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Calf digestive

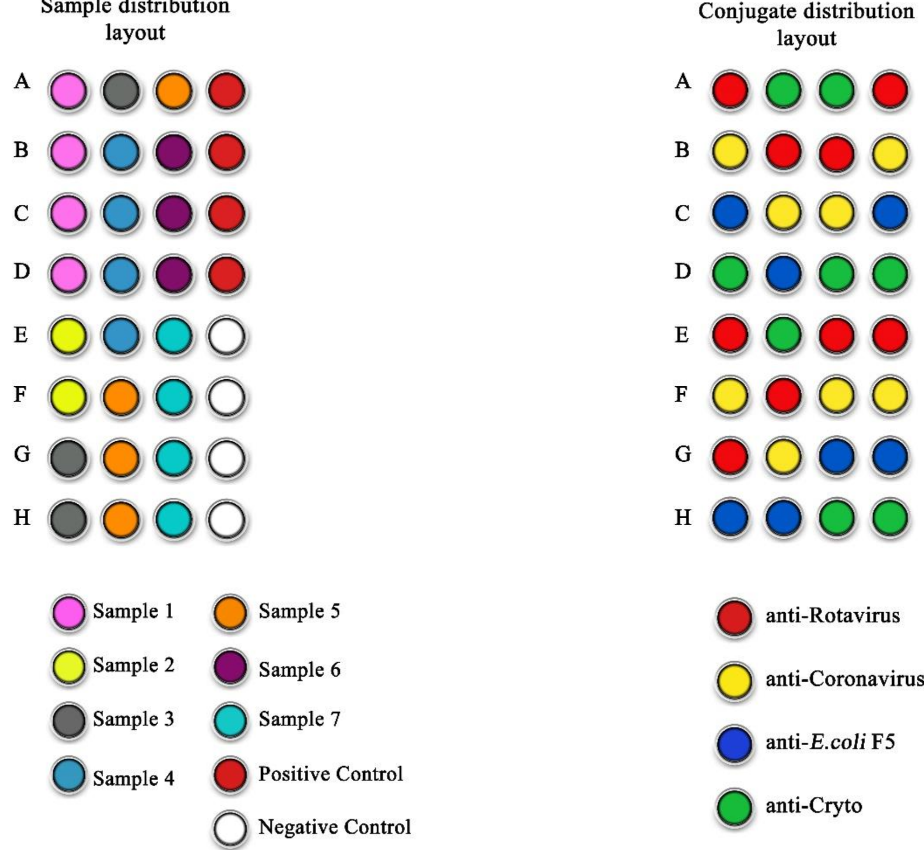
ELISA kit for antigenic diagnosis of Rotavirus, Coronavirus,
E. coli F5 attachment factor and *Cryptosporidium*
Sandwich test for faeces
Diagnostic test for cattle

I - INTRODUCTION

Diarrhoea is a major cause of mortality in calves in the first month of life. Bovine neonatal gastroenteritis is often a multifactorial disease. It can be caused by viruses (Coronavirus or Rotavirus), bacteria (*Salmonella* or *E. coli*F5), or protozoan microorganisms such as *Cryptosporidium*. The diagnosis of the aetiological agent of diarrhoea can be made only in the laboratory because it is not possible to differentiate between these different microorganisms on the basis of the clinical signs. The ELISA technique is simple to use requires little in the way of equipment and is particularly well suited to analysing large numbers of samples. The test is quick and reliable and can be evaluated by the naked eye if spectrophotometric equipment is not available.

II – PRINCIPLE OF THE TEST

In this test, the entire microtitration plate is sensitised with a mixture of antibodies that are specific for the four pathogens (see the diagram on the last page). These antibodies capture the corresponding pathogens in the faecal samples. The faecal material is diluted in dilution buffer and incubated on the microplate for 1/2 hour at 21°C +/- 3°C. Positive and negative controls are also deposited on the plate.
The plate is incubated and washed and then ready-to-use conjugates are added to the wells. The choice of conjugates is left up to the user. The diagram on the next page gives an example of the arrangement of samples and conjugates on the plate.
Following a second incubation for 1/2 hour at 21°C +/- 3°C, the plate is washed again and the chromogen tetramethylbenzidine (TMB), is added. This chromogen has the two advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If one or more of the pathogens being sought is present in the faeces, the corresponding conjugate or conjugates remain bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a blue compound. The intensity of the resulting colour is proportionate to the titre of the pathogen in the sample.



