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 0.2
- A sample is regarded as positive if its coefficient is above or equal to 0.3
- A sample is regarded as doubtful if its coefficient is between 0.2 and 0.3

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BIO-X BVDV ANTIGEN ELISA KIT (ONE STEP) BIO K 258

(Sandwich test for NS3 protein detection)

ELISA KIT FOR DETECTION OF BVDV ANTIGENS

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 1 hour incubation at room temperature 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** - 10 times concentrated solution for red cells haemolysis (100 ml) - contains bacteriostatic.
3- **Leucocytes lysis buffer** - Lysis solution for leucocytes (100 ml) - contains bacteriostatic (ready to use).
4- **Washing solution** - 20 times concentrated washing solution (100 ml). Contains bacteriostatic.
5- **Positive Reference** - Freeze-dried positive reference (inactivated C24V strain)- contains bacteriostatic. To be rehydrated with 1 ml distilled or demineralised water
6- **Negative Reference** – 1 vial of 1 ml ready to use
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 at 4°C protected from light. This solution is ready to use.
9- **Stopping solution** : 15 ml of phosphoric acid 1N solution.

IV - PRECAUTIONS FOR USE

1- This test should only be used for in-vitro diagnosis and for veterinary use only
2- Reagents must be kept between 4 °C and 8 °C. After reconstitution, positive reference is stable for 15 days at 4 °C. For longer preservation, - 20 °C storage in aliquots is recommended.
The peroxidase conjugate can not be frozen.
3- All reagents should be put for 30 minutes at room temperature before use.
4- Do not use reagents from other kits.
5- Stopping solution contains phosphoric acid 1M and must be handled with care. Avoid contact with skin or eyes.
6- Chromogen solution must be colourless before use into the wells. If a blue colour appears at this stage, the solution is contaminated with peroxidase. If this occurs, the chromogen solution must be discarded and a new solution made up using absolutely clean glassware and equipment.
7- Solutions must be clear of bacteria or algae.
8- Samples are suceptible to transmit diseases. Handle with special care.
9- Material must be perfectly clean.
10- TMB solution should be stored away of light.
11- Never use mouth pipettes.
12- Avoid contact of the chromogen solution with water containing metals or oxyding agents.
13- In order to get satisfactory and accurate results, particular care must be taken in pipetting. Procedure (incubation time and temperature) must also be followed carefully.
14- Do not use reagents after the expiry date.

V – PROCEDURE

A. PREPARATION OF SAMPLES

<u>Leucocytes preparation:</u>

1/ Dilute 10 times the haemolysis buffer in distilled or demineralized water, i.e. 1 ml concentrated haemolysis buffer 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 buffer in an identified glass tube.
3/ Add 2 ml of whole blood and mix thoroughly.
4/ Incubate for 5 to 15 minutes at room temperature 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 leucocytes lysis solution.
7/ Distribute 100 µl of so-prepared leucocytes into wells without any other dilution.

B. PREPARATION OF REAGENTS

1- Dilute 20 times the washing buffer in distilled or demineralized water, i.e. 1 ml concentrated washing buffer with 19 ml distilled or demineralised water.
2- Rehydrate the positive control with 1 ml distilled water. This control as well as negative control should be 2 times diluted in the leucocytes lysis solution (V/V). One recommends to process it two times in the plate.

C. CARRYING OUT THE TEST

All steps of the test are to be carried out at room temperature.

1/ Distribute prepared samples, positive and negative control into the plate.
2/ Incubate the plate covered with a lid at room temperature for an hour.
3/ Quickly turn the plate upside down over a sink in order to empty out its content. Hold the frame firmly on the long sides to keep the structures in place.
4/ Soak the plate in the washing solution checking all the wells are properly rinsed (avoid bubbles). Turn the plate upside down over a sink to pour out the liquid. This operation should be repeated at least three times until all colored residue is eliminated.
An automatic plate washer could be useful provided the depth of syringes is carefully checked. Dry the plate on blotting paper.
5/ Using a pipet distribute 100 µl of anti-BVDV peroxidase conjugate in each well and then incubate once again with a lid for an hour at room temperature.
6/ Once it is over, properly wash the plate as shown at paragraphs 3 and 4.
7/ Quickly distribute 100 µl of chromogen solution per well.
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 and read the plate with a spectrophotometer at 450 nm as soon as possible.

VI – 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.

$$O.D.av\ NC < 0.2 \times O.D.\ av\ PC$$