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*FAST*est® E.coli-K99 Strip

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In vitro diagnosticum

Test-kit for the qualitative detection of *E. coli* ssp. K99 (F5) antigens in faeces of the cattle

INSTRUCTIONS FOR USE

Supplied Excusively To The UK
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1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® E.coli-K99** Strip contains:
- 2 or 10 dipsticks coated with monoclonal antibodies
- 2 or 10 sample tubes with 2.0 ml buffer diluent each
- 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

TL – TEST line, **CL** – 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 96 %
Specificity 97 %
(Comparison Method: E.M., ELISA)

2. INTRODUCTION

The diarrhoea pathogen *Escherichia coli* (*E. coli*) K99 (F5) is a highly infectious bacterium occurring world-wide. Normally, the bacteria appear together with other pathogens of the Neonatal Calf Diarrhoea Complex, leading to severe diarrhoea in calves. The prevalences differ, depending on the stock, between 3 and 54 %. Mostly, calves of the age between 1 and 5 days are affected.

The different virulence factors (haemolysins, adhesins, enterotoxins (in ETEC) and Shiga toxins) of *E. coli* lead to various clinical symptoms of diarrhoea. *E. coli* K99 (F5) belongs to the enterotoxin producing *E. coli* bacteria. These show specific surface structures, so-called fimbria (F5 antigens) attaching to enterocytes of the intestinal mucosa, thus leading to massive diarrhoea, partially with lethal consequences.

The fecal-orally transmitted bacteria colonize in the distal small and large intestine. In young calves, small amounts of hydrochloric acid secretion in the abomasum prevent mortification of the *E. coli* bacteria, leading to a bacterial *E. coli* invasion of the intestine, together with secretory diarrhoea.

Caused by the high infectiveness, often a population problem arises. Here, next to aetiological diagnostics of all animals, especially older ones (asymptomatic chronic carriers!), the management of keeping and feeding shall be examined, too.

The ***FAST*est® E.coli**-K99 Strip offers the veterinarian a fast and reliable on-site proof of *E. coli* K99 (F5) for a fast and targeted initiation of suitable therapy and prophylaxis measures and therefore a reduction of the calf loss rate.

3. INFORMATION ON THE SPECIMEN MATERIAL

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

For the test, the required amount of faeces as described in point 4b/Specimen collection and preparation, is needed. The amount depends on the consistency of the sample. Use the attached spoon.

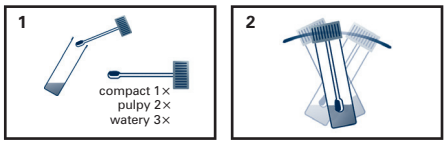
Non-cooled (**15–25 °C**), the sample should be tested within 4 hours! At **2–8 °C**, the sample can be stored up to 4 days, permanently **at minimum −20 °C**.

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. 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 the TL and CL.**

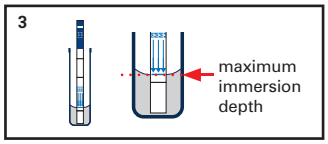
4. SPECIMEN COLLECTION AND PREPARATION

a. Open the sample tube with the buffer diluent.
b. Mix the faeces sample homogeneously (applicator, vortexer). Then mix the required sample volume (**compact: 1 level spoon, pulpy: 2 level spoons, fluid-watery: 3 level spoons of faeces**) steadily into the buffer diluent (fig.1).
c. Close sample tube tightly and rotate it easily to get the mixture as homogeneous as possible (fig.2).
d. For sedimentation of gross faeces particles place the sample tube on a flat and horizontal surface for 1–5 minutes.



5. TEST PROCEDURE

1. Remove the dipstick from its foil pouch shortly before use.
2. Introduce the dipstick vertically and with the arrows pointing downwards into the sample tube for at least 1 minute. The liquid level (meniscus!) must not exceed the blue horizontal line below the blue arrowheads (fig.3).
3. Remove the dipstick from sample tube as soon as the sample-buffer mixture (SBM) has reached the CL. If so, the pink-purple CL will appear slowly but surely (fig.4/5). If the CL does not appear after 5–10 minutes, a new SBM must be prepared and sedimented for at least 5 minutes. The dipstick must be held only in the supernatant until the LF has reached the CL (see also 7. Precautions for users*).
4. Place the dipstick on a flat and horizontal surface for incubation.



6. READING OF THE TEST RESULT

Read the test result after **5 (max. 10) minutes**. Positive test results may be observed earlier, depending on the concentration of antigen in the sample.

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 dipstick.

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 sample tube to ensure a precise assignment.
- Use a new sample tube and a new dipstick 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.

* To avoid an application error/external influence (e.g. too much sample material, too short sedimentation time, components in the faeces that clog the pores of the suction pad), the test can be repeated. Use a new dipstick and carefully observe the sample preparation. It is advisable to only hold the dipstick in the supernatant when repeating the test until the LF has reached the CL.

8. TEST PRINCIPLE

The ***FAST*est® E.coli-K99** Strip is based on latest rapid immunochromatographic technique.

The *Escherichia coli* (*E. coli*) antigens in the faeces sample will react at the conjugate pad with mobile monoclonal antibodies bound to gold particles. Migrating ("lateral flow", **LF**) along the nitrocellulose membrane, these specific antigen-antibody complexes are bound by fixed monoclonal anti-*E. coli* antibodies (mAbs) producing a pink-purple TEST line (**TL**). These mAbs guarantee a high level of specificity for the aetiologic detection of *E. coli*.

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

Please note the two light-green quality control lines on **TL** and **CL**. They indicate that the test membrane is of good quality and can be used.

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 within the indicated incubation time or after more than 10 minutes (e.g. greyish, shadow-like lines) has to be considered as unspecific reaction and therefore as negative test result.
- TL can vary both in intensity (from weak to intense pink-purple) and width. Therefore, any pink-purple line appearing within the required incubation time is to be interpreted as a positive test result.
- Because of intermittent antigen shedding, with ongoing diarrhoea a single negative test result should be confirmed by testing a **serial faeces sample (individual testing of at least three consecutive faeces samples)**.