Version 07/2025

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*FAST*est® C. diff 2T

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***In vitro* diagnosticum**

Test-kit for the qualitative detection of GDH and of Toxins A/B from *Clostridioides difficile* in feces of the dog, cat, horse and pig

INSTRUCTIONS FOR USE

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1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® C. diff 2T** contains:
- 10 revolver test tubes **R** with 2 dipsticks each, coated with monoclonal antibodies (purple: Toxin A+B, blue: GDH)
- 10 sample tubes **P** with 1.0 ml buffer diluent each
- 10 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

TL – TEST line, **CL** – CONTROL line, **LF** – Lateral flow
R – Revolver test tube, **P** – sample tube

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 96 % (GDH), 99 % (Toxin A/B)
Specificity 98 % (GDH), 96.3 % (Toxin A/B)
(Comparison Method: ELISA [GDH], Cytotoxicity [Toxin A/B])

2. INTRODUCTION

Clostridioides difficile is a gram positive anaerob spore former. It causes diarrhoea in various species. Studies prove the incidence of *C. difficile* in animal food. Therefore, a zoonotic potential for humans (diarrhoea, colitis) must be implied. Additionally there are hints of mutual transfer between dog/cat and human within a household.

Most important virulence factors for the development of *C. difficile* infection (CDI) are the enterotoxin A (TcdA) and cytotoxin B (TcdB).

Dog/cat: C. difficile can be proven in feces of healthy juvenile and adult animals as well as in animals with diarrhoea (single animals, nosocomial infections in animal hospitals and shelters). A significant correlation between C. difficile and diarrhoea could not be proven, but feces samples of animals with diarrhoea showed significantly higher TcdA (increased secretion of liquid into the intestinal lumen) and/or TcdB (lethal damage of the intestinal wall) detection as with healthy animals.

Horse: Both in single animals and with diarrhoea outbreaks in herds CDI (TcdA & TcdB) occur, especially in foals, partly associated with C. perfringens, then mostly with deathly course within 3 days. Clinically indicative are colic, partly without/before diarrhoea onset and massive antibiotic associated colitis.

Pig: In 1–7 days old piglets, CDI is one of the most important diarrhoea diseases (mortality up to 16 %). The prevalence decreases with increasing age. The fecal-oral colonisation with *C. difficile* happens in endemic areas at 100 % within 48 h, lactogenic via the sow (ca. 25 %) or aerogenic via surroundings. Clinical symptoms (yellow-watery diarrhoea, but also constipation) are not always visible. Risk factors for development of an acute CDI are age, provocation dose, but also associated toxins and the administration of antibiotics. Retarded growth, lower weaning weight and severe economical losses are the consequences.

Diagnosis of an acute CDI can be difficult due to the endemic nature of *C. difficile*. With a two-step diagnostics of GDH (Glutamate Dehydrogenase) and the Toxins A/B, the proof can succeed with high certainty. The proof of GDH is said to be very sensitive compared to culture (golden standard) and therefore can be used as so-called "exclusion test". On the other hand, the proof of the Toxins A/B is seen as highly specific (but less sensitive) compared to culture. Therefore, the double test can be optimally used as confirmation test.

In combination with anamnesis and clinic, the ***FAST*est® C. diff 2T** is suitable as on-site diagnostic test for the secure exclusion or proof of a *C. difficile* infection.

3. INFORMATION ON THE SPECIMEN MATERIAL

Due to the normally inhomogeneous or nest-like dissemination of antigens in the feces, the specimen material has to be mixed up homogeneously (spatula, vortex-mixer) before sampling.

For the test, the required amount of feces as described in issue 4b/Specimen collection and preparation, is needed. Use the attached spoon or pipette.

Non-cooled (**15–25 °C**), the sample should be tested as fresh as possible! At **2–8 °C**, the sample can be stored up to 2 days, permanently **at minimum −20 °C**.

Keep in mind that the sample material, as well as all used test-kit components, should have **room temperature** at the time of application.

Endogeneous and exogeneous interfering substances of the sample (e.g. proteases, mucosa components, blood, but also viscosity, pH value as well as grass and cat litter) can cause interferences (matrix effects) **that can influence the target measurement. These can lead to an impaired LF and/or unspecific reactions on TL and CL.**

4. SPECIMEN COLLECTION AND PREPARATION

a. Open the sample tube **P** with the buffer diluent.

pulpy-compact: 75 mg (½ level spoon)

fig.1

pulpy-watery: 3 drops

fig.2

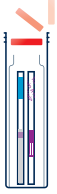

fig.3

b. **Homogenise the feces sample (applicator, vortexer)** and mix the required sample volume steadily into the buffer diluent (fig.1: **pulpy-compact feces: 75 mg** [½ level spoon] or **pulpy-watery feces: 3 drops** with the attached pipette ≙ **75 µl**).

c. Close **P** tightly. Rotate feces sample easily to get the mixture as homogeneous as possible (fig.2). No sedimentation required. Immediately start with the test procedure 5.1.

