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*FAST*est® ENCEPH

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***In vitro* diagnosticum**

Test-kit for the qualitative detection of antibodies against *Encephalitozoon cuniculi* in EDTA whole blood, EDTA plasma or serum of the rabbit

INSTRUCTIONS FOR USE

Supplied Exclusively To The UK Veterinary Market By
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1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® ENCEPH** contains:
- 2*, 6** or 25*** test cassettes coated with Encephalitozoon cuniculi antigens
- 1 dropper bottle A with *0.5 ml, **1.0 ml or ***3.0 ml buffer diluent
- 2, 6 or 25 disposable plastic pipettes (5 µl)
- 2, 6 or 25 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 98.5 % – Specificity 100 %
(Comparison Method: ELISA)

2. INTRODUCTION

The infection with *Encephalitozoon cuniculi* occurs via the extremely resistant spores by contaminated food or inhalation in contaminated surroundings. (Domestic) rabbits are primarily affected. Other strains of the pathogen cause disease in immunocompromised Old World mice and carnivores. Although very rare, it can occur in immunocompromised people (zoonosis).

Neurological symptoms, signs of kidney failure, and eye skin inflammation may occur due to lesions in the central nervous system, kidney and eye. Diseased rabbits can experience one or more of these symptoms. The most common is the so-called vestibular syndrome (head tilt, disorders of movement coordination and eye tremors). Other neurological symptoms can include seizures, incomplete paralysis, and loss of balance. In rare cases, increased aggression and loss of hearing or vision may also occur. In some animals, renal insufficiency with rather unspecific symptoms (loss of appetite or weight, dehydration, disorders of the mineral balance and bone metabolism as well as apathy) can be indicative.

The *in vivo* diagnosis of encephalitozoonosis in rabbits is problematic because of the large number of animals with chronic, asymptomatic infection. In this case, antibodies can be detected in the blood for years. The serological detection of antibodies against *E. cuniculi* is therefore considered the safest method since antibody formation begins 14–28 days after infection. A negative antibody titre after more than 14–28 days excludes an acute infection or a previous contact with *E. cuniculi* with high probability.

Therefore, the indirect detection of antibodies using ***FAST*est® ENCEPH** is of great diagnostic importance (exclusion diagnostics).

3. INFORMATION ON THE SPECIMEN MATERIAL

Exactly **10 µl (1 drop of attached plastic pipette) 15–25 °C warm EDTA whole blood (WB**, with anticoagulant) or exactly **5 µl (1 drop of attached plastic pipette) 15–25 °C warm EDTA plasma (P) or serum (S)** are needed. **Native blood without anticoagulant and the anticoagulants citrate and heparin can not be used.**

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. **S / P 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 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 test cassette from its foil pouch shortly before use. Place it on a flat surface.

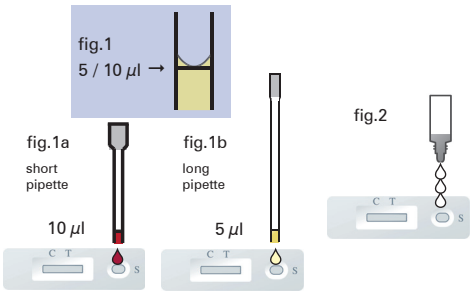
2a. **EDTA whole blood**:
10 µl sample volume / short plastic pipette (fig.1a)

2b. **EDTA plasma or serum**:
5 µl sample volume / long plastic pipette (fig.1b)

2c. Aspirate sample **up to the mark** using the respective disposable plastic pipette. **The meniscus must be above the black line** (fig.1). Place the **whole sample volume into the sample window S** of the test cassette (hold pipette vertically, fig.1a / b).

3. Hold the dropper bottle A vertically and express **3 drops of buffer diluent (ca. 120–150 µl)** into the sample window S (fig.2).

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.



6. READING OF THE TEST RESULT

10-15 min

Read the test result **10, maximum 15 minutes** after the three drops of the buffer diluent have been added into the sample window S.

POSITIVE TEST RESULT (fig.3)
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.4)
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.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 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® ENCEPH** is based on an immunochromatographic "sandwich principle".

The antibodies against *Encephalitozoon cuniculi* present in the sample will react in the conjugate pad with mobile monoclonal antibodies, which are conjugated to colloidal gold particles. These antibody complexes are migrating ("lateral flow", **LF**) along the nitrocellulose membrane and bind to fixed *Encephalitozoon cuniculi* antigens forming a pink-purple TEST line (**T**). These monoclonal antibodies guarantee a high level of specificity for the aetiologic detection of antibodies against *Encephalitozoon cuniculi* in the sample.

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

***FAST*est® ENCEPH** is based on purified, specially treated spores for the fast and reliable detection of antibodies against *Encephalitozoon cuniculi* in EDTA whole blood, EDTA plasma or serum of rabbits.

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) has to be considered as unspecific reaction and therefore as negative test result.
- 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.
- For the detection of antibodies, a two-step diagnosis is known to be standard. The first step starts with in-clinic IgG antibody screening test like ***FAST*est® ENCEPH**.

> A negative ***FAST*est® ENCEPH** 14–28 days post infection excludes with high probability an acute infection or previous contact with *E. cuniculi*. The following diseases should be examined for differential diagnosis: otitis media / interna (rabbit cold complex), otitis externa (*Psoroptes cuniculi*), trauma, toxoplasmosis, neoplasia, herpes infection, intoxication.

> The suspicion about an active encephalitozoonosis is substantiated by combination of ***FAST*est® ENCEPH and according clinic**. Furthermore, two serum samples at intervals of 2–4 weeks should be taken for quantitative antibody titre determination via indirect immunofluorescence test (**MegaFLUO® ENCEPHALITOZOON** cuniculi) or ELISA (**MegaELISA® ENCEPHALITOZOON** cuniculi) to determine the end titre or a 2- to 4-fold titre increase (seroconversion).