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BVDV ELISA KIT

ELISA kit for serodiagnosis of BVDV

Blocking test for blood sera (E0)

Diagnostic test for cattle

I - INTRODUCTION

BVD--bovine virus diarrhoea--and mucosal disease (MD) are two different clinical disorders caused by the same virus. BVD is the result of an acute infection in susceptible animals. Onset may occur at any time after birth. BVD has a brief course and low mortality. Mucosal disease, in contrast, is a deadly disease of low morbidity. It develops in viraemic animals that have been contaminated *in utero*. The characteristic of this *in utero* infection is the existence of specific immunotolerance that prevents the animals from producing antibody against the infective strain but not against another, antigenically different BVD strain. These persistent carriers which can live for years without developing clinical signs of the disease can only be detected by laboratory screening tests. While the only valid method for detecting animals with persistent viral infections remains identification of the BVD virus itself, it is possible to use a serotest in order to avoid to subject all animals of a farm to cumbersome testings as the detection of BVD virus in leucocytes. Indeed, one has the greatest chance of finding animals with persistent infections in a herd of perfectly seronegative animals. However, this group can also include animals that have never come in contact with the virus. Serotests also enable to monitor the serological status of a vaccinated herd and identify animals that have been contaminated by monitoring increases of their serum titres (seroconversions).

As it is a blocking test, it can be used in all animal species.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by monoclonal antibody specific to E0 protein from BVD virus. This antibody is used to purify the protein from lysate of the cells in which the virus was grown. The whole plate is coated with the viral protein. The operator deposits the previously diluted test sera in the microplate's wells. After 2 hours' incubation and a rinse step, the operator adds the conjugate, which is a specific monoclonal antibody against BVDV E0 protein coupled to peroxidase. After incubating and washing the preparation, the operator adds 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 sera are provided with the kit to be able to validate the test results.

III - COMPOSITION OF THE KIT

- **Microplates:** 96-well microtitration plate (12 x 8). The entire surface of each microplate has been sensitised with E0 BVDV protein.
- **Washing solution:** One 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:** One bottle of 5X concentrated buffer for diluting samples and conjugate. The bottle's content is to be diluted with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate:** One bottle of anti- E0 BVDV protein peroxidase conjugate (horseradish peroxidase-labelled anti- E0 BVDV protein monoclonal antibody).
- **Positive serum:** 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:** 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.
- **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.
- **Stopping solution:** One bottle of the 1 M phosphoric acid stop solution.

	BIO K 283/2	BIO K 283/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Dilution buffer	1 X 30 ml (5 X)	1 X 30 ml (5 X)
Conjugate	1 X 0,5 ml (50 X)	1 X 1,4 ml (50X)
Positive serum	1 X 0,5 ml (1 X) freeze-dried	2 X 0,5 ml (1 X) freeze-dried
Negative serum	1 X 0,5 ml (1 X) freeze-dried	2 X 0,5 ml (1 X) freeze-dried
Mono-component TMB	1 X 25 ml (1 X)	1 X 55 ml (1 X)
Stop solution	1 X 15 ml (1 X)	1 X 30 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. Remove the microplate from its wrapper.
- 2- Dilute the concentrated washing solution 20-fold in distilled or demineralised water. Be sure that all crystals have disappeared before dilution.
Dilute the concentrated dilution buffer 5-fold in distilled or demineralised water.
- 3- Dilute the blood sera twofold 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. Proceed in the same manner for the reference sera (positive and negative sera). Incubate the plate with a lid for 2 hours at 37°C.
- 5- 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.
- 6- Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.250 ml of diluent).
Add to each well used 100 µl of the conjugate. Incubate the plate with a lid for 1/2 hour at 37°C.
- 7- Rinse the plate with the washing solution as instructed in step 5.
- 8- 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.
- 9- Incubate for 10 minutes at 21°C +/- 3°C. This time 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 per microwell.
- 11- 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 crystallise 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:

$$\% \text{ inhib sample} = [(OD \text{ neg} - OD \text{ sample}) / OD \text{ neg}] * 100$$

$$\% \text{ inhib positive} = [(OD \text{ neg} - OD \text{ pos}) / OD \text{ neg}] * 100$$

VIII – VALIDATING THE TEST

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

- OD neg - OD pos > 0.7
- % inhib positive > 50%

IX – INTERPRETING THE RESULTS

Determine each sample's positivity using the following cut-off :

% inhibition > or = 50 % : positive

% inhibition < à 50 % : negative

X – ORDERING INFORMATION

BIO-X SEROLOGY BVDV ELISA KIT (E0):

2x96 tests

BIO K 283/2

5x96 tests

BIO K 283/5



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