III - COMPOSITION OF THE KIT

- Microplate: 96-well microtitration plate. The entire plate is sensitised by antibodies specific for the four pathogens for which the test is designed.
- Washing solution: One 100 ml bottle of 20 X 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 all crystals have disappeared. Mix the solution well and take up the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.
- Dilution buffer: One 50 ml bottle of 5x colored, 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.
- Conjugate: 4 X 12 ml vials of coloured conjugate.
  The specificity of each conjugate is indicated on the bottle. The reagents are ready to use.
- Positive control: 1 vial containing 3 ml of the positive control. The reagent is ready to use.
- Negative control: 1 vial containing 3 ml of the negative control. The reagent is ready to use.
- Single component TMB: One bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C. and +8°C.protected from light. This solution is ready to use.
- Stop solution: One bottle of 1 M phosphoric acid stop solution. This reagent is ready to use.

	BIO K 151/1	BIO K 151/2
Microplate	1	2
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Colored Dilution buffer	1 X 50 ml (5 X)	1 X 50 ml (5 X)
Conjugates	4 X 12 ml (1 X)	4 X 12 ml (1 X)
Positive control	1 X 3 ml (1 X)	1 X 3 ml (1 X)
Negative control	1 x 3 ml (1 X)	1 x 3 ml (1 X)
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stopping solution	1 X 6 ml (1 X)	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 faecal samples volume per volume into dilution buffer. 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.
3- Remove the microplate from its wrapper.
4- Pipette the diluted samples into the wells at the rate of 100 µl. Take care to change pipettes between two different samples. The arrangement of samples on the plate must be set by the user according to the number of faecal samples to test and the valences selected for each sample. Distribute the positive and negative controls over the plate as well (one well per valence tested). The control solutions are ready to use. If the distribution scheme for the samples and conjugates is complicated, fill out the layout forms.
5- Cover with a lid and incubate the plate at 21°± 3°C for 1/2 hour.
6- Rinse the plate with the washing solution prepared as instructed in the section “Composition of the Kit”. To do this, dispose of the microplate’s contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the

containment vessel. 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 the ready to use conjugates into the wells at the rate of 100 µl per well.
8- Cover with a lid and incubate the plate at 21°± 3°C for 1/2 hour.
9- Wash the plate as instructed in Step 6.
10- 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.
11- Incubate 10 minutes at 21°C +/- 3°C without covering and away from light
12- Interpret the results visually by checking for a blue colour, unless you want to record the signals using a plate reader. In the latter case, skip to Step 13 and stop the reaction with the stop solution (read in the yellow range).
13- Add 50 µl of the stop solution to each well directly from the bottle. The blue colour will change into a yellow colour.
14- Record the optical densities using a plate reader and a 450 nm filter. The readings must be made as soon as possible after applying the stop solution, for in the event of a strong signal the chromogen can crystallise and lead to incorrect measurements.

VII – INTERPRETING THE RESULTS

If spectrophotometer readings are made, 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 antigens.
The test is validated only if the positive control antigens yield difference in the optical densities at 10 minutes that are greater than the values:

Rotavirus	> 1,000
Coronavirus	> 1,000
E. coli F5	> 1,000
Cryptosporidium	> 1,000

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage.

