Diarrhoea is a major cause of mortality in young cattle under six weeks. Bovine neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses: coronavirus or rotavirus, by bacteria: *Salmonella* or by protozoan microorganisms such as *Cryptosporidium parvum*. In calves younger than three days, K99 (F5) positive enterotoxigenic *E.Coli* are frequently isolated, particularly in colostrum-deprived calves or in calves which received colostrum free of anti-K99 (F5) specific antibodies. The diagnosis of the etiological agent of diarrhoea can only be performed by laboratory technics because clinical signs do not allow to differentiate between the different microorganisms. The *E. coli* bacteria can be isolated from faeces on an appropriate growth medium. The Minca medium is often used because allows optimal expression of the attachment factor F5 (K99). However, the culture may be unsuccessful if the calf has previously undergone antibiotherapy. In this case, the ELISA test can be very useful because it will detect the attachment factor K99 (F5) even on dead bacteria. Moreover, when the ELISA test for the detection of the attachment factor K99 (F5) is performed directly on fecal samples, it will give more reliable results than the isolation of bacteria because it allows the quantification of the attachment factor K99 (F5) in the sample rather than the presence or absence of the factor in a limited number of isolated strains. The identification of *E. coli* bacteria on a growth medium is not sufficient on its own. It must be coupled to a detection test for the attachment factor K99 (F5) or the toxin. The ELISA test can be used to detect the attachment factor K99 (F5) produced by the *E. coli* bacteria in culture. The ELISA method is particularly useful when a large number of samples must be analyzed. The test is fast and reliable, and it can be evaluated at a glance if a spectrophotometer is not available.

**Reliable Results**
The use of monoclonal antibody for capture and as conjugate ensures excellent specificity and very reliable results.

**Ease-of-Use**
Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes for single or batch testing

**Flexibility**
Results can be read visually or spectrophotometrically.

**EIA Procedure**

1- Microplate coated with monoclonal antibody
2- Add samples and positive control. Incubate 1 hour at 21°C +/- 3°C. Wash
3- Add conjugate. Incubate 1 hour at 21°C +/- 3°C. Wash
4- Add chromogen + Substrate. Wait 10 minutes. Add stop solution. Read at 450 nm
E. coli F5 (K99) ELISA TESTING

<table>
<thead>
<tr>
<th>Isolated strain on Minca</th>
<th>ELISA (BIO K069) OD</th>
<th>Strips (BIO K154)</th>
<th>PCR (314 bp)</th>
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</table>

Specificity: 100 %  
Sensitivity: 90.9 %  
Results obtained by using 86 calf stool samples

COMPOSITION OF THE KIT

BIO K 069 E. coli F5 ELISA

- 2 plates of 12 strips for a total of 96 tests
- 2 vials of positive control
- 1 vial of conjugate
- 1 bottle of washing solution
- 1 bottle of dilution buffer
- 1 drop-dispenser bottle of chromogen
- 1 bottle of substrate solution
- 1 bottle of stopping solution

1 year of stability at 4°C

BIO-X Diagnostics s.p.r.l.  
Site du complexe des postes  
22, Rue Joseph Wauters  
B-5580 Jemelle (BELGIUM)
Diarrhoea is a major cause of mortality in young cattle under six months.

Bovine neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses: coronavirus or rotavirus, by bacteria: Salmonella or by protozoan microorganisms such as Cryptosporidium parvum. In calves younger than three days, F5 positive enterotoxigenic E.Coli are frequently isolated, particularly in colostrum-deprived calves or in calves which received colostrum free of anti-E.coli F5 specific antibodies. The diagnosis of the etiological agent of diarrhoea can only be performed in the laboratory because clinical signs do not allow to differentiate between the different microorganisms.

The E. coli bacteria can be isolated from feces on an appropriate growth medium. The Minca medium is often used because allows optimal expression of the attachment factor F5. However, the culture may be unsuccessful if the calf has previously undergone antibiotherapy. In this case, the ELISA test can be very useful because it will detect the attachment factor F5 even on dead bacteria. Moreover, when the ELISA test for the detection of the attachment factor F5 is performed directly on fecal samples, it will give more reliable results than the isolation of bacteria because it allows the quantification of the attachment factor F5 in the sample rather than the presence or absence of the factor in a limited number of isolated strains. The identification of E. coli bacteria on a growth medium is not sufficient on its own. It must be coupled to a detection test for the attachment factor F5 or the toxin. The ELISA test can be used to detect the attachment factor F5 produced by the E. coli bacteria in culture. The ELISA method is particularly useful when a large number of samples must be analyzed. The test is fast and reliable, and it can be evaluated at a glance if a spectrophotometer is not available.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by a monoclonal antibody specific for an antigenic determinant of E. coli K 99. This antibody allows specifically the capture of the corresponding pathogen which is present in the faeces samples. Rows A, C, E, G have been sensitized with this antibody and rows B, D, F, H are containing non specific antibody. These control rows allow the differentiation between specific immunological reaction and non specific bindings. A large number of false positives are eliminated.
Faeces are diluted in dilution buffer and incubated on the microplate for 1 hour at room temperature; After this first incubation step, the plate is washed, then the conjugate, a peroxidase labelled anti- E. coli F5 specific monoclonal antibody, is added to the wells. Plate is incubated for 1 hour at room temperature. After this second incubation step, the plate is washed again and the enzyme substrate (hydrogen peroxide) and the chromogen (tetrathylbenzidine) are added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If F5 positive E.Coli is present in the tested faeces, the conjugate remains bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of F5 in the sample. Enzymatic reaction can be stopped by acidification and resulting optical density at 450 nm can be recorded using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells.
A control antigen is provided with the kit so as to validate the test results. This control antigen is composed of a lyophilised F5 E. coli culture.

