VIII – 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	++++

Bio-X Diagnostics

BIO-X CORONAVIRUS ELISA KIT (BIO K 127)

(Competitive test for serum)

ELISA KIT FOR SERODIAGNOSIS OF BOVINE CORONAVIRUS

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 coronavirus can infect the calve and cause sometimes severe diarrhoea. Corronavirus is one of the pathogens associated with gastrotenteritis in young calves. Coronavirus 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 coronavirus ELISA kit enables one to measure the suckling calf's specific protection against coronavirus. 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 monoclonal antibody specific for bovine coronavirus. A bovine coronavirus culture was then added to these microplates. The kit's user deposits the previously diluted test sera in the microplate's wells, then adds the conjugate, which is a specific monoclonal antibody against coronavirus coupled to peroxidase. After incubating and washing the preparation, the operator adds the enzyme's substrate – hydrogen peroxide – and the chromogen tetramethylbenzidine (TMB). 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.

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III - COMPOSITION OF THE KIT

- **Microplates**: Two 96-well microtitration plates. The entire surface of each microplate has been sensitised with coronavirus.
- **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 1.25 ml vial of anti-coronavirus-peroxidase conjugate (horseradish peroxidase-labelled anti-coronavirus monoclonal antibody). The reagent must be diluted 1:20 with the dilution buffer.
- **Positive serum**: 1 bottle containing the positive serum. Reconstitute this serum with 0.5 ml distilled or demineralised water. The reconstituted serum must be kept at -20°C. Divide this reagent into several portions before freezing it to avoid repeated freeze-thaw cycles. If these precautions are taken, the reagent may be kept for several months.
- **Negative serum**: 1 bottle containing the negative serum. Reconstitute this serum with 0.5 ml distilled or demineralised water. The reconstituted serum must be kept at –20°C. Divide this reagent into several portions before freezing it to avoid repeated freeze-thaw cycles. If these precautions are taken, the reagent may be kept for several months.
- **Chromogen solution**: One 2-ml drop-dispenser bottle of the chromogen tetramethylbenzidine. 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. Reference serum must be kept at -20°C once reconstituted. 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.
- 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 the blood sera twenty-fold with the dilution buffer. Proceed in the same manner for the reference sera (positive and negative sera).
4- Distribute the dilute samples over the plate at the rate of 100 µl per well (two wells per sample). Proceed in the same manner for the reference sera (positive and negative sera).
5- 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. Incubate the plate for 1 hour at room temperature.
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- 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.
8- 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.
9- Add 50 µl of stop solution per microwell.
10- 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.

VI – INTERPRETING THE RESULTS

Calculate the mean 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

VII – VALIDATING THE TEST

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

- OD neg – OD pos > 0.7
- % inh positive > 50%

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