MONOSCREEN Ab ELISA

Bovine rotavirus

ELISA Kit for serodiagnosis of Bovine rotavirus
Competitive test for blood sera, plasma and colostrum
Diagnostic test for cattle
Monowell

I - INTRODUCTION

Diarrhoea is one of the leading causes of death in young calves under one month old. Since Mebus's 1969 discovery that viruses could be detected in the faeces of calves with diarrheoa, it has been proven that rotavirus can infect the calve and cause sometimes severe diarrhoea. Rotavirus is one of the pathogens associated with gastrotenteritis in young calves. Rotavirus is ubiquitous. As a result, most of the animals coming from intensive livestock farms have specific antibodies against this pathogen. The antibodies produced by the cow in response to natural immunisation or vaccination are transmitted to her calf at birth via the colostrum. The colostrum immunoglobulins frequently are not transmitted to the calves correctly (poor quality colostrum, late administration, too small an amount, pre-calving mastitis, etc…). As a result, the calf will be insufficiently protected from infection. The rotavirus ELISA kit enables one to measure the suckling calf's specific protection against rotavirus. For this, a serum sample must be taken in the first few days after birth when the calf is still protected by the colostrum and has not yet developed active immunity against the virus. However, you must wait at least 24 hours after the first dose of colostrum before taking the control blood sample to allow intestinal resorption of the immunoglobulins to take place. The kit may also be used to test the efficacy of vaccines.

II - PRINCIPLE OF THE TEST

The 96-well microplates have been sensitised by a polyclonal antibody specific for bovine rotavirus. A bovine rotavirus culture was then added to these microplates. The kit's user deposits the previously diluted test sera, colostrum, plasma or milk in the microplate's wells, then adds the conjugate, which is a specific monoclonal antibody against rotavirus coupled to peroxidase. After incubating and washing the preparation, the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic The intensity of the colour is inversely proportionate to the sample's serum titre. Positive and negative control sera are provided with the kit to be able to validate the test results.

III - COMPOSITION OF THE KIT

- **Microplates**: 96-well microtitration plates. The entire surface of each microplate has been sensitised with rotavirus.
- **Washing solution**: 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 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**: bottle of 5x colored, concentrated buffer for diluting samples and conjugate. 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**: vial of anti-rotavirus-peroxidase conjugate (horseradish peroxidase-labelled anti-rotavirus monoclonal antibody). The reagent must be diluted 1:20 with the dilution buffer.
- **Positive serum**: 1 bottle containing the positive serum. Store this reagent between +2°C and +8°C.
- **Negative serum**: 1 bottle containing the negative serum. Store this reagent between +2°C and +8°C.
- **Single component TMB**: bottle of the chromogen tetramethylbenzidine. 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 126/2
Microplates	2
Washing solution	1 X 100 ml (20 X)
Colored Dilution buffer	1 X 50 ml (5 X)
Conjugate	1 X 1,250 ml (20 X)
Positive serum	1 X 0,5 ml (1 X)
Negative serum	1 X 0,5 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, microplates dilution, 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 components to 21°C +/- 3°C before use. Remove the microplate from its wrapper.

2- DILUTION OF SAMPLES
Dilute the samples using the recommended dilutions and listed in the table below:

		Sample dilution plan advised			
Matrice	Total dilution	Individual dilution	Number of dilution	Sample volume to be transferred	Dilution buffer volume
Sera, plasma, reference sera	1/20	1/20	1	10 µl	190 µl
Colostrum	1/200	1/14,14	2	25 µl	325 µl
Milk*	1/1	1/1	1	/	/

*Use undiluted whole milk (without skimming) samples.

3- Distribute the dilute samples over the plate at the rate of 100 µl per well. Proceed in the same manner for the reference sera (positive and negative sera).
4- Add to each well used 100 µl of the conjugate diluted twenty-fold in the dilution buffer. When adding the conjugate take care to avoid contaminating the microtip by dipping it into the sera. Cover with a lid and incubate the plate at 21°± 3°C for one hour.
5- 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.
6- Add 100µl 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 for 10 minutes at 21°C +/- 3°C and away from the light. Do not cover. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
7- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.
8- 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 cristallize in wells with strong signals and distort the results accordingly.

VII – CALCULATING THE RESULTS

Measure the optical densities of the positive and negative sera (OD pos and OD neg) and those of all the samples (OD samples).
Calculate the percent inhibition (% inhib) for each tested sample and the positive serum by means of the following formulas:

% inh sample = [(OD neg – OD sample)/OD neg]*100
% inh positive = [(OD neg – OD pos)/OD neg]*100

VIII – VALIDATING THE TEST

The test may be validated only if the following two conditions are met:

- OD neg – OD pos > 0.5
- % inh positive > 40%

IX – INTERPRETING THE RESULTS

Determine each sample's positivity using the scale shown in Table 1.

Table 1	Calculated value	Degree of positivity
	% inh < 20	0
	20 <= % inh < 40	+
	40 <= % inh < 60	++
	60 <= % inh < 80	+++
	80 <= % inh	++++

X – ORDERING INFORMATION

Monoscreen AbELISA Bovine rotavirus 2X 96 tests BIO K 126/2