DRG® Heartworm Ag (Canine) (EIA-4795)

Revised 24 May 2011 rm (Vers. 2.1) **For Veterinary Use Only**

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

INTRODUCTION

Dirofilaria Immitis (Nematode Filaridae) has been reported parasitizing a variety of wild canids, foxes and felids (9% positive animals found in Australia). Prevalence of infection may vary with geographic location, habitat, densities of mosquito vectors and definitive hosts and climatic conditions. In diseased dogs Dirofilaria Immitis are found in heart, longs, pulmonary arterities or thoracic vena cava. The amount of Microfilaraemia correlates with the number of adult filariae which also correlates with the age and weight of the dog. Dirofilaria antigen titers correlates best with weight and worms present (R=91) adjusted to female worm equivalents (four male worms equal to one female worm).

Epidemiological studies on the canine population have demonstrated that Dirofilaria Immitis is the predominant species prevalence between (0,6% -34%) have been reported (Valladres et all 1984/Perez-Sanches et all 1989) important areas are Italy, France, Spain, Portugal, Australia and Brazil. Different potential vectors have been studied, the ability is attributed to Aedes Vexans, Aedes caspuis and Aedes longitubes (blood sucking mosquitoes).

Several studies demonstrate **Human Dirofilariosis** (± 250 cases are reported) in high prevalence area the majority of this infections are subclinical. A small percentage of patients with symptoms (Malaise, Thoracic aches, low fever, cough) all have pulmonaire ”coin lesions”. Some ELISA antigen test also detects cross-reactive Dirofilaria SPP (rapens/Dipetalonema).

The life cycle of the Dirofilaria Immitis is as follows: Microfilaria develops (inside mosquito) within 14 days (10-16) are transferred by the mosquito to the host (dog/fox etc.) by biting. This L3 stage in the definitive host moults five times and migrates to the heart area (venous part) within 6 months. Ivermectin and diethylcarbamazine can be used as treatment for infected dogs/cats (0,006mg/kg) once a month.

PRINCIPLE OF THE TEST KIT

The heartworm (Dirofilaria Immitis) ELISA antigen test kit detects heat stable *Dirofilaria Immitis* antigens, which is the major course of canine Heartworm in serum samples.

The principle of the test is based on the reaction of two monoclonal antibodies with an antigenic determinant against Dirofilaria Immitis. One monoclonal antibody, coated to the plate, catches the Dirofilaria in the serum or plasma sample after which the other, enzyme-labeled antibody detects the bound antigen.

After incubation and rinsing, the substrate is then added and the optical density is measured at 450 nm.

CONTENTS

- 12 x 8-well **microtitre strips** coated with monoclonal anti-Dirofilaria antibody.
- 1 x **strip holder**
- 1 x 11 ml **HRPO-conjugated** monoclonal **antibody**
- 1 x 1 ml **positive control** (Ready to use)
- 1 x 1 ml **negative control** (Ready to use)
- 1 x 20 ml **wash solution** 200 x concentrated *(must be diluted in deionized water before use!)*

- 1 x 15 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**

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 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.

TEST PROTOCOL

1. Open the packet of strips, 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.

2. Wash the microtiter strip(s) with washing solution, according to the washing protocol.
3. Add 100 µL positive control to one well.
   Add 100 µL negative control to the next well.
4. Add 50 µL buffer to all remaining wells and thereafter
   add 50 µL serum of each serum sample to be tested to the subsequent well.
5. Seal and incubate for 60 min. at 37°C.
6. Wash as pointed out in wash protocol.
7. Dispense 100 µL anti-Heartworm antibody conjugate to all wells.
8. Seal and incubate for 60 min. at 37°C.
9. Wash as pointed out in wash protocol.
10. Mix equal parts of buffer A and buffer B together with gentle shaking. Prepare immediately before use!
    Dispense 100 µL substrate solution to each well.
    Incubate 10-15 min. at room temperature.
11. Add 50 µL stop solution to each well.
12. Read the absorbency values immediately (within 10 min.!) at 450 nm ref. 620 nm

PRECAUTIONS

- Handle all biological materials as though capable of transmitting infectious diseases.
- 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, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expiry 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 microtiter plate and protect it from damage and dirt.

INTERPRETATION OF TEST RESULTS

A sample is considered positive when the measured extinction is higher than the OD of the negative control, but at least ≥ 0,300.

All signals below 0,300 are considered to be negative.

The OD of the positive control must be at least > 0.800.

In case of a negative result, the presence of Microfilaria should be tested by microscope.

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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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