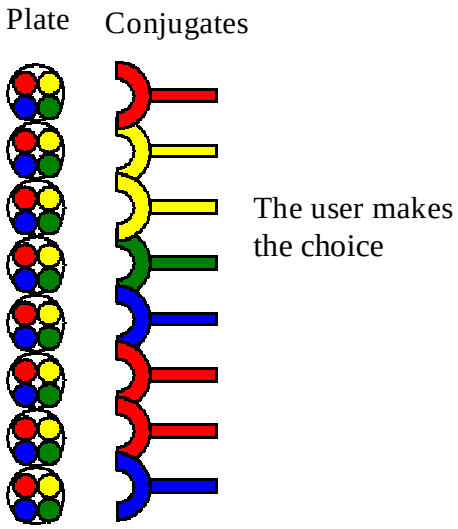
VI – INTERPRETING THE RESULTS

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 given on the quality control data sheet attached to the package insert.
The limit of positivity for each antigen is 0.150. Any sample that yields a difference in optical density that is greater than or equal to 0.150 is considered positive for the valence in question. Conversely, any sample that yields a difference in the optical density that is less than 0.150 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.



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Bio-X Diagnostics

BIO-X EASY-DIGEST (BIO K 151)

(Direct test for faecal material)

ANTIGENIC ELISA DIAGNOSTIC KIT FOR ROTAVIRUS, CORONAVIRUS, *E. COLI* ATTACHMENT FACTOR F5 AND CRYPTOSPORIDIUM PARVUM IN 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 parvum*. 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 symptoms. 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 +/- 5°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 +/- 5°C, the plate is washed again and the indicator mixture, which contains the enzyme's substrate (hydrogen peroxide) and the chromogen tetramethylbenzidine (TMB), are 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.

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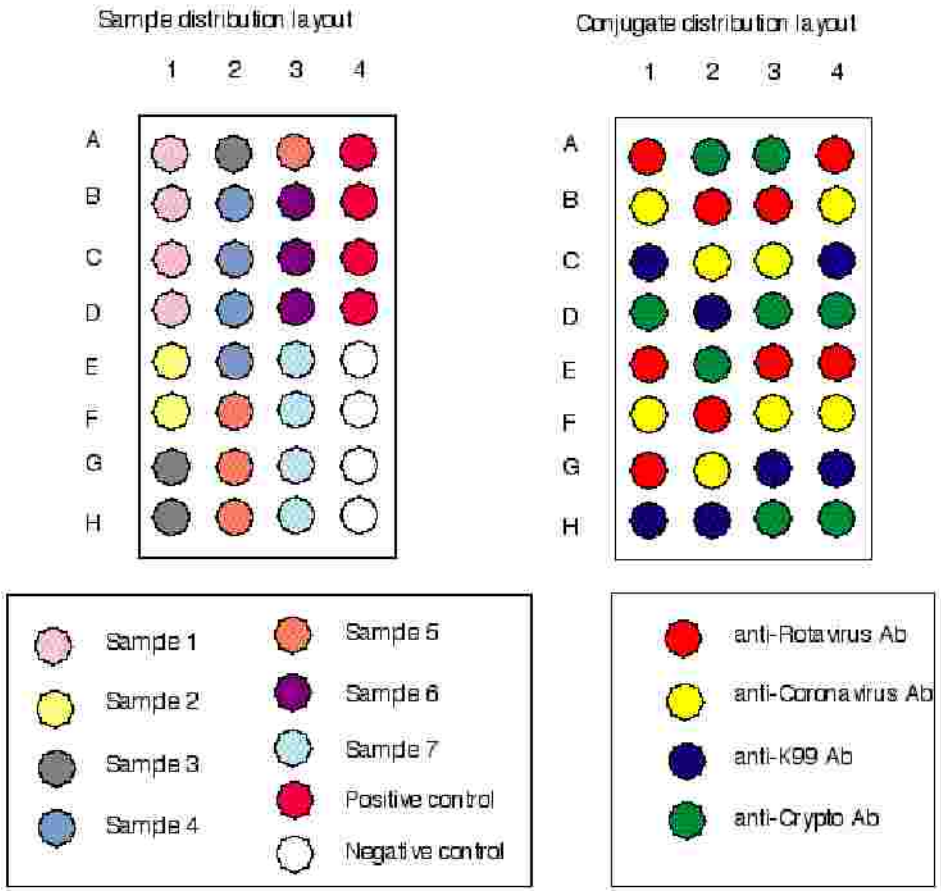

III - COMPOSITION OF THE KIT

- **Microplate**: one 96-well microtitration plate (12 X F8 strips). 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 +/- 5°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. Store the diluted solution at 4°C.
- **Dilution buffer**: One 100-ml bottle of buffer for diluting samples. The reagent is ready to use. Store the solution at 4°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate**: 4 X 12.5-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 the positive control. The reagent is ready to use.
- **Negative control**: 1 vial containing the negative control. The reagent is ready to use.