5. TEST PROCEDURE

1. Remove the revolver test tube **R** from the test-kit shortly before use.
2. Open **R**, remove the red desiccant disk (fig.3) and introduce **P** containing the sample-buffer mixture (SBM) vertically into **R** (fig.4).
3. Turn the cap of **R** until hearing a clicking noise twice (fig.5).The SBM of **P** will run into **R**. To ensure a proper LF, the liquid level must not exceed the white sucking pad of the dipsticks (fig.6, see also 7. Precautions for users*).
4. Place **R** on a flat and horizontal surface for incubation.

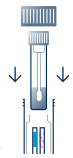

fig.4

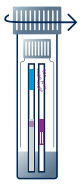

fig.5

fig.6

6. READING OF THE TEST RESULT

Read the test result after **exactly 15 minutes**. Positive test results may be observed earlier, depending on the concentration of antigen in the sample.

POSITIVE TEST RESULT (fig.7+8)
One (GDH) or two (Toxin A/B) red TEST line/s **of any intensity** (varying from very weak to strongly intensive) and a blue CONTROL line appear.

NEGATIVE TEST RESULT (fig.9)
Only a blue 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 **P** and **R** *.

POSITIVE TEST RESULT

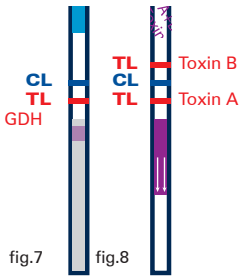

fig.7 fig.8

NEGATIVE TEST RESULT

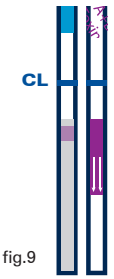

fig.9

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, associated sample tube **P** and revolver test tube **R** to ensure a precise assignment.
- Use a new sample tube **P** and revolver test tube **R** 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.

* If the LF does not appear one minute after closing the cap of **R** completely, swing **R** carefully 1–2× in a circle.

If the LF still does not appear, maybe a too large amount of feces was used. The test must be completely repeated/rescheduled. Carefully observe the advice for sample preparation (also see 4.b/ Specimen collection and preparation and fig.1).

8. TEST PRINCIPLE

The ***FAST*est® C. diff 2T** is based on latest rapid immunochromatographic "sandwich technique".

Glutamate dehydrogenase (GDH) or Toxin A/B from *C. difficile* present in the sample are extracted from the feces by the buffer diluent and will react in the conjugate pad area with mobile monoclonal antibodies against GDH or Toxin A/B, bound to red latex particles, and form antigen-antibody complexes. These complexes migrate anlong the membrane ("lateral flow", **LF**) and are bound by fixed specific monoclonal antibodies against GDH or Toxin A/B, producing a red TEST line (**TL**).

The correct test procedure will be indicated by a second, blue CONTROL line (**CL**).

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 TL and CL (e.g. greyish, shadow-like lines) has to be considered as unspecific reaction and therefore as negative test result.
- **ATTENTION:** In case of feces samples with high blood content, especially with negative samples, unspecific reactions on TL and CL can occur. Frequently, the CL changes colour from light blue to dark blue or purple.
- A sample overload can lead to delayed or incomplete lateral flow (no formation of CL). The test must be repeated with a reduced sample volume (considering the sample-buffer diluent ratio).

Test interpretation

- GDH positive and Toxin A and/or Toxin B positive
  With a high probability, an acute infection with toxigenic *C. difficile* is going on.
- GDH negative and Toxin A and Toxin B negative
  With a high probability, no acute infection with toxigenic *C. difficile* is going on.
- The TL can vary both in intensity (certain correlation to toxin or GDH concentration in the sample) and in time of appearance.
  – Samples with very low TL can be repeated with higher sample volume (considering the sample-buffer diluent ratio).
  – Positive test results may be observed before end of incubation time. After end of incubation time, TL are not interpretable!
- Inspite of the high negative predictive value of the GDH test, a *C. difficile* infection cannot be excluded completely. In case of consisting clinical suspicion diagnosis (increasing intensity of diarrhoea), a test repetition after 12–24 hours is recommended.
- A negative GDH and positive Toxin A/B test result from solid feces must be seen critical and be evaluated in connection with anamnesis and clinic (*C. difficile* actually causes diarrhoea!).
- Cross reactions with *E. histolytica* are possible (Toxin A negative, Toxin B weak positive).