

User's Manual

Heartworm Antigen One-Step

*For the detection of Heartworm (*Dirofilaria Immitis*) antigen in serum or plasma samples of dogs*

REF D1013-AG01

Σ 6

January 2022

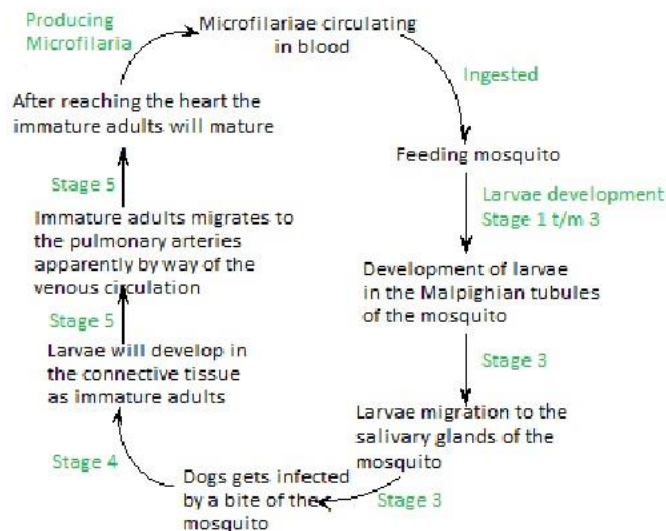
Please use only the valid version of the package insert provided with the kit.

1. Table of Contents

1.	Table of Contents	2
2.	Introduction.....	3
3.	Intended use of the test kit	3
4.	Principle of the test kit	4
5.	Contents	4
6.	Handling and storage of specimens	4
7.	Sample material.....	4
8.	Precautions.....	5
9.	Test protocol	5
10.	Validation of the test.....	6
11.	Interpretation of the test results.....	6
12.	Symbols used with EVL ASSAYS	7

2. Introduction

Dirofilaria immitis has been to infect a variety of wild canines, foxes and felines (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, lungs, pulmonary arteries or thoracic vena cava. The amount of microfilariae correlates with the number of adult filariae which also correlates with the age and weight of the dog. *Dirofilaria* antigen titres correlate best with weight and worms present adjusted to female worm equivalents (four male worms equal to one female worm).



Lifecycle of Dirofilaria Immitis in a dog:

Mature worms may survive and produce Microfilariae for ± 5 years in their host. Microfilariae circulate in the blood of the dog, where they may be ingested by a feeding mosquito. Larval development occurs in the Malpighian tubules. In the Malpighian tubules the Microfilariae will develop in larvae (stage 1 up to 3). When the larvae have reached his third stage he will migrate to the salivary glands of the mosquito. The third stage larvae enter the bite wound when the mosquito feeds on the dog. Within 3 days the molt from third stage to fourth stage occurs after the bite of the mosquito. Fourth stage larvae remain in the connective tissue for several months (2-3 months) to develop as immature adults (stage 5). The immature adults migrates to the pulmonary arteries apparently by way of the venous circulation. After reaching the heart, the young adults mature and start producing Microfilariae (6-9 months after infections)

3. Intended use of the test kit

This One-Step test is intended to use as practical/routine screening test that can be done in a few minutes. This test kit is designed to detect *Dirofilaria Immitis* antigen by use of a rapid immunochromatic assay.

4. Principle of the test kit

The Heartworm One-Step test is based on a chromatographic principle in which a monoclonal antibody reacts with the *Dirofilaria Immitis* antigen produced by the adult worm. A monoclonal antibody is conjugated to colloidal gold particles and a monoclonal antibody is immobilized on the test strip in the test zone "T". *Dirofilaria Immitis* antigen in the sample that is applied to the test strip at the sample zone "S", will bind to the colloidal gold particles which then migrate to zone "T". A colour change in zone "T" indicates a positive test.

Labelled colloidal gold particles are also immobilized on the test strip in the control zone "C", to indicate that the test is working properly.

5. Contents

- 6x Pouches, each containing 1 test strip and 1 pipette
- 6x Buffer vial
- 1x Protocol

6. Handling and storage of specimens

The One-Step should be stored at room temperature ($\pm 21^{\circ}\text{C}$). An unopened package can be used until the expiry date. An opened package must be used immediately. If the conditions are no longer fulfilled the test can no longer be used. Avoid freezing and heating as this will contribute to destruction of the test. Samples may be used fresh or may be kept frozen below -20°C before use.

