INTERPRETATION

Validation of the Test

The test is considered valid if:

- the OD of the positive control minus the OD of the negative control is higher than 0.8
- the OD of the negative control is lower than 0.5

Interpretation of the Results

Sample value calculation

For each sample, calculate a sample value using the following formula:

$$\text{Sample value:} \quad \frac{\text{OD sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}}$$

Interpretation

Serum and leucocytes	Ear notches
Sample with value ≥ 0.3 is considered positive *	Sample with value ≥ 0.1 is considered positive *
Sample with value ≥ 0.2 and < 0.3 is considered as suspicious *	Sample with value ≥ 0.05 and < 0.1 is considered as suspicious *
Sample with value < 0.2 is considered negative.	Sample with value < 0.05 is considered negative

* To identify PI animals, a second blood sample has to be examined three to four weeks after the first sample using this assay (animals older than 6 months) or a PCR assay (animals younger than 6 months). PI animals will produce two consecutively positive results.

PRECAUTIONS AND WARNINGS

- For *in vitro* veterinary use only.
- Store all kit components at 2-7°C.
- Do not use the kit after the expiry date indicated on the package.
- Do not intermix instructions or components from different serials.
- Reagents should be stored back at appropriate temperature as soon as possible after use.
- The material used in this kit must be considered as potentially infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not expose the substrate to either light or oxidizing agent. Always keep the substrate in a plastic container. This solution may cause skin or eye irritation.
- The stop solution contains a strong acid and must be manipulated with caution to avoid contact with skin or mucous membranes.
- Dispose of the substrate and the stop solution according to local regulations for chemicals.

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Detection of BVDV antigen in persistently infected cattle

Bovine Viral Diarrhea Virus Antigens Test Kit, ELISA

Insert – 2 plates

2015-04-09

This kit is based on an immunoenzymatic assay intended for the detection of bovine viral diarrhea virus (BVDV) antigens in cattle persistently infected with BVDV.

BVDV is a pestivirus that is widely spread into cattle populations and is responsible for a variety of economically important disorders. Two major antigenically distinct genotypes of BVDV exist, type 1 and 2.

Foetal infections with BVDV during the first trimester of pregnancy can result in the birth of immunotolerant and persistently infected (PI) calves. Such animals demonstrate high levels of BVDV in their tissues and excrete large quantities of the virus throughout their entire life. PI animals are mainly responsible for maintenance of the virus in cattle populations. Removal of PI animals is the corner stone to efficiently control BVDV infections.

PI animals may be identified by a variety of techniques such as virus isolation, polymerase chain reaction (PCR) or immunohistochemistry (IHC). These methods are effective but they require skilled personnel and highly equipped laboratories. The BVDV Antigen Test Kit is an easy, fast and inexpensive alternative tool to detect BVDV in blood or ear notch samples. The test detects both BVDV type 1 and 2 strains.

PRINCIPLE OF THE TEST

This kit is based on an enzymatic immunoassay (antigen capture Elisa) which has been developed to detect BVDV antigens in PI animals. The assay may be performed on whole blood (heparin or EDTA), serum, plasma (heparin or EDTA), leucocytes extracts or ear notch extracts. **The sensitivity of the test on whole blood, serum and plasma is affected by the presence of maternally derived BVDV antibodies. Therefore, in animals of less than 6 months of age, the test should be done on leucocytes or ear notch extracts only.**

Samples (serum, plasma, or leucocytes or ear notch extracts) and controls are incubated in wells coated with polyclonal antibodies specific to BVDV. These antibodies capture the BVDV antigens eventually present. After several washes to eliminate unbound substances, BVDV-specific biotin-conjugated polyclonal antibodies are added. These antibodies bind to BVDV antigens that have been captured by the BVDV antibodies adsorbed in the wells. After incubation, the excess of biotinylated antibodies is eliminated by serial washings. An avidin – horseradish peroxydase (HRPO) conjugate is then added and allowed to react with the biotinylated antibodies bound to the BVDV captured antigens. After washings, a chromogenous substrate is added. During incubation, the enzymatic portion of the conjugate, if present, reacts with the substrate and a blue color develops. The reaction is then stopped (the color changes from blue to yellow) and the optical densities are read. The intensity of the color is proportional to the quantity of BVDV antigen present.

