MONOSCREEN Ag ELISA

BVDV

ELISA kit for antigenic diagnosis of Bovine Virus Diarrhoea
Sandwich test for the detection of NS3 protein in leukocytes
Diagnostic test for cattle
Monowell

I - INTRODUCTION

BVDV virus is known to induce a wide variety of foetal and postnatal disorders. Two pathologies are most commonly related to this virus in cattle: bovine viral diarrhea (BVD) and mucosal disease (MD). The latter is always lethal. It arises from a surinfection of immunotolerant persistenly infected (IPI) animals. These animals produce continuously the virus in a non-cytopathic form et are therefore mainly responsible for disease spreading. A persistenly infected animal surinfected with a cytopathic strain identical to the non-cytopathic one will develop the mucosal disease.
Disease prevention against this pathology must be performed through immunotolerant persistenly infected animal detection.
The ELISA technique allows a diagnosis easier to perform than in vitro sample culture. It has been shown that results from these two methods are similar although ELISA do not always allow transient infected animal detection.

II - PRINCIPLE OF THE TEST

The BVDV antigen kit is based on a single well immunoenzymatic technique type ELISA (enzyme linked immunosorbent assay). A specific monoclonal antibody directed against BVDV NS3 protein is adsorbed in the microplate wells. This antibody catches this viral protein present in purified leucocytes samples. After 1hour incubation at 21°C +/- 3°C and a washing step, the conjugate (a monoclonal antibody anti-NS3 BVDV protein) is applied to each well for the same period of time. Following this step and another wash, the chromogen (TMB) is added to the plate.
Formation of immunocomplex elements is detected by the appearance of a blue colour. The intensity of the colour resulting from the enzymatic activity is proportional to the content of pathogenic elements in samples. Blocking the reaction with an acid solution allows reading at 450 nm.
A positivity threshold is given as a percentage of the optical density of the provided positive control.
This ELISA test allows detection of immunotolerant persistenly infected (IPI) animals, with restriction for transient viraemic animals which are not systematically detected.

III - COMPOSITION OF THE KIT

1- **Microplates** Two ninety-six-well-plates. 12 strips of 8 wells. All wells are sensitized with the specific anti-BVDV monoclonal antibody.
2- **Haemolysis solution** – One 100-ml bottle of 10x concentrated solution for red cells haemolysis - contains bacteriostatic.
3- **Lysis solution** – One 100-ml lysis solution for leucocytes - contains bacteriostatic (ready to use).
4- **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 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. Contains bacteriostatic.
5- **Positive Control** - Freeze-dried positive reference (inactivated C24V strain)- contains bacteriostatic. To be rehydrated with 1 ml distilled or demineralised water
6- **Negative Control** – 1 vial of 1 ml.
7- **Conjugate**: One 25-ml bottle of anti-BVDV monoclonal antibodies peroxidase conjugate. The reagent is ready to use.
8- **Single component TMB**: One 25-ml bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use.
9- **Stopping solution**: 15 ml of phosphoric acid 1M solution.

	BIO K 258/2
Microplates	2
Washing solution	1 x 100 ml (20 x)
Haemolysis solution	1 x 100 ml (10 x)
Lysis solution	1 x 100 ml (1 x)
Conjugate	1 x 25 ml (1 x)
Positive control	1 x 1 ml (1 x) Freeze-dried
Negative control	1 x 1 ml (1 x)
Single component TMB	1 x 25 ml (1 x)
Stopping solution	1 x 15 ml (1x)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, glass 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. After reconstitution, positive control is stable for 2 weeks between +2°C and +8°C. For longer preservation, - 20°C storage in aliquots is recommended.
  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 haemolysis solution 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

A. PREPARATION OF SAMPLES

Leucocytes preparation:

1/ Dilute 10 times the haemolysis solution in distilled or demineralized water, i.e. 1 ml concentrated haemolysis solution with 9 ml distilled or demineralised water. One recommends to prepare only the needed quantity, i.e. 3 ml per sample.
2/ Distribute 3 ml of the prepared haemolysis solution in an identified glass tube.
3/ Add 2 ml of whole blood and mix thoroughly.
4/ Incubate for 5 to 15 minutes at 21°C +/- 3°C in order to lyse completely red cells.
5/ Spin down 15 minutes at 1000 g.
6/ Discard thoroughly supernatant and resuspend the pellet with 200 µl of the lysis solution.
7/ Distribute 100 µl of so-prepared leucocytes into wells without any other dilution.