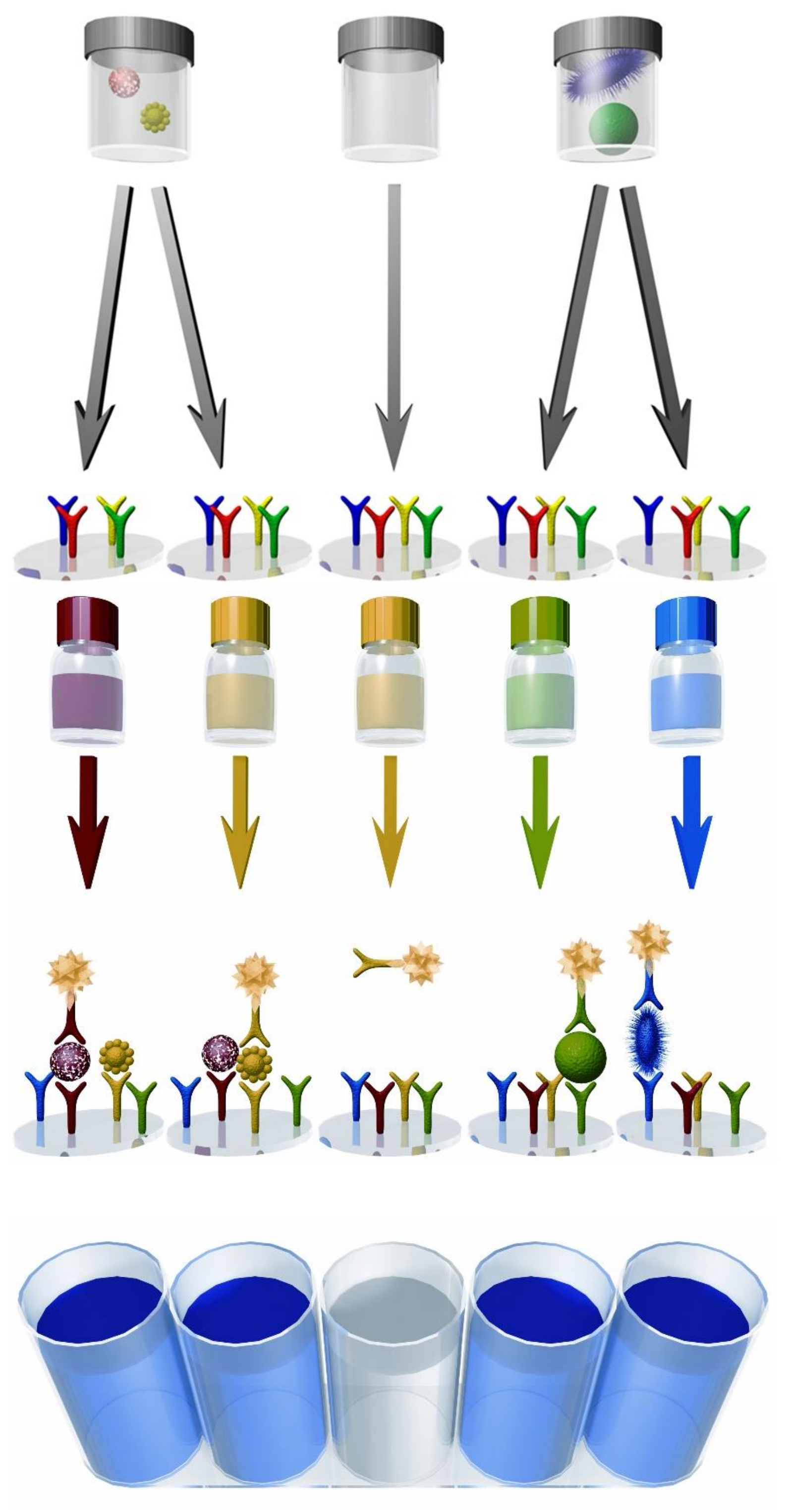
$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the following table, determine each sample's status (positive, negative).

Rotavirus	> = 6,00 %
Coronavirus	> = 7,00 %
E. coli F5	> = 6,00 %
Cryptosporidium	> = 6,00 %

Any sample that yields a difference in optical density that is greater or equal than the percentages above is considered positive for the valence in question.
Conversely, any sample that yields a difference in the optical density that is less than the percentages above is considered negative for the valence in question.

If the results are interpreted visually (reading of the blue colour), the samples that produce a more intense blue colour than the colour in the corresponding negative control wells are considered to be positive.



VIII – ORDERING INFORMATION

Multiscreen AgELISA Calf digestive	1 X 96 samples	BIO K 151/1
	2 X 96 samples	BIO K 151/2