SVANOVIR® BRSV-Ab

Bovine Respiratory Syncytial Virus
Antibody Test

Contents	**Art. No. SV-104888**
Microtitre plate Microtitre plates (96 wells) coated with non-infectious BRSV antigen (sealed and stored dry) Odd columns coated with viral antigen and even columns with control antigen	2 (Strips) 6 x 16
Conjugate Ready-to-use (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies)	1 x 24 mL
PBS-Tween Solution 20 x concentrate	1 x 125 mL
Sample Dilution Buffer Ready-to-use	1 x 25 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) - DANGER! 	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® BSRV-Ab ELISA kit: Article number SV-104888

Bovine Respiratory Syncytial Virus
Antibody Test

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

General information
Bovine respiratory syncytial virus (BRSV), a pneumovirus, has a counterpart in the human RSV, affecting the respiratory tract of mainly infants. Also, a related caprine RSV is known[1]. The virus name derives from large syncytial masses formed in infected cell cultures. Bovine RSV is associated with upper and lower respiratory tract diseases primarily affecting calves less than 12 months old and in milking cows. The initial symptoms of the disease include pyrexia, coughing, ocular, and nasal discharge. The occurrence of anorexia, trachypnoea, and dyspnoea indicates a more serious, advanced stage of the disease which may lead to fatal pneumonia. RSV is transmitted horizontally by direct contact with respiratory secretions (aerosol infection). Infection is facilitated by crowding during the milking process and when animals are housed during the winter months[2,3]. Newly acquired calves should be isolated and monitored for the presence of infection to prevent contamination of uninfected herds. The antibody response to RSV infection is well-documented and facilitates a rapid diagnosis[4].

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

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.

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

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

   Serum Samples

A. Add 100 µL of Sample Dilution 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 BRSV viral antigen and to corresponding wells coated with control 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 BRSV viral antigen and to 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.

   Continue at step #3.

   Milk Samples

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

B. Add 100 µL of skim milk sample to a selected well coated with BRSV viral antigen and to 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.

   Continue at step #3.

3. Shake the plate thoroughly. Seal the plate/strips and incubate at 37°C (98.6°F) for 1 hour.
4. Rinse the plate/strips 3 times with PBS-Tween Buffer. At each rinse cycle fill up the wells, empty the plate and tap hard to remove all remains of fluid.
5. Add 100 µL of HRP Conjugate to each well. Seal the plate/strips 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 wells coated with BRSV viral antigen are corrected by subtracting the OD values of the corresponding wells containing the control antigen.

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

   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:

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

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. Trudel, M. *et al*. (1989). Comparision of caprine, human and bovine respiratory syncytial virus. Arch. Virol. 107, 141-149.
2. Verhoeff, J., Van der Ban, M., and van Nieuwstadt, A.P.K.M.I. (1984). Bovine respiratory syncytial virus infections in young dairy cattle: clinical and haematological findings. Vet. Rec. 114, 9-12.
3. Kahrs, R.F. (1981). Respiratory syncytial virus. In Viral Diseases of Cattle. Edited by Robert F. Kahrs. The Iowa State University Press. Ames, Iowa, pp. 215-220.
4. Westenbrink, F. *et al*. (1989). Analysis of the antibody response to bovine respiratory syncytial virus proteins in calves. J. Gen. Virol. 70, 591-601.
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.
6. Beaudeau F. *et al*. (2010). Spatial patterns of bovine respiratory syncytial virus in the Swedish beef cattle population. Acta Vet Scand. May 21;52:33.

***DANGER: 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

INDICAL BIOSCIENCE GmbH
Deutscher Platz 5b
04103 Leipzig
Germany
www.indical.com

Customer Service
support@indical.com

Manual version: HB-2621-001
December 2023