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- **Single component TMB**: One 15-ml bottle of the chromogen tetramethylbenzidine (TMB). Store at 4°C protected from light. This solution is ready to use.
- **Stop solution**: One 15-ml bottle of 1 *M* phosphoric acid stop solution. This reagent is ready to use.

IV - PRECAUTIONS FOR USE

- This test may be used for *in vitro* diagnosis only. It is strictly for veterinary use.
- The reagents must be stored at between 4 and 8°C. The reagents cannot be guaranteed if the shelf-life dates have expired and/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.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 *M* phosphoric acid. Handle it carefully.

V – PROCEDURE

1- 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.
2- Take the microtitration plate out of its wrapper.
3- 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.
4- Incubate the plate at 21°C +/- 5°C for 1/2 hour.
5- Rinse the plate with the washing solution, prepared as instructed in the section 'Composition of the Kit', 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 wash 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.
6- Add the ready to use conjugates into the wells at the rate of 100 µl per well directly from the bottles.
7- Incubate 1/2 hour at 21°C +/- 5°C.
8- Wash the plate as instructed in Step 5.
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.
10- Incubate 10 minutes at 21°C +/- 5°C without covering.
11- 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 12 and stop the reaction with the stop solution (read in the yellow range).
12- Add 50 µl of the stop solution to each well directly from the bottle.
13- 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.

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EASY-DIGEST

BIO K 151

Rotavirus - Coronavirus - *E. coli* F5 - *Cryptosporidium*

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, pathogenic strains of E. coli) or by protozoa such as Cryptosporidium parvum. Coronavirus and rotavirus are often associated with episodes of neonatal diarrhoea. Cryptosporidium parvum is also frequently isolated in faeces, where it can be present in very high quantities. It can persist for a long period in the environment. F5-positive enterotoxigenic E. coli is frequently isolated in under-three-day-old calves, particularly in colostrum-deprived calves or in calves fed colostrum that is free of anti- E. coli F5 + specific antibody. 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.

Reliable Results

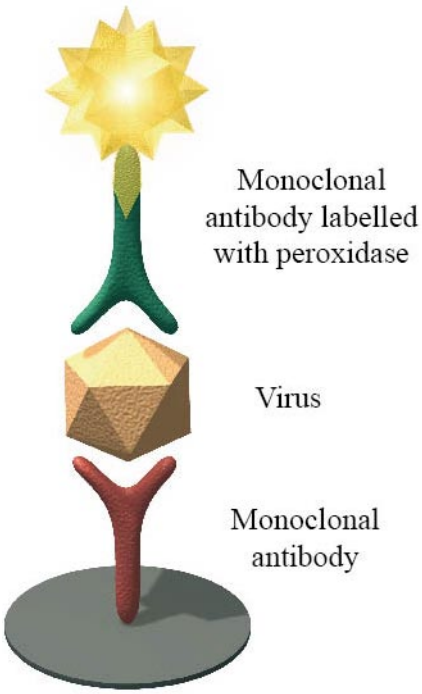
The use of monoclonal antibody as conjugate ensures excellent specificity and very reliable results.

Ease-of-Use

Minimal hands-on-time
Room temperature incubation
Results available in 70 minutes for single or batch testing

Flexibility

Results can be read visually or spectrophotometrically.



EIA Procedure

1- Microplate coated with monoclonal antibodies
2- Add samples and positive + negative controls.
Incubate 1/2 hour at 21°C +/- 3°C
Wash
3- Add conjugates.
Incubate 1/2 hour at 21°C +/- 3°C .
Wash
4- Add chromogen
Wait 10 minutes.
Add stop solution.
Read at 450 nm or visually

