Version 03/2023

Click Here For More Information About

*FAST*est® LEPTOSPIRA IgM

ad us. vet.

***In vitro* diagnosticum**

Test-kit for the qualitative detection of IgM antibodies against *Leptospira* spp. in plasma, whole blood supernatant or serum of the dog

INSTRUCTIONS FOR USE

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

1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® LEPTOSPIRA** IgM contains:
- 2 or 10 test cassettes coated with recombinant antigens
- 1 dropper bottle A with 1.0 ml or 3.0 ml buffer diluent
- 2 or 10 disposable plastic pipettes (10 µl)
- 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.

ACCURACY

Sensitivity 100 %
Specificity 95.3 %
(Comparison Method: MAT = Microscopic Agglutination Test)

2. INTRODUCTION

Leptospirosis (Weil's disease) is a world-wide spread bacterial infectious disease in various animals and humans (zoonosis!). Of the pathogen species *Leptospira interrogans* sensu lato, more than 300 serovars are known which are summarised in 24 serogroups of varying pathogenicity. Due to non-existence of a cross immunity of the vaccination serovars and an increasing "serovar shift", leptospirosis becomes more important (world-wide increasing prevalences especially for *L. icterohaemorrhagia, L. canicola, L. grippotyphosa, L. australis* and *L. pomona*).

Transmission is direct: horizontal (esp. infectious abortion material, urine, food animals [pathogen reservoir: rodents, small mammals], bites), vertical (lactogenic, placental, veneric) and indirect: contaminated soil, water.

Incubation time as well as symptoms are strongly depending on age, immune status, serovar type. Subclinical infections are rather the rule (higher seroprevalence than the prevalence of the clinical disease), proven by numerous studies. With present immunity due to past infection, normally quick antibody (ab) formation and pathogen elimination takes place. Typical are general symptoms like fever, apathy, anorexia, power drop, loss of weight, partially diarrhoea 3–7 days after infection as well as pale icteric mucous membranes. Other symptoms are late abortuses, dead births, birth of weak young animals. Within 48–72 h, a serious, partly deathly process can develop, depending on organ manifestation (especially kidneys [tubular persistence], liver and lung [hemorrhagic syndrome] dysfunctions, DIC).

A clinical suspicion for leptospirosis requires a quick, laboratory-ensured diagnosis, because the animals become a shedder and infection risk for humans and animals. Because the direct proof of the pathogen (dark field microscopy, culture, PCR [false negative through high antibiotic dose]) often is difficult, time consuming, expensive and only proving if positive, the ab detection, especially the IgM detection, has an important diagnostic relevance.

The actual reference method is microagglutination test (MAT). However, its sensitivity varies strongly (30–80 %) depending on the stadium of infection, and it does not distinguish between IgM and/or IgG antibodies. Therefore, a straight IgM detection (increase in the 1st week p.inf./maximum from week 2–3 on), can be of significant diagnostic benefit compared to MAT or straight IgG detection (detectable not until 3–4 weeks, persistence for months, vaccination-caused IgG antibody persistence).

The ***FAST*est® LEPTOSPIRA** IgM is an important diagnostic tool for the veterinarian for the fast and simple on-site detection of a leptospirosis in the early stage of infection. Therefore, further laboratory diagnostics as well as therapeutic and prophylactic measures can be started immediately.

3. INFORMATION ON THE SPECIMEN MATERIAL

Exactly **10 µl (defined volume of attached plastic pipette) of 15–25 °C warm plasma (P), whole blood supernatant (WBS: supernatant from whole blood**, with anticoagulant, without centrifugation**), or serum (S)** are needed. Mix the sample material well before use!

Non-cooled (**15–25 °C**), P, WBS and S should be tested within 4 hours! At **2–8 °C**, P, WBS and S can be stored up to 4 days. The 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.**

Whole blood as well as citrate or heparin blood should not be used in the *FAST*est® LEPTOSPIRA IgM.

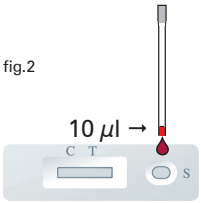
4. SPECIMEN PREPARATION

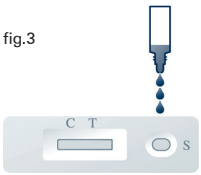
- No specimen preparation necessary.
- **Partially filled and/or insufficiently mixed EDTA tubes could create invisible microclots resulting in lateral flow delay and/or unspecific reactions** (e.g. greyish shadow-like lines).
- To obtain the **whole blood supernatant** from EDTA blood: Allow to sediment for 15 minutes at room temperature and use the supernatant as sample material.

5. TEST PROCEDURE

1. Remove the 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).
3. Place the whole sample volume **(10 µl) into the sample window S** of the test cassette (hold pipette vertically, fig.1).
4. Hold the dropper bottle **A** vertically and place **3 drops of the buffer diluent (ca. 120–150 µl)** into the sample window S (fig.2).
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.1





6. READING OF THE TEST RESULT

Read the test result **15 minutes** after the buffer diluent 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 test cassette.

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 test cassette to ensure a precise assignment.
- Use a new disposable plastic pipette and a new test cassette for each sample.
- The buffer diluent contains 0.1 % ProClin™ 950 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® LEPTOSPIRA** IgM is based on an immunochromatographic "sandwich principle".

In the conjugate pad, the sample IgM antibodies against *Leptospira* will react to mobile monoclonal anti-dog antibodies which are bound to colloidal gold particles. Migrating ("lateral flow", **LF**) along the nitrocellulose membrane, these specific antigen-antibody complexes are bound by fixed *Leptospira* antigens producing 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 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) within the incubation time has to be considered as unspecific reaction and therefore as negative test result.
- Due to 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.
- **Two-step diagnostics** as method of choice.
  Step 1: IgM ab screening via ***FAST*est® LEPTOSPIRA** IgM.
  A: Test positive + clinic ➔ highly suspicous for acute leptospirosis
  B: Test negative + clinic ➔ IgM ab level too low (< 2 weeks p.inf.) ➔ Repetition of ***FAST*est® LEPTOSPIRA** IgM after 1 week.
  Step 2 after positive ***FAST*est® LEPTOSPIRA** IgM: Serum pair at an interval of 2–4 weeks for quantitative IgM and/or IgG ab titre determination via **MegaFLUO® LEPTOSPIRA** ➔ 2–4-fold titre increase ➔ confirmation of an acute infection. Alternative choice: MAT [titre or serovar determination: no distinction between IgG and IgM!].

  Advantage of this combined IgM/IgG 2-step diagnostics is a better differentiation (IFAT) of infection and vaccination induced ab titres and of the state of infection.
- Due to serologic cross reaction between the various serovars and sero groups, a leptospirosis vaccination in early phase of immune answer (IgM > IgG) or up to Ø 2–3 months can lead to a positive ***FAST*est® LEPTOSPIRA** IgM.