Version 04/2025

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*FAST*est® HW Antigen

ad us. vet.

***In vitro* diagnosticum**

Test-kit for the qualitative detection of *Dirofilaria immitis* specific antigens in whole blood, plasma or serum of the dog and cat

INSTRUCTIONS FOR USE

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1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® HW** Antigen contains:
- 2*, 10**, 25*** or 50**** test cassettes coated with monoclonal antibodies
- 1 dropper bottle **A** with *1.0 ml, **2.0 ml, ***4.0 ml or ****8.0 ml buffer diluent
- 2, 10, 25 or 50 disposable plastic pipettes
- 1 instructions for use

STABILITY AND STORAGE

15–25°C Store at 15–25 °C

Expiry date – see label

APPLICATION AND ABBREVIATIONS

For veterinary use only

LOT Lot number

In vitro diagnosticum

! Do not use test-kit components from different kits, lot numbers or beyond stated expiry date.

Follow instructions for use precisely

S – TEST line, **C** – CONTROL line, **LF** – Lateral flow

LIABILITY

The entire risk due to the performance of this product is assumed by the purchaser. The manufacturer shall not be liable for indirect, special or consequential damages of any kind resulting from the use of this product.

ACCURACY

Sensitivity 98.6 % – Specificity 99.1 %
(Comparison Method: Knott's Test)

2. INTRODUCTION

The dirofilariosis of the dog, cat, ferret as well as other carnivores, is caused by the so-called "heart worm", a nematode of the filaria family named *Dirofilaria immitis*. Infection of humans (dead end host) is possible (zoonosis). The filaria manifest especially in lungs and conjunctive tissue, but are rarely diagnosed.

The transmission happens via infected, haematophagous mosquito species (Culicidae), releasing infectious *D. immitis* larvae (stage L3) in the host blood with the sting. After development of the larva (stage L4) in the hypodermis of the host (about 8 days post infection), they migrate into the blood circulation. The establishment of the adult worms (macrofilaria: up to 1 mm thick, 20–30 cm long) takes place earliest 80 days p. inf., most of all in the pulmonary artery and in the right heart chamber. The female adult parasite of the bisexual macrofilaria produce new larvae (stage L1, microfilaria) at first after 6 (dog) to 7 (cat) months. These are released together with antigens of the female reproductive tract to the peripheral blood (microfilaraemia) and are ingested again by mosquitoes at sucking action. In the mosquitoes, the larva 1 develops into an infectious larva 3.

The degree of disease depends on the quantity of adult worms ("worm burden"), localization, duration of infection and the host's immunological reaction. Concerning the worm burden, dogs and cats differ a lot. In cats, normally there are less than 5 worms, in dogs more than 30.

First of all, dirofilariosis is a cardiopulmonary disease beginning without symptoms. In advanced stage, right-sided heart failure and Cor pulmonale occur with symptoms like cough, dyspnoea, heart and lung murmurs, oedemas as well as fast fatigue. Particularly in small dogs, in case of large "worm burden" the "vena-cava syndrome" (obturation stenosis because of massive worm cluster in the posterior vena cava and the right atrium of the heart) occurs and thus leads to intravascular haemolysis, shock, kidney failure and sudden death.

In cats, rather the lungs are affected and the symptoms are not always typical for heart worms. Nevertheless, only one *D. immitis* can be lethal.

Due to the rather difficult clinical diagnosis and the short and transient proceeding microfilaraemia, a repeated testing with ***FAST*est® HW** Antigen is recommended.

Being fast and reliable, the ***FAST*est® HW** Antigen detects group-specific antigens of the active reproductive tract of the female adult *D. immitis* worm. Due to the long incubation time of 6 (dog) and 7 (cat) months p. inf. (after stay in dirofilariosis regions), testing with ***FAST*est® HW** Antigen should be carried out earliest 6 and 7 months after stay in dirofilariosis regions, respectively.

3. INFORMATION ON THE SPECIMEN MATERIAL

Approximately **25 µl (1 drop of attached plastic pipette) 15–25 °C warm freshly obtained whole blood (WB**, with or without anticoagulant like Heparin or EDTA**), plasma (P) or serum (S)** are needed. **Native blood without any anticoagulant should be avoided due to the potential risk of microclots.** Mix the sample material well before use!

Non-cooled (**15–25 °C**), WB, P and S should be tested within 4 hours! At **2–8 °C**, WB with anticoagulant, P and S can be stored up to 3 days. **Serum and / or plasma samples** can be permanently stored **at minimum −20 °C**.

Keep in mind that the sample material, as well as all used test-kit components, should have reached **room temperature** at the time of application.

Endogeneous and exogeneous interfering substances of the sample (e. g. albumin, fibrinogen, lipids, CRP, heterophilic antibodies, especially type IgA, as well as viscosity, pH-value and excess EDTA) **as well as native blood can cause interferences** (matrix effects) **that can influence the target measurement. These can lead to an impaired LF and / or unspecific reactions on S and C.**

4. SPECIMEN PREPARATION

Normally no specimen preparation necessary!

