

INTRODUCTION

Calf diarrhoea poses a major health problem in herds, and therefore is a significant economic risk in cattle breeding. The causes for diarrhoea are multiple. Infectious and non-infectious factors can trigger the onset of calf scours. Non-infectious factors that may trigger diarrhoea include, among others: hygienic conditions; the vitality of the calf's immune system and the calf's physical constitution.

Infectious factors include viruses, bacteria, single-cell parasites and other causative organisms. The most common causative organisms were identified:

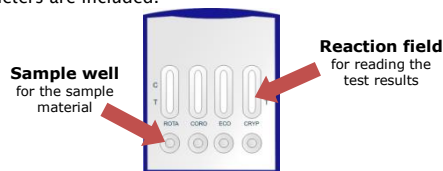
- Rotavirus
- Coronavirus
- Escherichia Coli K99
- Cryptosporidium parvum

These organisms, among other things, alter the structure of epithelial cells in the gastrointestinal tract, disturbing digestion and generally cause diarrhoea.

This causes the calf to excrete an increased amount of faeces with elevated content of water, causing disequilibrium in fluid balance. When a calf suffers from diarrhoea, immediate treatment is required. It is imperative that the cause of the illness is quickly and accurately identified.

TESTING PRINCIPLE

The CALF DIARRHOEA TESTING KIT is a sandwich Immunoassay. Gold-labeled antibodies, antigens from the specimen and immobilized antibodies form a sandwich construction which shows as the test line on the test strip. The CALF DIARRHOEA TESTING KIT is a highly sensitive immunoassay that comes in a user convenient test cassette, wherein test strips for the parallel detection of four parameters are included.



EXPLANATION OF THE TESTING PROCESS

When the specimen is put into the sample well, it will be absorbed by the absorbing pad of the test strip. The fluid mixes with the gold labeled antibodies of the conjugate pad. Due to capillary action the fluid starts to run up the test strip, crossing the test line region and afterwards the control line region. The control line always should appear to show the right functioning of the test. If the specimen contains the pathogen the respective test strip is testing for, a line will show in the test line region. The test line forms by building a sandwich between the gold labeled antibodies from the conjugate pad, the antigen from the specimen and the immobilized antibody, in the test line region. If no pathogen is in the specimen, the gold labeled antibodies cannot connect to the immobilized antibodies in the test line region and therefore no test line appears. Then the test result is negative.

QUALITY CONTROL

In order to ensure the proper functioning of the kit, external controls are utilized as a matter of good laboratory practice. The controls should consist of a negative and positive control with minimal analytic content. It can be determined through the use of a weak positive control that a test was not negatively impacted and that the analytic can be detected with the given sensitivity of the test system.

REAGENTS, MATERIALS, INSTRUMENTS

I. Package Contents

- 5 multi-test cassettes incl. pipettes and drying pads
- 5 extra pipettes
- 5 test tubes incl. 1.5 ml dilution buffer
- 1 detailed instruction manual

II. Possible additional materials

- Timer

CAUTION

- Only for professional use.
- For one-time usage only.
- Use the test cassette within 10 minutes after opening the pouch.
- Do not place sample solution in the reaction well.
- Use a new pipette and sample test tube for each new sample to avoid cross reactions.
- Do not touch the reaction field.
- Only use the original dilution buffer provided in the kit.
- Sample material could be infectious. Wear gloves while carrying out the test and discard afterwards. Care should be taken with waste disposal not to spread any infectious organisms.
- Do not use the test after expiry date printed on the test pouch.
- Do not use the test if the packing is damaged.
- Consider the test results as invalid after the specified read-out time if the control line has not appeared.

SAMPLE PREPARATION

The sample should be tested as quickly as possible after it is collected. If this is not possible, the tube including the sample can be stored at temperatures between 2 °C and 8 °C for a period of up to 24 hours. If it is necessary for the specimen to be stored longer, it must be kept at a temperature below -20 °C. Make sure that the sample is not contaminated with formaldehyde solutions or derivatives.

If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

Useful Tip: Sample taking should be performed either with the pipette or with the sample stick. This depends on the consistency of the faeces. In the case of nearly liquid, very watery faeces, use the pipette – please use only the liquid faeces. If the faeces are slightly grainy or it contains larger particles, the sample stick for sample taking should be used.

SAMPLING

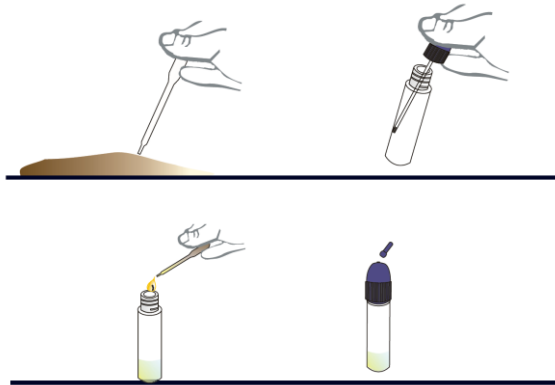
A. For nearly liquid / watery faeces

1. Use the pipette provided to draw up liquid material.
2. Open the purple lid of the sample test tube and remove the sampler.
3. Add 3 drops from the pipette of the faecal sample into the test tube. Close the sample tube and then shake the tube gently.