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MATERIAL

Components	Quantity
12 strips of 8 wells coated with anti-BVDV polyclonal antibodies *	2
Positive BVDV control antigen (freeze dried)	1 vial
Dilution solution (ready to use)	1 x 10 mL
Hemolysis solution (10X)	**On request****
Leucolysis solution (ready to use)	**1 x 10 mL*****
Biotinylated anti-BVDV polyclonal antibodies (ready to use)	1 x 25 mL
Avidin-peroxydase conjugate (ready to use)	1 x 25 mL
Concentrated wash solution (20X) ****	**2 x 100 mL*****
Substrate (ready to use)	1 x 25 mL
Stop solution (ready to use)	1 x 25 mL

*Once the bag is opened, unused wells are stable for at least 3 months if stored at 2-7°C in a closed bag with a desiccant.

****The hemolysis solution is provided separately on request.**

*****Additional quantities of leucolysis solution and concentrated wash solution are available on request.**

****Crystals may form when wash solution is kept at 2-7°C. This will not affect the efficiency of the product. However, crystals have to be dissolved by bringing the solution to room temperature and agitating.

The materials provided are sufficient for testing up to 184 samples (2 plate format).

Material required but not provided:

- Purified water
- Phosphate buffered saline (for ear notch samples only)
- Pipettes and containers for preparation of solutions
- Adjustable single- and multi-channel micropipettes (from 100 to 300 µl)
- Single-use micropipette tips
- ELISA microplate washer (optional)
- ELISA 96-well microplate reader equipped with 450 nm filter
- Centrifuge (for leucocytes extracts only)

EXECUTION

Bring all reagents to room temperature before use. Liquid reagents and samples have to be well mixed. All steps of the test have to be done at room temperature (23 ± 2°C).

A. Preparation of wash solution

After homogenizing the concentrated wash solution (no evidence of crystals), dilute at 1/20 with purified water (e.g., 10 mL of 20X concentrated wash solution in 190 mL purified water). Once diluted, the solution (1X) is stable for 3 months at 2-7°C.

B. Preparation of hemolysis solution

After homogenizing the concentrated hemolysis solution, dilute at 1/10 with purified water (e.g., 1 mL of 10X concentrated hemolysis solution in 9 mL purified water). Prepare fresh solution before use (3 mL are needed per sample).

C. Preparation of positive control

Rehydrate the positive BVDV antigen control with 1 mL of purified water. Distribute the reconstituted solution into several aliquots and keep them at -20°C in order to avoid repeated freezing-thawing cycles. Under these conditions the positive control is stable for several months. Immediately before use, the control has to be diluted with the leucolysis solution according to the dilution indicated on the Final Control Sheet.

D. Preparation of negative control

Dilute the leucolysis solution 1/2 with the dilution solution (e.g., 125 µL of leucolysis solution in 125 µL of dilution solution).

E. Preparation of samples

- **Whole blood, serum and plasma**
  These samples do not require special preparation and are used undiluted.
- **Leucocytes extracts**
  Dispense 3 mL of the freshly prepared hemolysis solution in a tube. Add 2 mL of whole blood. Incubate for 15 minutes in order to completely lyse the erythrocytes. Spin down the cells for 15 minutes at 1000 g. Discard the supernatant and suspend the pellet with 200 µL of the leucolysis solution. The resulting solution is used for testing.
- **Ear notch extracts**
  Dispense 2 mL of phosphate buffered saline in a tube. Place the ear notch sample into the tube and mix gently. Allow to soak for a minimum of 10 minutes. The resulting solution is used for testing and must be mixed well beforehand.

F. Test procedures

1. Dispense 100 µL of positive control, negative control or samples (whole blood, serum, plasma, leucocytes extracts or ear notch extracts) into appropriate wells. It is recommended to run controls in duplicates.
2. Cover the plate and incubate for either 1 hour or 16 to 18 hours (overnight) at 23 ± 2°C.
3. Wash each well 3 times **thoroughly**. Fill the wells with 1X wash solution (see section A). During the last wash, allow the wells to stand for 2 minutes before emptying. Throw away all liquid contained in the wells after each wash. After the last wash dry the plate by tapping onto an absorbent paper. Do not keep the wells dry more time than strictly needed.
4. Dispense 100 µl of anti-BVDV biotinylated antibodies into each well.
5. Cover the plate and incubate for 1 hour at 23 ± 2°C.
6. Wash each well 3 times following the procedure described in step 3.
7. Dispense 100 µl of the avidin – peroxydase conjugate into each well.
8. Cover the plate and incubate for 1 hour at 23 ± 2°C.
9. Wash each well 3 times following the procedure described in step 3.
10. Dispense 100 µL of substrate solution into each well. Before being added to the plate, the substrate solution has to be completely colourless. If a blue color appears at this stage, this indicates that the solution is contaminated and has to be discarded.
11. Keep the plate for 5 minutes in the dark at 23 ± 2°C.
12. Dispense 100 µl of stop solution into each well following the same order in which the substrate was added.
13. Measure optical densities (OD) at 450 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.

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