SVANOVIR® BCV-Ab

Bovine Coronavirus
Antibody Test

Contents	**Art. No. 104887**
Microtitre plate Microtitre plates (96 wells) coated with non-infectious BCV antigen (sealed and stored dry) Odd columns coated with viral antigen and even columns with control antigen	2 (Strips) 12 x 8
Conjugate Lyophilised (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies)	2
PBS-Tween Solution 20 x concentrate	1 x 125 mL
Substrate Solution (Tetramethylbenzidine in substrate buffer containing H_2O_2) - STORE IN THE DARK!	1 x 20 mL
Stop Solution Contains sulphuric acid (2M) - CORROSIVE! 	1 x 10 mL
A. Positive Control Serum - Contains preservatives	1 x 0.1 mL
B. Negative Control Serum - Contains preservatives	1 x 0.1 mL

This manual covers the following SVANOVIR® BCV-Ab ELISA kit: Article number 104887

Bovine Coronavirus
Antibody Test

Name and Application
SVANOVIR® BCV-Ab is an Enzyme Linked Immunosorbent Assay (ELISA) test for the detection of BCV specific antibodies in serum and milk samples.

General information
Bovine Coronavirus (BCV), a member of the family Coronaviridae, is a major cause of neonatal calf diarrhoea on dairy farms, cow-calf ranches, and veal-producing operations[1]. The virus is also associated with respiratory tract disease[2]. Besides, BCV has also been identified as the agent of 'winter dysentery' – an illness in adult cattle, accompanied by diarrhoea and reduced milk yields[3]. In both the enteric and respiratory diseases, BCV is either a sole agent or participant of a mixed infection. Transmission occurs when faecal material bearing the virus is ingested leading to infection of the absorptive epithelium of the gut. After a brief incubation period of 19 to 24 hours calves may develop symptoms including excess salivation, weakness, lethargy, dehydration, and acute diarrhoea. Severe diarrhoea may lead to hypovolemic shock and death. Pulmonary congestion, pneumonia, and predisposition to bacterial infections are secondary features of BCV infection. Newborn calves may obtain partial protection from the virus via colostrum. Resistance depends upon the antibody titre of the mother and protection lasts only a few days. Clinically, BCV infections are not readily distinguishable from other causes of neonatal calf diarrhoea but diagnosis is possible through laboratory analysis of faecal material, blood serum, or milk[1].

Principle
The kit procedure is based on a solid phase indirect ELISA. In this procedure, samples are exposed to non-infectious BCV antigen coated wells on microtitre plates or strips. BCV antibodies (if present in the test sample) bind to the antigens in the well. HRP conjugate added subsequently forms a complex with the BCV 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.

Materials needed but not provided

1. Precision pipettes
2. Disposable pipette tips
3. Distilled water, deionised or any similar hight quality water
4. Wash bottle, multichannel pipettor or plate washer
5. 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. Fresh, refrigerated or previously frozen milk may be tested. Milk samples must be centrifuged for 15 minutes at 2000 x g to remove the lipid layer, or leave the milk samples until the fat layer is formed on top of the sample. Pipette under the fat 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 PBS-Tween 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 on a shaker. 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 2-8°C (36-46°F).
3. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction manuals 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 expiry.
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 controls on each plate or test strip series.
12. Use only distilled, deionised or any similar high quality water for preparation of reagents.
13. When preparing the buffers, etc., measure the required volume.
14. The Stop Solution contains sulphuric acid, which is corrosive.*
15. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!

The volume of the reagents is sufficient for at least 8 separate test occasions.
Strips with broken seal can be stored at 2-8°C (36-46°F) for up to 4 weeks.
Reconstituted conjugate may not be stored in refrigerator.

Procedure

1. All reagents should equilibrate to room temperature 18-25˚C (64-77˚F) before use. Label each strip with a number.
2. Add samples:

   Controls

   The provided negative and positive control sera are used for both serum and milk testing.