With suspicion of "false negative" and clinical dirofilariosis symptoms (see issue 9 / Information for the interpretation), heat treatment of plasma / serum before testing is recommended to increase the sensitivity of the antigen detection.

Heat the plasma / serum in a block thermostat up to 103 °C for 10 minutes. Immediately centrifuge the resulting coagulate for 5 minutes at 16 000 × g (optimum, alternatively 8–12 000 × g). The supernatant won hereof can be used as sample material for the test procedure.

5. TEST PROCEDURE

1. Remove the test cassette from its foil pouch shortly before use. Place it on a flat surface.
2. Take the disposable plastic pipette and express **1 drop (ca. 25 µl) of whole blood, plasma or serum** into the round sample window of the test cassette (fig.1). Hold the pipette vertically!
3. Hold the dropper bottle **A** vertically and express **3 drops of buffer diluent (approx. 100–120 µl)** into the sample window of the test cassette (fig.2).
4. Add 1 additional drop of buffer diluent into the sample window if there is no beginning pink-purple LF visible within 2 minutes after adding the buffer diluent.

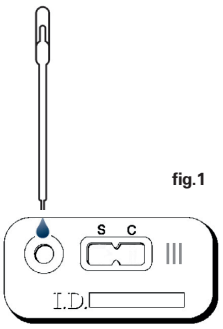

fig.1

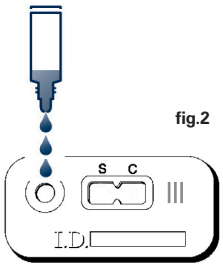

fig.2

6. READING OF THE TEST RESULT

15 min

Read the test result **15 minutes** after the three drops of buffer diluent have beed added into the sample window.

POSITIVE TEST RESULT (fig.3)
A pink-purple TEST line (S) **of any intensity** (varying from very weak to stronly intensive) and a pink-purple CONTROL line (C) appear.

NEGATIVE TEST RESULT (fig.4)
Only a pink-purple CONTROL line (C) appears. This line indicates, irrespective of its intensity, that the test has been performed properly.

INVALID TEST RESULT
No CONTROL line visible. The test should be repeated using a new test cassette.

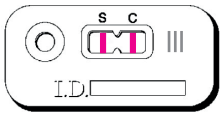

fig.3
POSITIVE TEST RESULT

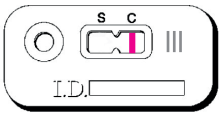

fig.4
NEGATIVE TEST RESULT

7. PRECAUTIONS FOR USERS

- The guidelines for working in medical laboratories must be observed. It is recommended to wear disposable gloves and other personal protective equipment (protective clothing, possibly a face mask). Wash and disinfect hands after completing the test.
- Label sample material and associated test cassette to ensure a precise assignment.
- Use a new pipette and a new test cassette for each sample.
- The buffer diluent contains low concentrations of toxic sodium azide as a preservative, therefore avoid skin / eye contact and / or ingestion.
- The sample material must be seen as potentially infectious and disposed of accordingly, together with the used test-kit components.

8. TEST PRINCIPLE

The ***FAST*est® HW** Antigen is based on an immunochromatographic "sandwich principle" detecting specific *Dirofilaria immitis* group antigens of the adult female reproductive organ.

The *Dirofilaria immitis* antigens of the sample bind to mobile monoclonal anti-*Dirofilaria immitis* antibodies which are bound to colloidal gold particles. Migrating along the nitrocellulose membrane ("lateral flow", **LF**), these antigen-antibody complexes are captured by immobilised antibodies forming a pink-purple TEST line (**S**). The intensity or width of the TEST line (S) depends on the concentration of *Dirofilaria immitis* antigens in the tested sample.

A correct test procedure will be indicated by a second, pink-purple CONTROL line (**C**).

The ***FAST*est® HW** Antigen shows no cross reactivity to *Angiostrongylus vasorum* (Internal study at the Institute for Parasitology of the Vetsuisse Faculty, University of Zurich, March 2020)

9. INFORMATION FOR THE INTERPRETATION

- The interpretation of the test result should always be based on anamnestic and clinical data as well as the therapy and prophylaxis possibilities.
- Any non-described colour or contour variation of S and C (e. g. greyish, shadow-like lines) has to be considered as unspecific reaction and therefore as negative test result.
- Positive test results may be observed in as short as 1 minute depending on the severity of the infection.
- Due to anticoagulated whole blood and / or red hemoglobin background of the test membrane caused by hemolysed blood samples, the visibility of S, especially in case of weak positive samples, could be from bad to not visible.

Possible reasons for false positive test results
- Dog with dead adult dirofilaria remains antigen-positive for approx. 3–4 months → a second test after 4 months is recommended!

Possible reasons for false negative test results
- Dog or cat with an infection period less than 6 / 7 months
- Very low worm load, especially in cats
- Infection with only male *Dirofilaria*, infection with non-gravid female *Dirofilaria* (single sex infection)
- Antigen masking by immune complexes (long-term medication with macrocyclic lactone preventives ["slow-kill medication"], inflammation). Recommendation: heat inactivation of plasma / serum (also read issue 4 / Specimen preparation).