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# ANTIGENIC DIGESTIVE ELISA KIT

**Sandwich ELISA test**

**Direct test for faeces**

**Diagnosis test of Rotavirus, *E. Coli* F4, F5, F18, F41, *Clostridium perfringens,***

***Clostridium difficile* and *Cryptosporidium***

**for piglets**

## *I - INTRODUCTION*

Diarrhoea is a major cause of mortality in piglets under three weeks.
Porcine neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses (coronavirus or rotavirus), by bacteria: (*Clostridium*, *Salmonella*, pathogenic strains of *E. coli*) or by protozoa such as *Cryptosporidium*.
The diagnosis of the etiological agent of diarrhoea can be performed only in the laboratory because the clinical signs do not suffice to distinguish between these different microorganisms. It is possible to identify these agents by means of different techniques, including culture, staining, electron microscopy and floating techniques. However, these techniques are labour intensive, impractical and time consuming.
These classical techniques have rapidly been replaced by the ELISA technology because of its simplicity and limited laboratory equipment requirements.
The sensitivity and specificity of the ELISA technique for detecting these pathogens is at least as good as that of the more classic techniques, and the results are very similar. The ELISA technique is rapid and reliable and is particularly suited to the analysis of large numbers of samples.

## *II - PRINCIPLE OF THE TEST*

Specific antibodies produced against pathogens responsible of digestive diseases have been immobilized on 6 x 16-well microtitre plates. These antibodies allow the specific capture of the corresponding pathogens in the faecal samples. Columns 1, 3, 5, 7, 9, 11 have been sensitised with these antibodies and columns 2, 4, 6, 8, 10, 12 contain non-specific antibodies. These columns allow the differentiation between specific immunological reactions and non-specific binding so as to eliminate false positives.
The faeces are diluted in dilution buffer and incubated on the microplate for 1 hour at 21°C +/- 3°C.
After this first incubation step, the plate is washed, and then conjugates, peroxidase-labelled anti-pathogen monoclonal antibodies, are added to the wells. The plate is then incubated for 1 hour at 21°C +/- 3°C.
After this second incubation step, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic.
If specific pathogens are present in the tested faeces, conjugates remain 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 titres of the specific pathogens in the sample. The enzymatic reaction can be stopped by acidification and the 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' optical densities.
Positive control is provided with the kit so as to validate the test results.

## *III - COMPOSITION OF THE KIT*

- **Microplates**: 96-well microtitration plates. The odd columns are sensitised by specific antibodies, the even columns by non specific antibodies. (See picture)
  Row A: anti-Rotavirus
  Row B: anti- *E. Coli* F4
  Row C: anti- *E. Coli* F5
  Row D: anti- *E. Coli* F18
  Row E: anti-*E. Coli* F41
  Row F: anti-*Clostridium perfringens*
  Row G: anti-*Clostridium difficile*
  Row H: anti-*Cryptosporidium*
- **Washing solution:** One bottle of concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.
- **Dilution buffer:** One bottle of colored and concentrated buffer for diluting samples. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugates**: vial of colored conjugates. **These solutions are ready to use.** Rotavirus (red), *E. Coli* F4 (yellow), *E. Coli* F5 (blue) , *E. Coli* F18 (purple) , *E. Coli* F41 (orange), *Clostridium perfringens* (turquoise), *Clostridium difficile* (pink) and *Cryptosporidium* (green).
- **Positive Control:** The reagent is ready to use.
- **Single component TMB** bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use.
- **Stopping solution:** bottle of the 1 *M* phosphoric acid stop solution.

| | BIO K 326/2 |
|---|---|
| Microplates | 2 |
| Washing solution | 1 X 100 ml (20 X) |
| Colored Dilution buffer | 1 X 50 ml (5 X) |
| Conjugates | 8 X 3 ml (1 X) |
| Positive control | 1 X 4 ml (1 X) |
| Single component TMB | 1 X 25 ml (1 X) |
| Stopping solution | 1 X 15 ml (1 X) |

## *IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED*

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

## *V - PRECAUTIONS FOR USE*

- This test may be used for “in vitro” diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope’s seal airtight. If these precautions are taken, the strips’ activity can be conserved up to the kit’s shelf-life date.
- 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.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.

- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

## *VI – PROCEDURE*

1- Bring all the reagents at 21°C +/- 3°C 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.
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 of column 3 and 4, sample 2 in wells of column 5 and 6, etc. Do not forget positive control: 8 wells of column 1 and 2 (100 µl/well)
5- Incubate the plate at 21°C +/- 3°C for 1 hour. Cover with a lid.
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- Add 100 µl of conjugate solutions per well :
   Row A: anti-Rotavirus (red)
   Row B: anti- *E. Coli* F4 (yellow)
   Row C: anti- *E. Coli* F5 (blue)
   Row D: anti- *E. Coli* F18 (purple)
   Row E: anti-*E. Coli* F41 (orange)
   Row F: anti-*Clostridium perfringens* (turquoise)
   Row G: anti-*Clostridium difficile* (pink)
   Row H: anti-*Cryptosporidium* (green)
   Incubate at 21°C +/- 3°C for 1 hour. Cover with a lid.
8- Wash the plate as described in step 6 above.
9- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated. Incubate at 21°C +/- 3°C and away from light for 10 minutes. Do not cover. This time interval is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
10- Add 50 µl of stop solution to each well. The blue colour will change into a yellow colour.
11- Read the optical densities by means of a microplate spectrophotometer with a 450 nm filter. The results must be read as quickly as possible after the stop solution has been applied, for in the case of a strong signal the chromogen can crystallise and lead to incorrect measurements

## *VII – INTERPRETING THE RESULTS*

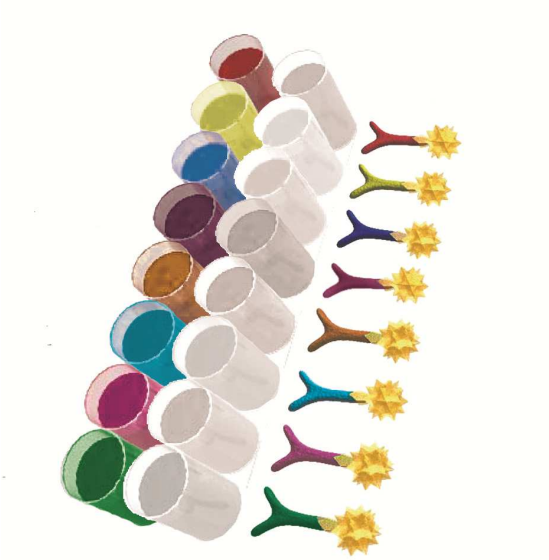
Calculate the net optical density for each sample by subtracting from the reading for each sample well the optical density of the corresponding negative well.
Proceed in the same way for the positive control antigen.
The test is validated only if the positive control antigen yield differences in optical density at 10 minutes that are greater than the values given on the QC data sheet.
Divide each resulting value by the corresponding value obtained for the corresponding positive control and multiply this result by 100 to express it as a percentage.

$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the first table in the quality control procedure, determine each sample's status (positive, negative).

## *VIII – ORDERING INFORMATION*

BIO-X DIGESTIVE ELISA KIT: 2 X 6 Tests BIO K 326/2



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