Version 04/2023

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# *FAST*est®
# EHRLICHIA-LEISH

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***In vitro* diagnosticum**

**Test-kit for the qualitative detection of antibodies against *Ehrlichia canis* and *Leishmania infantum* 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® EHRLICHIA-LEISH** contains:
- 2*, 6**, 15***, 25*** or 50**** twin test cassettes, coated with recombinant antigens
- 1 dropper bottle **A** with *1.0 ml, **3.0 ml or ***7.5 ml or ****2 dropper bottles **A** with 7.5 ml buffer diluent
- 2, 6, 15, 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

**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

Ehrlichia: Sensitivity 94.3 % – Specificity 93.3 %
Leish: Sensitivity 98.0 % – Specificity 97.0 %
(Comparison Method: IFAT)

## 2. INTRODUCTION

Both the canine Leishmaniosis (*Leishmania infantum*) and the canine monocytic Ehrlichiosis (CME, *Ehrlichia canis*) count among the most important vector-transmitted infectious diseases/travel diseases of the dog. Coinfections play a major role, because these dogs are more likely to become ill. In the literature, there are hints that an upstream *Ehrlichia* infection possibly sensibilises for a *Leishmania* infection.

The seroprevalences for the Ehrlichiosis and Leishmaniosis vary strongly depending to country (endemic or non-endemic) and study. In addition, indigenous infections in dogs with symptoms of canine Leishmaniosis, but without a travel report, are increasingly being described in non-endemic areas (e. g. Germany).

The canine Ehrlichiosis is characterised by a very long incubation period (Ø 4–5 to 12–13 years) and a non-specific clinic and is therefore also referred to as "silent killer"!

Dogs with clinical Leishmaniosis show typical clinical symptoms and/or clinic-pathologic findings. Subclinically infected dogs (infected, but clinically healthy) do not show any symptoms during clinical examination and no clinical-pathologic findings.

The consequences of such coinfection from an immunological, therapeutic and diagnostic point of view are known to very few veterinarians. Therefore, ***FAST*est® EHRLICHIA-LEISH** is useful as rapid qualitative antibody detection test in dogs suspected of having leishmaniosis, ehrlichiosis or a coinfection.

## 3. INFORMATION ON THE SPECIMEN MATERIAL

**Exactly 20 µl (of attached plastic pipette) 15–25 °C warm whole blood (WB**, with anticoagulant**), plasma (P) or serum (S)** per test cassette are needed. **Native blood without any anticoagulant must not be used due to the potential risk of microclots** (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 insufficiently 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 (≙ 20 µl sample volume)** using the disposable plastic pipette. **The meniscus must be above the black line** (fig.1).

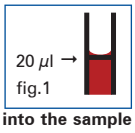


fig.1

3. Place the whole sample volume **(20 µl) into the sample window S** of the Ehrlichia test cassette (hold pipette vertically, fig.2). **Repeat the whole procedure for the Leish test cassette.**
4. 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 Ehrlichia and the Leish test cassette (fig.3).
5. 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

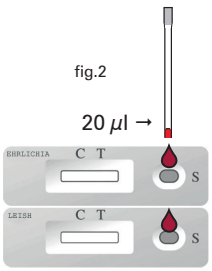
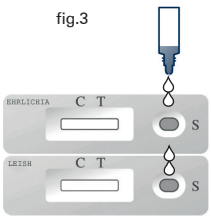



fig.3




## 6. READING OF THE TEST RESULT


Read the test result **15 minutes, maximum 20 minutes** after the 2 drops of buffer diluent have been added into the sample window S.

**POSITIVE Ehrlichia and/or Leish TEST RESULT** (fig.4–6)
A pink-purple TEST line **of any intensity** (varying from very weak to stronly 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 **Ehrlichia positive, Leish positive**

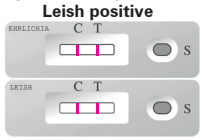



fig.5 **Ehrlichia negative, Leish positive**

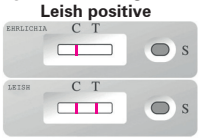



fig.6 **Ehrlichia positive, Leish negative**

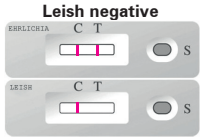
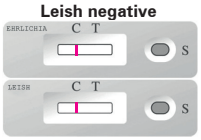



fig.7 **Ehrlichia negative, Leish negative**




## 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 of accordingly, together with the used test-kit components.

## 8. TEST PRINCIPLE

The ***FAST*est® EHRLICHIA-LEISH** is based on an immunochromatographic "sandwich principle" technique.

The antibodies in the sample bind in the area of the conjugate pad to recombinant *Ehrlichia* or *Leishmania* antigens, conjugated with gold particles. These antigen-antibody complexes are migrating along the nitrocellulose membrane ("lateral flow", **LF**) and bind to membrane-bound capture antibodies, 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 in endemic areas, tick bites) and clinical data as well as the 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 reactions and therefore as negative test result.
- Positive test results may be observed even before the end of incubation, depending on the antibody concentration of the sample. 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, especially in case of weak positive samples, could be from worse to not visible.
- T can vary both in intensity (from weak to strong pink-purple) and in width. Therefore, any pink-purple line which appears within the required incubation time has to be interpreted as a positive test result.
- Due to the innovation of a Leishmania vaccine, it is required to determine the antibody titre status of the dog **before vaccination** to get a decision "vaccination or no vaccination" according to the guidelines of the vaccine manufacturer.
- 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® EHRLICHIA-LEISH**. The suspicion about an active leishmaniosis/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).

### *FAST*est® EHRLICHIA

**Positive test result**
- Dog was very likely in contact with *Ehrlichia canis* (antibody formation)
- Dog from endemic ehrlichiosis area

**Negative test result**
- With high likeliness, dog had no contact with *Ehrlichia canis*
- Dog in early (< 2–3 weeks post infection) stage of infection (antibody titre < cut off ***FAST*est®**)

### *FAST*est® LEISH

**Positive test result**
- Dog was very likely in contact with *Leishmania infantum* (antibody formation)
- Dog from endemic leishmaniosis area
- Dog with vaccination anamnesis (no typical Leish clinics or laboratory changes, negative PCR)

**Negative test result**
- With high likeliness, dog had no contact with *Leishmania infantum*
- Dog in early (< 2–3 weeks post infection) stage of infection (antibody titre < cut off ***FAST*est®**). The incubation time (symptomless animal) can last months up to 8 years.
- Dog with Th1-mediated (cellular) immune response → subclinically infected dog (Leish stadium I with negative to low grade positive antibody titre < cut off ***FAST*est®**, mild clinic with single lymphadenomegaly or papular dermatitis and inconspicuous laboratory)
- In case of suspicion, follow-up with ***FAST*est® EHRLICHIA-LEISH** every 3–6 months