7. Sample material

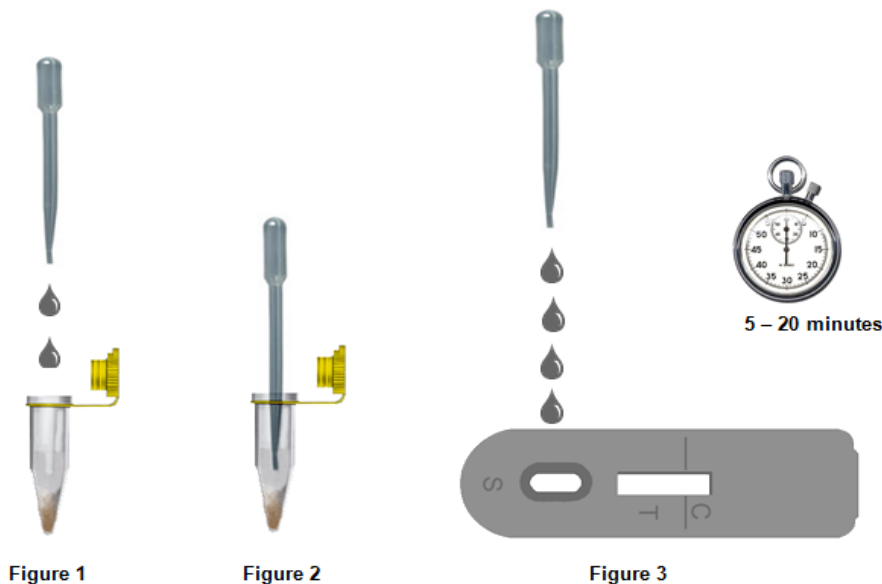
It is advised to test fresh samples. Do not use haemolytic or lipemic serum.

8. Precautions

- Handle all biological materials as though capable of transmitting infectious diseases.
- Do not pipette by mouth
- Do not eat, drink, smoke, prepare foods or apply cosmetics within the designated work area.
- Do not use components which passed the expiry date and do not mix components from different serials lots together.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and sampling throughout this procedure are necessary to maintain precision and accuracy.
- Each test strip is ultimately used as an optical reference. Therefore, do not touch the surface of the test strip and protect it from damage and dirt.

9. Test protocol

1. Unpack the test strip, swab and pipette. Only open the amount of pouches to be used. An opened package should be used immediately.
2. Add **2 drops** sample to the buffer vial by using the pipette (Figure 1).
3. Mix well by using the pipette (Figure 2).
4. Add **4 drops** of the buffer vial containing the sample, with the included pipette **slowly** to the sample zone “S” (Figure 3).
5. Read the result after 5 – 20 minutes (for the interpretation of the test result see chapter 10 and chapter 11).



10. Validation of the test

To validate an EVL One-Step a control line should always be visible at control zone “C”. If no control line is visible the test should be considered invalid.

Results should be read in the given time. Results read after the given time should be considered invalid. Invalid tests should be repeated with a new test.

11. Interpretation of the test results

Positive:

Two lines are visible in zone “T” and in zone “C” (Figure A). The sample contains *Dirofilaria immitis* antigen.

Positive results may vary in optical density due to variations in viral concentrations in the sample.

Weak positive:

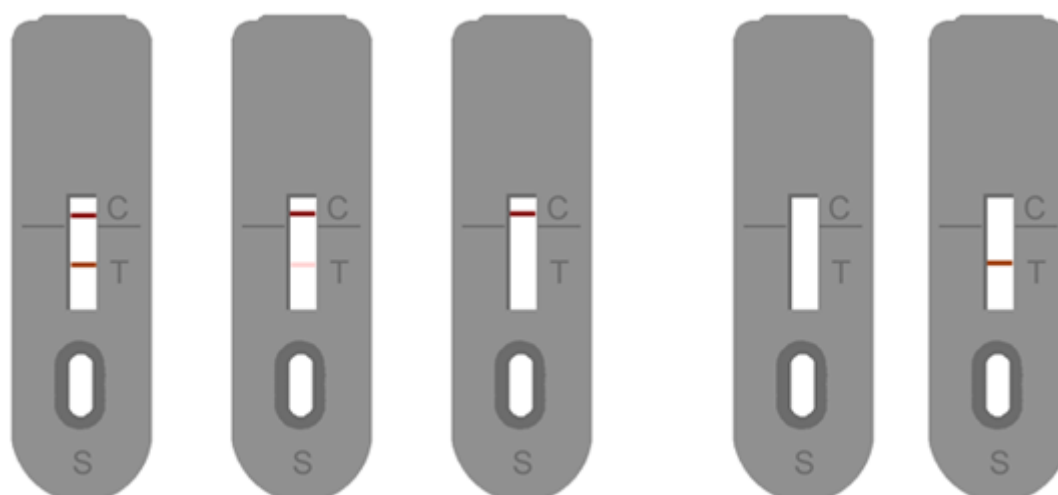
Two lines are visible, a weak line in zone “T” and a line in zone “C” (Figure B). The sample contains low concentrations *Dirofilaria Immitis* antigen.

Negative:

Only one line is visible in zone “C” (Figure C). The sample does not contain *Dirofilaria Immitis* antigen.

Not valid:

No line is visible in zone “C” (fig. D). Repeat the test procedure with a new test cassette.



**Figure A:
Positive**

**Figure B:
Weak positive**

**Figure C:
Negative**

**Figure D:
Not Valid**

12. Symbols used with EVL ASSAYS



<u>Symbol</u>	<u>English</u>
	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.

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