Version 10/2023

Click Here For More Information About

*FAST*est® ANAPLASMA -EHRLICHIA

ad us. vet.

***In vitro* diagnosticum**

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

INSTRUCTIONS FOR USE

Supplied Exclusively To The UK Veterinary Market By
Vetlab Supplies Ltd
Visit Our Website
www.vetlabsupplies.co.uk
Telephone: 01798 874567
email us: info@vetlabsupplies.co.uk

Manufacturer:
DIAGNOSTIK MEGACOR
6912 Hörbranz – AUSTRIA
www.megacor.com

1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® ANAPLASMA-EHRLICHIA** contains:
- 2*, 10** or 50*** twin test cassettes coated with recombinant *Anaplasma* spp. or *Ehrlichia canis* antigens
- 1 dropper bottle **A** with *1.0 ml or **3.0 ml or ***2 dropper bottles **A** with 7.5 ml buffer diluent
- 2, 10 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

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.

ACCURACY

Anaplasma: Sensitivity 99 % – Specificity 96.4 %
Ehrlichia: Sensitivity 94.3 % – Specificity 93.3 %
(Comparison Method: IFAT)

2. INTRODUCTION

Canine Granulocytic Anaplasmosis (GA, *Anaplasma phagocytophilum* [*A.p.*], Thrombocytic Anaplasmosis (TA, *Anaplasma platys*) and Canine Monocytic Ehrlichiosis (CME, *Ehrlichia canis*) belong to the most important vector-borne infectious diseases induced by canine parasites. Co-infections play a major role in both pathogens, therefore, depending on the animals' place of origin and whereabouts, potential other pathogens (e. g. *Borrelia* spp., *Babesia* spp., *Leishmania* spp.) or laboratory values indicative of these pathogens (e. g. anaemia, leucocytosis/neutrophilia, hypoalbuminaemia/hypergammaglobulinaemia, kidney values etc.) should be clarified.

In principle, tick territories (endemic area) are potential breeding grounds. Seroprevalences for GA/TA and CME vary widely, depending on country (endemic or non-endemic) and study.

With an incubation time of 2–20 days, *A. phagocytophilum* infections are often subclinical or self-limiting. Clinical symptoms are fever, apathy, stiff muscle, polyarthritis with joint pain/swelling, lameness, weight loss, thrombocytopenia, anaemia, petechial haemorrhages and increasing inflammatory values (CRP, haptoglobin). Subarachnoid haemorrhage could lead to central nervous disorders. The cause of TA is *A. platys*. It occurs worldwide, mainly in the Southern Hemisphere. In most cases, the infection is asymptomatic with mild fever, uveitis, petechiae and ecchymoses. In the laboratory diagnostics, a thrombocytopenia is shown.

CME is characterised by a very long incubation period (Ø 4–5 up to 12–13 years) and an unspecific clinic. It is therefore also referred to as a "silent killer"!

Very few veterinarians are aware of the consequences of such a co-infection from an immunological, therapeutic and diagnostic point of view. Therefore, ***FAST*est® ANAPLASMA-EHRLICHIA** is suitable as a quick, qualitative antibody (ab) detection if an anaplasmosis, ehrlichiosis or a co-infection is suspected. This enables to start immediately further diagnostic investigations as well as therapeutic and prophylactic measures.

3. INFORMATION ON THE SPECIMEN MATERIAL

Exactly 10 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)!

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, 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 twin test cassette from its foil pouch shortly before use. Place it on a flat surface.
2. Draw sample **up to the mark (≙ 10 µl sample volume)** using the disposable plastic pipette. **The meniscus must be above the black line** (fig.1). Place the **whole sample volume (10 µl) into the sample window S** of the Anaplasma test cassette (hold pipette vertically, fig.2). **Repeat the whole procedure for the Ehrlichia test cassette.**

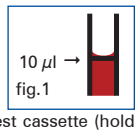


fig.1

3. Hold the dropper bottle **A** vertically and place **2 drops (ca. 80–100 µl) of the buffer diluent** into the sample window S of the Anaplasma and the Ehrlichia test cassette (fig.3).
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.2

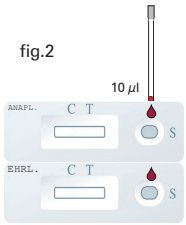
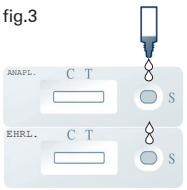


fig.3



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 *Anaplasma* and/or *Ehrlichia* TEST RESULT (fig.4–6)
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.5–7)
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 twin test cassette.

fig.4 **Anaplasma positive, Ehrlichia positive**

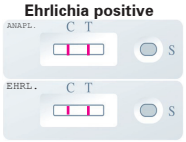


fig.5 **Anaplasma negative, Ehrlichia positive**

fig.6 **Anaplasma positive, Ehrlichia negative**

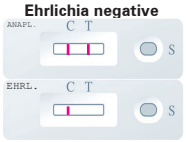


fig.7 **Anaplasma negative, Ehrlichia negative**

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 twin test cassette to ensure a precise assignment.
- Use a new pipette and a new twin 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® ANAPLASMA-EHRLICHIA** is based on an immunochromatographic "sandwich principle".

The antibodies against *Anaplasma* spp. and/or *Ehrlichia canis* present in the sample react in the conjugate pad with mobile antibodies, which are conjugated to colloidal gold particles. These antibody complexes are migrating along the nitrocellulose membrane ("lateral flow", **LF**) and bind to fixed recombinant *Anaplasma* spp. or *Ehrlichia* spp. antigens, 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.
- 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® ANAPLASMA-EHRLICHIA**. The suspicion about an active anaplasmosis /ehrlichiosis 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 proof of ab against *Anaplasma* spp./*Ehrlichia canis*, together with anamnesis and clinic, shows with a high likelihood that *Anaplasma* spp./*Ehrlichia canis* can be considered as cause of the acute disease.
- Dog has had contact with *Anaplasma* spp./*Ehrlichia canis* (ab formation!)
- Ab can persist over months to years inspite of therapy (potential chronic carriers).
- Asymptomatic, but anti-*Anaplasma* spp./*Ehrlichia canis* ab positive animals have been infected with *Anaplasma* spp./*Ehrlichia canis* at a particular time. Therefore, they are potential carriers of *Anaplasma* spp./*Ehrlichia canis*. Subclinical animals could develop clinical symptoms at some indefinite future date, especially in case of coinfection with another vector-transmitted agent.
- The decision starting an antibiotic therapy should be based on indirect immunofluorescence test (coupled serum test in an interval of 2–3 weeks with 3-fold titre increase) combined with clinical symptoms. Persistence of abs could be stable over a long term.

Negative test result
- With high likeliness, dog had no contact with *Anaplasma* spp./*Ehrlichia canis*.
- Early stage of an *Anaplasma* spp./*Ehrlichia canis* infection (< 2–3 weeks post infection!). Dog has not formed abs in detectable concentration. Animals could still be seronegative during acute infection (ab increase from 14 days post infection) resulting in a negative test result.