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*FAST*est® CVBD 4T

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***In vitro* diagnosticum**

Test-kit for the qualitative detection of antibodies against *Anaplasma* spp. *(A. phagocytophilum, A. platys)*, *Ehrlichia canis* and *Leishmania infantum* as well as *Dirofilaria immitis* antigens in whole blood, plasma or serum of the dog

INSTRUCTIONS FOR USE

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1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® CVBD 4T** contains:
- 2*, 6**, 15***, 25**** or 50***** quadruple test cassettes coated with synthetic *A.* spp., *Ehrlichia canis* or *Leishmania infantum* antigens or specific antibodies against *D. immitis*
- 1 dropper bottle **A** with *1.0 ml, **3.0 ml, ***7.5 ml, ****12.5 ml or *****2 dropper bottles **A** with 12.5 ml buffer diluent
- 2, 6, 15, 25 or 50 disposable plastic pipettes
- 1 instructions for use

STABILITY AND STORAGE

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

T – 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.

2. INTRODUCTION

Vector-borne parasitic diseases (e.g. leishmaniosis, anaplasmosis, ehrlichiosis and dirofilariosis) are of increasing diagnostic importance in dogs and cats due to climatic, ecological and cultural changes. In addition to vector-borne transmission, mainly by arthropods (especially ticks) and dipterans (especially mosquitoes and sandflies/butterflies), these can also be transmitted directly via blood (especially transfusions). The spread of these vectors and pathogens into previously non-endemic areas is constantly increasing. Therefore, testing for these pathogens (antigen detection and/or antibody detection) is recommended in suspected cases (routine check in case of travel/import history, presence of clinical symptoms, persistent infections, etc.).

Further detailed background information on these diseases can be found under the individual diseases or under the headings "Vector-borne diseases", "CVBD", e.g. the ESCCAP, the ABCD guidelines, the WHO and others.

Co-infections are not uncommon in vector-borne diseases. In suspected cases of (travel) parasitosis, the ***FAST*est® CVBD 4T** is therefore suitable for the rapid, qualitative detection of antigens (dirofilariosis) or antibodies (leishmaniosis, anaplasmosis, ehrlichiosis). Therefore, it enables further diagnostic, therapeutic and prophylactic measures to be taken immediately.

ACCURACY

Anaplasma: Sensitivity 93.6 % – Specificity 97.6 %
Ehrlichia: Sensitivity 92.5 % – Specificity 96.7 %
Dirofilaria: Sensitivity 94.1 % – Specificity 100 %
Leishmania: Sensitivity 95.2 % – Specificity 100 %
(Comparison Method: ELISA)

3. INFORMATION ON THE SPECIMEN MATERIAL

Exactly 20 µl each (1 drop of attached plastic pipette) 15–25 °C warm whole blood (WB, with anticoagulant**), plasma (P) or serum (S)** are needed. **Native blood without anticoagulant must not be used due to potential micro agglutination** (e.g. migration delay on the membrane, unspecific reaction).

Homogenise 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, P and S can be stored up to 4 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 T and C.**

4. SPECIMEN COLLECTION AND PREPARATION

- No specimen preparation necessary.
- **ATTENTION:** Partially filled and/or insufficient mixed EDTA, Citrate or Heparin tubes could create invisible microclots resulting in lateral flow delay and/or unspecific reactions (e.g. greyish shadow like lines).

5. TEST PROCEDURE

1. Remove the quadruple test cassette from its foil pouch shortly before use. Place it on a flat surface.
2. Draw sample **up to the mark (≙ 20 µl sample volume)** without air bubbles, using the disposable plastic pipette. **The meniscus must be above the black line** (fig.1). Place the **whole sample volume (20 µl) into the sample window S** of the Anaplasma test cassette (ANAPL AB, hold pipette vertically, fig.2). **Repeat the whole procedure for E.canis, Diro and Leish.**
3. Hold the dropper bottle **A** vertically and place **2 drops (ca. 80–100 µl) of the buffer diluent** into each of the four sample windows S of the test cassette (fig.3). Ensure pressure equalisation after every 2 drops (air flow by turning the bottle 180° to the starting position.
4. Add 1 additional drop of buffer diluent into the sample window S if there is no beginning LF visible within 1 minute after adding the buffer diluent.