III - COMPOSITION OF THE KIT

- **Microplates**: Two 96-well microtitration plates. Rows A, C, E, G are sensitised by anti – E. coli F5 specific antibodies, while rows B, D, F, H are sensitised by the non specific antibodies.
- **Washing solution**: One 100-ml bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to room temperature until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water. Store the diluted solution at 4°C.
- **Dilution buffer**: One 50-ml bottle of 5x concentrated buffer for diluting samples and conjugate. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. Store the diluted solution at 4°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate**: One vial of anti- E. coli F5 -peroxidase conjugate (horseradish peroxidase-labelled anti – E. coli F5 monoclonal antibody).
- **Positive reference**: 2 vials containing the E.Coli F5 control antigen. Reconstitute this antigen with 0.5 ml of distilled or demineralised water. The reconstituted reagent may be kept at -20°C. Divide the reconstituted antigen into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Chromogen solution**: One 2-ml drop-dispenser bottle of the chromogen tetrathylbenzidine. Store at 4°C.
- **Substrate solution**: One 30-ml bottle of the hydrogen peroxide substrate solution. Store this reagent at 4°C.
- **Stopping solution**: One 15-ml bottle of the 1 M phosphoric acid stop solution.

IV - PRECAUTIONS FOR USE

- This test may be used for in vitro diagnosis only. It is strictly for veterinary use.
- The reagents must be kept at between 4 and 8°C. The conjugate must be kept at 4°C. The positive reference must be kept at -20°C once reconstituted. The reagents cannot be guaranteed if it their shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.

- The stop solution contains 1 M phosphoric acid. Handle it carefully.

V - PROCEDURE

1- Bring all the reagents at room temperature at least half an hour before use.
2- Dilute the concentrated washing solution 20 fold in distilled water. Be sure that all crystals have disappeared before dilution. Dilute the concentrated dilution buffer 5 fold in distilled water. Keep these solutions at 4°C when not used.
3- Dilute faecal samples volume per volume into dilution buffer prepared as instructed in step 2. This is a qualitative dilution only, which must allow the pipetting of faecal suspensions. Discard any gruds by natural decantation for about 10 minutes. Do not centrifuge the suspensions.
4- Add 100-µl aliquots of the diluted samples to the wells as follows: sample 1 in wells A1 and B1, sample 2 in wells C1 and D1, etc. Proceed in the same manner for the negative reference (ex.: G1 and H1).
5- Incubate the plate at room temperature for 1 hour.
6- Rinse the plate with the washing solution, prepared as instructed in step 2, as follows: empty the microplate of its contents by flipping it over sharply over a sink. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill all the used wells with the washing solution using a spray bottle or by plunging the plate in a vessel of the right dimensions, then empty the wells once more by turning the plate over above a sink. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.
7- Dilute the conjugate 1:50 with the buffer for dilution (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.25 ml of diluent). Add 100 µl of the diluted conjugate solution to each well. Incubate at room temperature for 1 hour.
8- Wash the plate as described in step 6 above.
9- Prepare 10 ml of indicator solution extemporaneously as follows: add 12 drops (500µl) of chromogen to 9.5 ml of the substrate solution (enough for 1 plate). Mix thoroughly, then pipette onto the plate immediately in volumes of 100 µl per microwell. At the time of distribution of the chromogen-substrate mixture on the plates the solution must be completely colourless. If a blue colour appears at this stage, this solution must be discarded and a new one made up using clean glassware and equipment.
10- Incubate for 10 minutes at room temperature. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
11- Add 50 µl of stop solution per microwell.
12- Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may crystalize in wells with strong signals and distort the results accordingly.

VI - INTERPRETING THE RESULTS

Calculate the net optical density of each sample by subtracting from the reading for each sample well the optical density of the corresponding negative control. Proceed in the same way for the positive control antigen.

The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than or equal to 0,150 is considered positive. Conversely, any sample that yields a difference in the optical density that is less than 0,150 is considered negative.