Remarks: At first add 3 drops of the faecal material into the sample tube, shake it gently. If the liquid in the tube is slightly brown or yellow, no more drops of the sample material are necessary. If the colour of the buffer still appears clear with no significant colour change, please add more drops of the specimen until the colour of the buffer changes. If the faecal sample is very watery, add up to 5 drops more into the tube (up to 8 drops total can be added).

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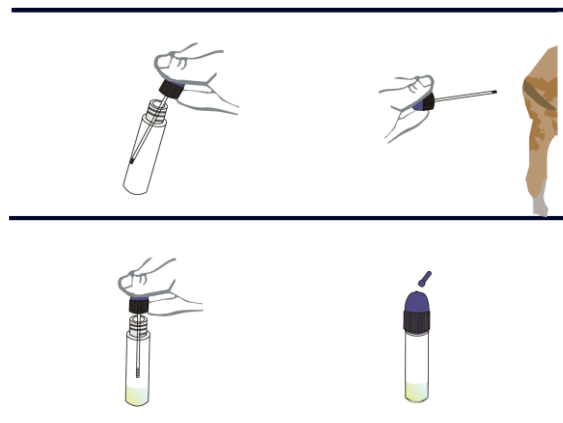
Unit 3, Grousehall, Loughduff, Co. Cavan, Ireland.



B. Grainy faeces / with larger particles

1. Use the sample stick for sample taking. Open the sample tube and use the stick (the stick is attached to the purple lid of the sample tube).
2. Prick with the sample stick into three different places of the faecal material; return the stick with the sample material into the sample tube. Close the sample tube and shake it well.
3. The reagent has to change colour to slightly brown or yellow otherwise not enough of the specimen was taken. If so, repeat step 2.

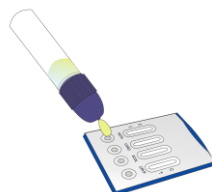
Remarks: You need sufficient material for testing, if the specimen is rather liquid, use the pipette. No large amount of faecal material is necessary, just pricking into the specimen is sufficient; nevertheless if the faecal material is more liquid, the use of the pipette is mandatory. The sample stick also can be used to take a faecal sample from the anus.



TEST PROCEDURE

Remark: All materials used to perform the test should be at room temperature.

1. Take a test cassette out of the protective pouch.
2. Shake the closed sample tube with the sample fluid again gently.
3. Break off the purple pin of the sample tube.
4. Apply 3-4 drops of the sample fluid into each sample well by slightly pressing the sample tube.



Remarks: Be careful. No big particles should get into the sample wells. In this case the liquid can stop running. Please use the pipette for removing the particles from the sample well and press with the tip of the pipette directly in the sample well for activating the run. Also add an additional drop of the sample fluid into the respective sample well supports the further run on the test strip.

Proceed with the test evaluation.

TEST EVALUATION

The results of the test can be first read within 5 to 10 minutes after starting the test. Positive test results can also appear later. The maximum read out time of the tests is 20 minutes after reaching the control line of each test.

Positive Result:

Two red lines appear in the reaction field.

Also faint test lines are considered to be positive.



The illustration on the right shows a positive test result for rotavirus and E.coli with a clear test line. Coronaviruses and Cryptosporidia were not detected in this example.

Remarks: If a high level of antigens is in the sample material, it is possible that the test line appears very strong with only a very faint control line. This result is valid; the test shows a positive test result.

Negative Result:

Only the control lines appear on the membranes. No test lines are visible.

In this illustration all parameters show clear negative test results. No rotaviruses, coronaviruses, E. coli K99 bacteria or cryptosporidia were detected.



Invalid Result:

If no control line is visible after the test was performed, the test is invalid. In this case, the test might not have been carried out properly, the expiration date has already passed or the test was exposed too long to ambient air outside of the sealed pouch.

STORAGE

- The test must remain in the sealed pouch until use.
- The kit should be stored at 4–30°C.
- Do not use after the expiry date printed on the sealed pouch.
- Care should be taken to protect the components of the kit from contamination.
- Do not freeze.






SHELF LIFE

- 18 month after manufacturing.

DISPOSAL

- No special disposal necessary.

SYMBOLS USED

	Only for one use		Read user instruction carefully
	Content	+4°C +30°C	Storage temperature
	Lot number		Expiry date

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