Useŕs Manual

Heartworm (Dirofilaria immitis) Antigen SRE

A monoclonal antibody-mediated SRE test to detect Heartworm antigen (Dirofilaria immitis) in serum or plasma samples

REF D3213-AG01

Σ 32

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

1. Table of Contents

2. 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 host 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 er 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 arches, low fever, cough) all have pulmonaire “coin lesion”. 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 bitting. This L3 stage in the definitive host moults five times and migrates to the heart area (venous part) within 6 months.

3. Intended use of the test kit

The heartworm (Dirofilaria immitis) SRE antigen testkit detects Dirofilaria immitis antigens, which is the major course of canine heartworm in serum or plasma samples.

4. Principle of the test kit

The principle of the test is based on the reaction of two monoclonal antibodies with an antigenic determinant of Dirofilaria immitis. One monoclonal antibody, coated to the plate, catches the Dirofilaria antigen in the serum or plasma after which the other, enzyme-labeled antibody detects the bound antigen.
The colour reaction in the wells is directly related to the concentration of heartworm antigen in serum/plasma samples.

5. Contents

- 4x 8 Microtiter strips coated with monoclonal anti-Dirofilaria antibody
- 2x ELISA buffer (white bottle + green cap)
- 1x HRPO conjugated monoclonal antibody (black bottle + red cap)
- 1x Positive control (ready to use) (yellow cap)
- 1x Negative control (ready to use) (brown cap)
- 1x 4ml Substrate A (white bottle + white cap)
- 1x 4ml Substrate B (black bottle + blue cap)
- 1x User's manual

Supplies needed (not included)

- Precision pipette 10-200µl (EVL)
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader (the results can be interpreted by eye, but for a more accurate and objective reading the use of the ELISA plate reader is strongly recommended)

6. Handling and storage of specimens

- The kit should be stored at 4°C.
- An open strip packet should be used within 28 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.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ±15 min. at room temperature (±21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solutions.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol **qualitative**

1. Before starting this test read **"preparations**".
2. Open the packet of strips and take out the amount of wells needed from the test strip, 1 for each sample and 2 extra wells for the controls. Cover the remaining strips with a part of the provided seal and store them at 4°C and use them within 10 days.
3. Use the precision pipette 10-200µl and use a clean pipette tip **before** pipetting the buffer, standards, samples, conjugate and substrate.
4. Before testing make sure all reagents are at room temperature.

5. Wash the test strips with running tap water.
    - Fill all wells to the rim.
    - Empty the wells.
    - Repeat 5 times.
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

6. Add 120µl of the negative control to the first well.
7. Add 120µl of the positive control to the second well.
8. Add 60µl of buffer to the remaining sample wells.
9. Add 60µl of sample (serum/plasma) to the remaining sample wells.
10. Mix the reagents gently (see **"preparations**").
11. Incubate for 40 minutes at room temperature (±21°C).

12. Wash the test strips with running tap water.
    - Fill all wells to the rim.
    - Empty the wells.
    - Repeat 5 times.
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

13. Add 120µl of conjugate to each well.
14. Incubate for 40 minutes at room temperature (±21°C).
15. Turn on the analyser (when available).

16. Wash the test strips with running tap water.
    - Fill all wells to the rim.
    - Empty the wells.
    - Repeat 5 times.
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

17. Add 60µl of substrate A to each well.
18. Add 60µl of substrate B to each well.
19. Mix the reagents gently (see **"preparations**").
20. Incubate for 10-15 minutes in the dark (e.g. cover the wells with a sheet of paper).

21. Read the absorbency values immediately (**within 10 min!**) at 620 nm on the analyser or by eye.
    Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

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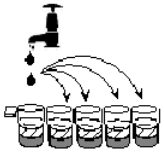
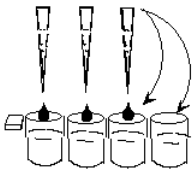
9. Illustrated Test protocol

STEP: 5



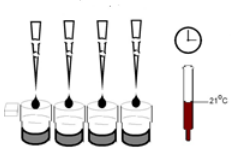
- Wash procedure
- Repeat 5 times

STEP: 6 & 7



- Add 120µl of the negative control to the first well.
- Add 120µl of the positive control to the second well.
- Add 60µl of buffer to the remaining sample wells.

STEP: 8 t/m 11

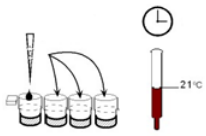


- Add 60µl of sample (serum/plasma) to the remaining sample wells.
- Mix the reagents gently (see “preparations”).
- Incubate for 40 minutes at ±21°C.

STEP: 12

- Wash procedure
- Repeat 5 times

STEP: 13 & 14

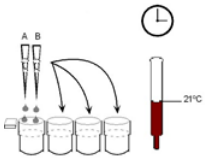


- Add 120µl of conjugate to each well.
- Incubate for 40 minutes at ±21°C.

STEP: 16

- Wash procedure
- Repeat 5 times

STEP: 17 t/m 21



- Add 60µl of substrate A to each well.
- Add 60µl of substrate B to each well.
- Mix the reagents gently (see “preparations”).
- Incubate for 10-15 minutes in the dark at ±21°C.

Read the absorbency values immediately (within 10 min!) at 620 nm on the analyser or by eye
Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

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10. Precautions

- Handle all biological material as through 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 B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix 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 optimal cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

11. Interpretation of the test results

The analyser will give the results as positive, weakly positive or negative, but always double-check the outcome by observing the intensity of colour development.

- **Positive**
  - A sample is scored positive if the sample colour is dark blue, at least as blue as the positive control.

- **Weakly positive**
  - A sample is scored weakly positive if the sample colour is blue, with an intensity between that of the negative and positive control.

- **Negative**
  - A sample is scored negative if the sample colour is equally blue or less blue than the negative control.

In summary

- **Colourless**
  - (Negative) no antigen found
    - The animal has no measurable D.immitis antigen in serum/plasma.

- **Dark blue or blue**
  - (Positive/weakly positive) antigen found
    - The animal has D.immitis antigen in serum/plasma.

12. Symbols used with EVL ASSAYS

Symbol	Definition
	Consult instructions for use
	European Conformity
	In vitro diagnostic device
	For research use only
	Catalogue number
	Lot/ No. / Batch code
	Contains sufficient for <n> tests
	Storage Temperature
	Expiration Date
	Legal Manufacturer
Distributed by	Distributor
Content	Content
Volume/No.	Volume / No.

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