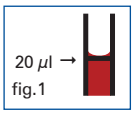


fig.1

fig.2

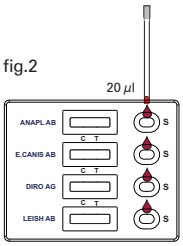
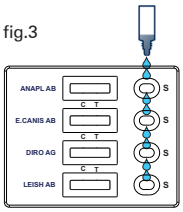


fig.3



6. READING OF THE TEST RESULT

Read the test result **10 minutes** after the buffer solution has been added into the sample window S.

POSITIVE TEST RESULT (fig.4)
A pink-purple TEST line **of any intensity** (varying from very weak to strongly intensive) and a pink-purple CONTROL line appear.

NEGATIVE TEST RESULT (fig.5)
Only a pink-purple CONTROL line 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 quadruple test cassette.

Note: Different combinations of positive and negative test results can occur.

fig.4 **Positive test result**

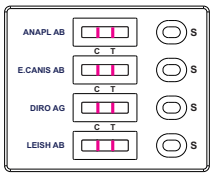
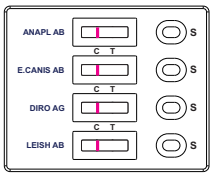


fig.5 **Negative test result**



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 quadruple test cassette to ensure a precise assignment.
- Use a new pipette and a new quadruple 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 accordingly, together with the used test-kit components.

8. TEST PRINCIPLE

The ***FAST*est® CVBD 4T** is based on an immunochromatographic "sandwich principle".

The antibodies against *A.* spp., *E.c.* or *L.inf.* resp. the *D.i.* antigens present in the sample react in the conjugate pad with mobile antigens or antibodies, which are conjugated to colloidal gold particles. These antigen-antibody complexes are migrating along the nitrocellulose membrane ("lateral flow", **LF**) and bind to fixed recombinant *A.* spp., *E.c.* or *L.inf.* antigens or to monoclonal antibodies against *D.i.*, forming a pink-purple TEST line (**T**).

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

9. INFORMATION FOR THE INTERPRETATION

- The interpretation of the test result should always be based on anamnestic (stay abroad, tick infestation) and clinical data as well as therapy and prophylaxis possibilities.
- Any non-described colour or contour variation of T and C (e.g. greyish, shadow-like lines) has to be considered as unspecific reaction and therefore as negative test result.
- T can vary both in intensity (from weak to strong pink-purple) and in width. Therefore, any pink-purple line appearing within the required incubation time has to be interpreted as positive test result.
- Positive test results may be observed even before the end of incubation. Beyond this time, test results should not be interpreted.
- Due to anticoagulated whole blood and/or red hemoglobin background of the test membrane caused by hemolytic blood samples, the visibility of T could be from worse to not visible.

Antibody detection (Anaplasma, Ehrlichia, Leish):

For the detection of antibodies, a two-step diagnostics is known to be standard. The first step starts with in-clinic IgG antibody screening test like ***FAST*est® CVBD 4T**. The suspicion about an active anaplasmosis/ehrlichiosis/leishmaniosis is substantiated by combination with according clinic. Furthermore, a quantitative antibody testing via IFAT (coupled serum samples at intervals of 2–4 weeks) should be taken to determine the end titre or the titre increase (seroconversion).

Positive test result
- The detection of ab against *Anaplasma* spp./*Ehrlichia canis*/*Leishmania*, with a matching anamnesis/clinic indicates a high probability of a clinical infection.

Negative test result
- There is a high probability that the dog has had no contact with *Anaplasma* spp./*Ehrlichia*/*Leishmania*.

Antigen detection (Diro):

Positive test result
- The detection of *D. immitis* antigen indicates a high probability of clinical dirofilariosis.
- Exception: Dogs with dead adult dirofilariae remain antigen-positive for approx. 3–4 months → a second test every 4 months is recommended.

Negative test result
- Dog most probably had no contact with *D. immitis*.
- Exceptions: Infection duration shorter than 6/7 months // very low worm burden // infection with only male dirofilariae, infection with non-gravid female dirofilariae (single sex infection).