Bovine Virus Diarrhoea Virus (BVDV-Ab)

**SVANOVIR™**

ELISA test for the detection of BVDV antibodies in serum and milk

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**General information**

The Bovine Virus Diarrhoea Virus (BVDV), a pestivirus, is associated with a range of diseases affecting the alimentary tract of cattle. Clinical signs include pyrexia, diarrhoea and reduced milk yield; there is a high morbidity but low mortality of infected animals. Infection of pregnant cows may result in transplacental foetal infection. Foetuses may be aborted, mummified, stillborn or born with severe anomalies; however, in many instances calves, if immunotolerant, are born unaffected but with persistent viraemia and become transmission sources of the virus. Moreover, recurrent infection of such calves may result in mucosal disease—a condition with low morbidity but high mortality; it is characterized by extensive erosions in the oral and gastrointestinal mucosa. BVDV has also been shown to have immunosuppressive effects which predisposes animals to infection by other microorganisms. Economic losses can be high due to these factors which is why herds should be monitored for the presence of infection.

**Principle**

The Bovine Virus Diarrhoea Virus BVDV-Ab ELISA Kit is designed to detect BVDV specific antibodies in serum or milk samples. The kit procedure is based on a solid phase indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA). In this procedure, samples are exposed to non-infectious BVDV antigen coated wells on microtitre plates or strips. BVDV antibodies (if present in the test sample) bind to the antigen in the well. HRP conjugate added subsequently forms a complex with the BVDV antibodies. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of a blue colour. The reaction is stopped by addition of the stop solution; the colour changes to yellow. The result can be read visually or by a microplate photometer, where the optical density (OD) is measured at 450 nm.

**References**


**Manufacturer**

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**Interpretation Serum/Milk**

- All samples with an OD value greater than Cut-off, are considered as positive.
- All samples with an OD value lower than Cut-off, are considered negative.

It is recommended to retest all samples with a positive reaction in an ELISA with control antigen, to eliminate any doubtful reacting samples.

**Calculation of Cut-off**

\[
A = OD_{\text{value negative control}} \times 2.0
\]

If \(A \geq 0.1\): Use \(A\) as your Cut-off
If \(A < 0.1\): Use 0.1 as your Cut-off
A. Positive Control Serum – 0.05% merthiolate
B. Negative Control Serum – 0.05% merthiolate
C. Positive Control Milk – 0.05% merthiolate
D. Negative Control Milk – 0.05% merthiolate

Materials needed but not provided
1. Precision pipets (range from 4 to 200 µl)
2. Disposable pipet tips
3. Distilled water
4. Wash bottle
5. 1 container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm filter

Specimen information
Serum: 4 µl of blood serum or plasma is needed for each sample well. Fresh, refrigerated, or previously frozen serum or plasma may be tested.

Milk: 100 µl of skim milk is required for each sample well. Milk samples must be centrifuged for 15 minutes at 2000 x g to remove the lipid layer.

Preparation of reagents
PBS-Tween Buffer: Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 ml per plate by adding 25 ml PBST solution to 475 ml distilled water and mix thoroughly. N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

Anti-Bovine IgG Conjugate: Reconstitute the lyophilized HRP Conjugate with 11.5 ml PBS-Tween Buffer. Add the buffer carefully to the bottle. Leave the solution one minute and mix thoroughly. Prepare immediately before use. The remaining reconstituted conjugate can be stored at -20°C and thawed and refrozen up to 3 times.

Precautions
1. Carefully read and follow all instructions.
2. Store the kit and all reagents at +2 to +8°C (35 to 45°F)
3. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction booklets from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expire.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipet tip for each sample.
10. Do not pipet by mouth.
11. Include positive and negative serum and/or milk controls on each plate.
12. Use only distilled water for preparation of reagents.
13. The Stop Solution contains sulphuric acid, which is corrosive.
14. All unused biological materials should be disposed according to the local, regional and national regulations.

Interpretation of the results
Criteria for test validity Serum
To ensure validity the Positive Control Serum should have an OD value greater than 0.6 and the Negative Control Serum should have an OD value of less than 0.2. For invalid tests, technique may be suspect and the assay should be repeated.

Calculation of Cut off
\[ A = \text{OD value negative control} \times 2.0 \]
If \( A \geq 0.2 \): Use \( A \) as your Cut-off
If \( A < 0.2 \): Use 0.2 as your Cut-off

Criteria for test validity Milk
To insure validity the Positive Control Milk should have an OD value greater than 0.3 and the Negative Control Milk should have an OD value of less than 0.15. For invalid

Procedure
1. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.

Serum Samples
A. Add 100 µl of PBS-Tween Buffer to each well that will be used for serum samples and serum controls.
B. Add 4 µl of Positive Control Serum (Reagent A) and 4 µl of Negative Control Serum (Reagent B) respectively, to selected wells coated with BVDV antigen. For confirmation purposes it is recommended to run the control sera in duplicates.
C. Add 4 µl of serum sample to a selected well coated with BVDV antigen. For confirmation purposes it is recommended to run the samples in duplicates.

Milk Samples
A. Add 100 µl of Positive Control Milk (Reagent C) and 100 µl of Negative Control Milk (Reagent D) respectively, to selected wells coated with BVDV antigen. For confirmation purposes it is recommended to run the control sera in duplicates.
B. Add 100 µl of skim milk sample to a selected well coated with BVDV antigen. For confirmation purposes it is recommended to run the samples in duplicates.
C. Add 4 µl of serum sample to a selected well coated with BVDV antigen. For confirmation purposes it is recommended to run the samples in duplicates.

4. Shake the plate thoroughly. Seal the plate and incubate at 37°C (98.6°F) for 1 hour.

5. Rinse the plate 3 times with PBS-Tween Buffer. Fill up the wells at each rinse, empty the plate and tap hard to remove all remains of fluid.

6. Add 100 µl of HRP Conjugate to each well and incubate at 37°C (98.6°F) for 1 hour.

7. Repeat step # 5

8. Add 100 µl Substrate Solution to each well and incubate for 10 minutes at room temperature. Begin timing after the first well is filled.

9. Stop the reaction by adding 50 µl of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #8.

10. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Recommendation!
Reconstituted conjugate may not be stored in refrigerator.