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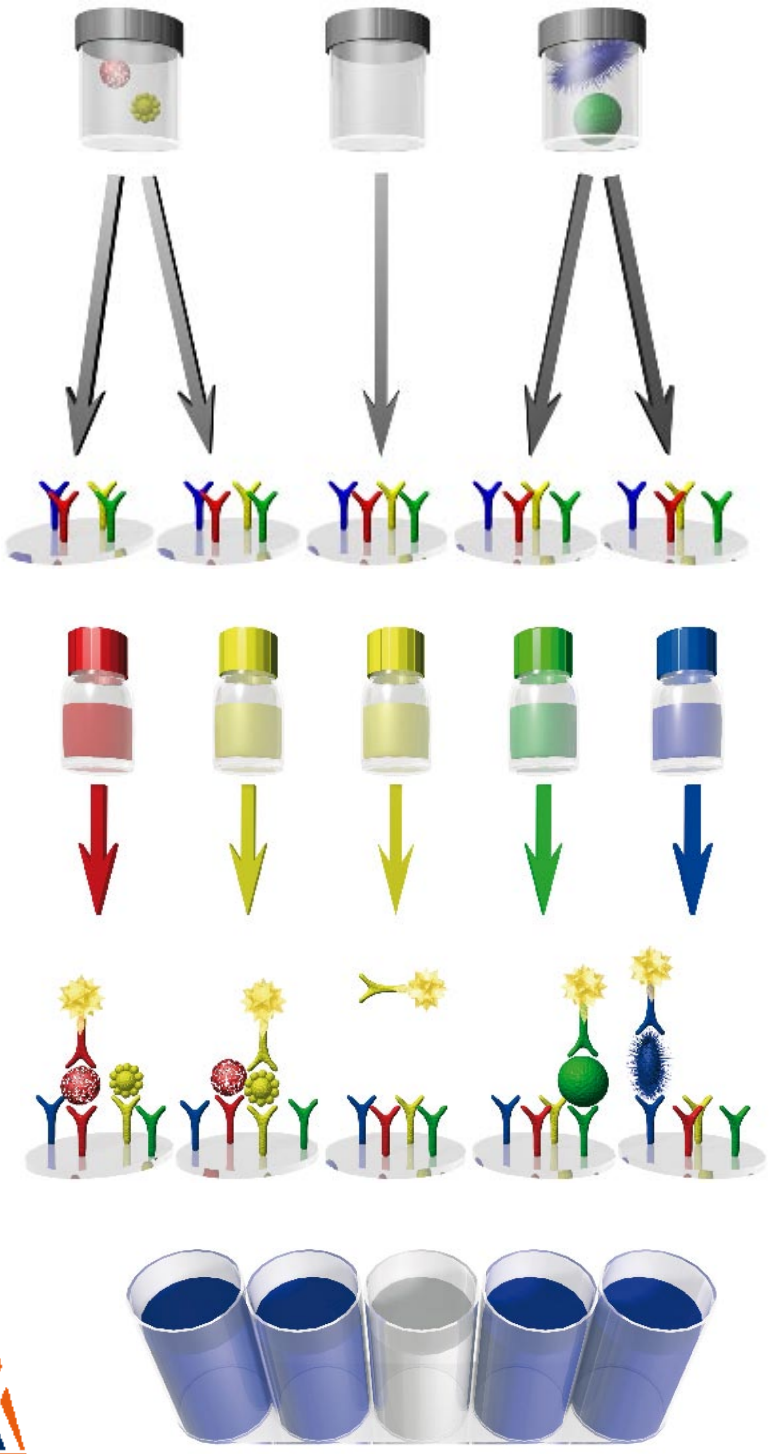
EASY-DIGEST

BIO K 151

Rotavirus - Coronavirus - *E. coli* F5 - *Cryptosporidium*



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- All of the solutions are ready to use, so you have no dilutions to perform.
- The four conjugates are stabilised in different coloured solutions to eliminate the risk of mix-ups.
- A single positive and s single negative control, both ready to use. These reagents are common to the four valencies, for ease of application.
- A single plate on which each well is saturated with four monoclonal antibodies specific to the four causal agents that the kit covers, i.e., rotavirus, coronavirus, E. coli attachment factor F5, and Cryptosporidium parvum. The user chooses which conjugate(s) to use in line with the customer's request.
- Enough of each conjugate is supplied to test up to 94 samples for each valence or 22 samples for all four valences. You can also "mix 'n match".
- The results can be determined with the naked eye, without using a plate reader.
- The reagents are stable for one year.

The Easy Digest kit's reliability has been confirmed by comparing its results with those produced by our kit Digestive BIO K 071.

Example of results for Rotavirus

Digestive BIO K 071

ELISA BIO K 151

	+	-	
+	34	0	34
-	1	52	53
	35	52	87

Example of results for Coronavirus

Digestive BIO K 071

	+	-	
+	13	3	16
-	1	70	71
	14	73	87

Example of results for E. coli F5

Digestive BIO K 071

ELISA BIO K 151

	+	-	
+	3	0	3
-	1	83	84
	4	83	87

Example of results for Cryptosporidium

Digestive BIO K 071

	+	-	
+	29	7	36
-	0	51	51
	29	58	87

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Example of results

Days after birth

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Calf 1	Crypto						+	+	+	+	+	+	+	+	+			
	Rota																	
	Diarrhoea						+	+	+	+	+	+	+					
Calf 2	Crypto							+	+	+	+	+	+	+	+	+	+	+
	Rota					+	+											
	Diarrhoea					+	+	+	+	+	+	+	+	+	+		+	

BIO K 151 EASY-DIGEST ELISA KIT

1 plate of 12 strips for a total of 22 to 94 tests
1 vial of positive control (ready to use)
1 vial of negative control (ready to use)
4 vials of conjugates (ready to use)
1 bottle of washing solution (20 X)
1 bottle of chromogen (ready to use)
1 bottle of stopping solution (ready to use)

1 year of stability at 4°C

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