B. PREPARATION OF REAGENTS

1. Make up the necessary amount of wash solution by diluting the concentrated solution twenty-fold with distilled or demineralised water.
2. Reconstitute the positive control by adding 1 ml of distilled or demineralised bottle to the vial. The reconstituted positive control may be kept for two weeks at between +2°C and +8°C, or, even better, in aliquots frozen at -20°C. The positive control must be used in a 1:2 solution with the lysis solution (50 µl of lysis solution + 50 µl of positive control). Depositing it on the plate in duplicate is recommended.
3. Set the plan for depositing the samples on the plate either singly or, better yet, in duplicate.
4. Distribute the samples and positive and negative controls over the plate.
   Each well should contain one of the following
   - 100 µl of purified leucocytes or
   - 100 µl of positive control diluted 1:2 in the lysis solution or
   - 100 µl of negative control diluted 1:2 in the lysis solution.

C. CARRYING OUT THE TEST

All steps of the test are to be carried out at 21°C +/- 3°C.

1/ Distribute prepared samples, positive and negative control into the plate.
2/ Cover with a lid and incubate the plate at 21°± 3°C for one hour.
3/ 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 particular care to avoid bubble formation in the wells. After these three rinses, go on to the next step.
   Using a plate washer (whether automatic or manual) is also recommended. However, the depth of the needles’ immersion must be set so as not to disturb the layer of reagents adsorbed to the bottom of each well.
4/ Using a pipet distribute 100 µl of anti-BVDV peroxidase conjugate in each well.
5/ Cover with a lid and incubate the plate at 21°± 3°C for one hour.
6/ Once it is over, properly wash the plate as shown at paragraph 3.
7/ 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.
   Incubate at 21°C +/- 3°C and away from light for 10 minutes. Do not cover.
8/ Note the appearance of a blue colour which usually occurs in the positive wells after a few minutes. After 10 minutes incubation period, stop the reaction by adding 50 µl of the stopping solution, the blue colour will change into a yellow colour, and read the plate with a spectrophotometer at 450 nm as soon as possible.

VII – INTERPRETING THE RESULTS

The optical density average of each sample, positive (O.D. av PC) and negative (O.D. av NC) control should be calculated, if processed two times on the plate.
Results are validated if the optical density of the positive control (PC) is between the two values given on the QC data sheet
If a negative control is used, its O.D. average must be located below a value calculated as 0,2 x O.D. of the positive control (PC) average.

$$\text{O.D.av NC} < 0.2 \times \text{O.D. av PC}$$

Calculate for each unknown sample a coefficient using the following formula:

$$\text{sample coefficient} = \frac{\text{O.D. sample - O.D. av NC}}{\text{O.D. av PC - O.D. av NC}}$$

Sample assessment should be regarded as follows:

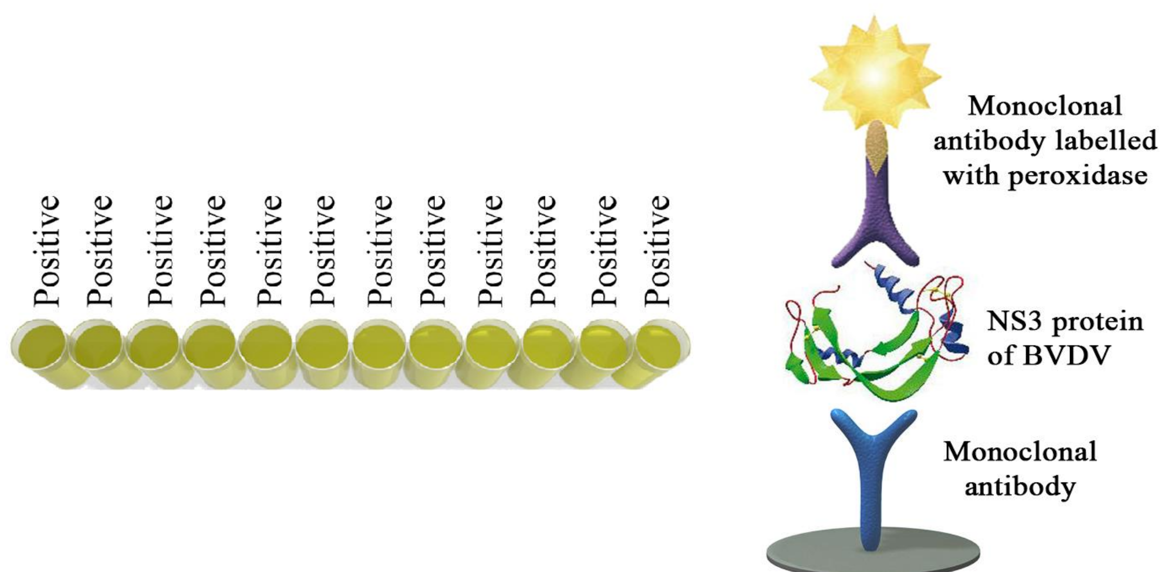
- A sample is regarded as negative if its coefficient is below 0.08

- A sample is regarded as positive if its coefficient is above or equal to 0.12

- A sample is regarded as doubtful if its coefficient is between 0.08 and 0.12

VIII – ORDERING INFORMATION

Monoscreen AgELISA BVDV	2 X 96 tests	BIO K 258/2



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