react with the attached antigen. After the antigen/ antibody reaction, the attached antibodies can be detected by use of a polyclonal conjugate.

CONTENTS

- 2 x 6 8-well microtiter strips coated with monoclonal anti distemper virus antibody
- 1 x strip holder
- 2 x 6 ml anti dog IgM HRPO conjugate
- 3 x 5 ml ELISA buffer (freeze-dried)
- 1 x 100 ml wash solution, 10 x concentrated, which must be diluted in deionized water before use!
- 1 x 0,5 ml positive control serum
- 1 x 1 ml negative control serum (freeze-dried)
- 1 x 8 ml substrate A
- 1 x 8 ml substrate B
- 1 x 8 ml stop solution
- 1 x plastic cover seal

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.

WASHING PROTOCOL

In ELISA's un-complexed components must be removed efficiently between each immunological 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 follow the washing procedures outlined below carefully.
Both manuals 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 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 any residual washing solution from 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.

TEST PROTOCOL

1. We advise that you pre-incubate all your sera with a solution of αIgG or protein-G in order to eliminate any IgG that might prevent IgM from reacting with the antigen on the microtiter plate.
2. Reconstitute the ELISA buffer directly before use with 5 ml PBS. Make 3 steps dilutions of each sample (1:30; 1:90; 1:270; 1:810) in an other round bottomed microtiter plate. Reconstitute the positive with 0,5 and the negative control with 1 ml PBS directly before use. Make the following dilutions of the positive (1:5, 1:15, 1:45, 1:135) and negative controls (1:30; 1:90; 1:270 and 1:810). Add 100 µl of each dilution to a well of the coated microtiter plate.
3. Seal and incubate for one hour at 37°C.
4. Wash according to washing protocol, with washing solution. Dilute washing solution 1:10 before use!
5. Add 100 µl of the HRPO-conjugated antibodies to each well.
6. Seal and incubate for one hour at 37°C.
7. Wash as in 4.
8. Mix equal parts of buffer A and B under gentle shaking (Prepare immediately before use!). Dispense 100 µl of this substrate solution to each well. Incubate for 10-20 min. at room temperature (21° C).
9. Add 50 µl of stop solution to each well.
10. Read the absorbency values immediately (within 10 min.!) at 450 NM. Use a wavelength of 620NM as a reference.

PRECAUTIONS

- Handle all biological materials as though capable of transmitting CDV.
- 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, in contact with skin and is swallowed; observe care when handling the substrate.
- Do not use components past expiration date and do not intermix components from different serial lots.
- 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 microtitre plate and prevent it from damage and dirt.

VALIDATION OF THE TEST

The negative control should give an OD < 0.200. The end point titer of the positive control should be approximately 1:150 according to the instructions for interpretation of test results.

INTERPRETATION

The titer of the sample is the dilution which gives an extinction above 0,200 OD units (450 nm).
The test is valid if the first two dilutions of the positive control are above 0,200 (OD 450 nm).

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