A. Add 100 µL of PBS-Tween Buffer to each well that will be used for 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 BCV viral antigen and to corresponding wells coated with control antigen. For confirmation purposes it is recommended to run the control sera in duplicates.

   Serum Samples

A. Add 100 µL of PBS-Tween Buffer to each well that will be used for serum samples.

B. Add 4 µL of serum sample to a selected well coated with BCV viral antigen and to a corresponding well coated with control antigen. The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

   Milk Samples

A. For addition of controls, see "Controls" (point A and B).

B. Add 100 µL of skim milk sample to a selected well coated with BCV viral antigen and to a corresponding well coated with control antigen. The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

3. Shake the plate thoroughly. Seal the plate/strip and incubate at 37˚C (98.6˚F) for 1 hour.
4. Rinse the plates/strips 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.
5. Add 100 µL of HRP Conjugate to each well and incubate at 37˚C (98.6˚F) for 1 hour.
6. Repeat step #4.
7. Add 100 µL Substrate Solution to each well. Incubate for 10 minutes at room temperature 18-25˚C (64-77˚F). Begin timing when the first well is filled.
8. 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 #7.
9. 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.

Calculations

Calculation of results are done in two steps as described below.

1. **Corrected OD Values (OD_{Corr})**

   The optical density (OD) values in well coated with BCV viral antigen are corrected by subtracting the OD values of the corresponding wells containing the control antigen.

   $$OD_{BCV} - OD_{Control} = OD_{Corr}$$

   Calculate the mean OD_{Corr} value for each of the controls and samples.

2. **Percent Positivity Values (PP)**

   All Corrected OD Values for the test samples as well as the Negative Control are related to the corrected OD value of the positive control as follows:

$$PP = \frac{OD_{Corr\ (Sample/Negative\ Control)}}{OD_{Corr\ (Positive\ Control)}} \times 100$$

Interpretation of the results

Criteria for test validity

To ensure validity, the duplicate of the OD values of the positive control should not differ more than 25 % from the mean value of the two duplicates. Additionally, the control values should fall within the following limits:

OD_{Corr}	**Positive control**	**> 0.5**
PP	**Negative control**	**< 10**

Should any of these criteria not be fulfilled, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of serum and milk samples

PP	**Interpretation**
< 10	**Negative**
≥ 10	**Positive**

References

1. Kahrs, R.F. (1981) Coronavirus. In Viral Diseases of Cattle. The Iowa State University Press, Ames, Iowa, pp. 107-113.
2. McNulty, M.S. *et al.* (1984). Coronavirus infection of the bovine respiratory tract. Vet. Microb. 9, 425-434.
3. Espinasse, J. *et al.* (1982). Winter dysentery: a coronavirus-like agent in the faeces of beef and dairy cattle with diarrhoea. Vet. Rec. 110, 385.
4. Alenius, S., Niskanen, R., Juntti, N., and Larsson, B. (1989/90) Bovine coronavirus as the causative agent of winter dysentery: serological evidence. Acta Vet. Scand. (In press).
5. Ohlson A., *et al.* (2010). Risk factors for seropositivity to bovine coronavirus and bovine respiratory syncytial virus in dairy herds. Vet. Rec. Aug 7; 167(6); 201-6.

***WARNING: Stop solution (sulphuric acid)**
May be corrosive to metals. Causes skin irritation. Causes serious eye irritation.

Keep only in original container. Wear eye protection/ face protection. Wear protective gloves.
IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. If eye irritation persists: Get medical advice/ attention.
IN CASE OF CONTACT WITH SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse. If skin irritation occurs: Get medical advice/attention. Absorb spillage to prevent material damage.

Symbols

REF	Article No.
LOT	Serial (batch) No.
	Temperature limit
	Expiry date
	Number of tests
	See manual
	Manufacturer
	Telephone
	Fax


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