Bovine Corona Virus (BCV-Ab)
SVANOVIR®
ELISA test for the detection of BCV antibodies in serum and milk

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. The virus is also associated with respiratory tract disease. Besides, BCV has also been identified as the agent of ‘winter dysentery’ – an illness in adult cattle, accompanied by diarrhoea and reduced milk yields. 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.

Principle
The SVANOVIR® Bovine Coronavirus-Ab ELISA Kit is designed to detect BCV 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 noninfectious 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.

Contents
- BCV viral and control antigen coated microtitre plates, odd columns coated with viral antigen and even columns with control antigen.
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 temperature 18 to 25°C (64 to 77°F) before use.
3. Handle all materials according to the Good Laboratory Practice.
4. Do not mix components or instruction booklets from different test kit batches.
5. Care should be taken to prevent contamination of kit components.
6. Do not use test kit beyond date of expire.
7. Do not eat, drink, or smoke where specimens or kit reagents are handled.
8. Do not pipet by mouth.
9. Include positive and negative serum and/or milk controls on each plate or test strip series.
10. 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.

Calculations
The optical density (OD) values in wells coated with BCV are corrected by subtracting the OD values of the corresponding wells containing the control antigen, (OD\text{cont}). All control and samples OD values should be corrected before results are interpreted.

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\text{OD}_{\text{BCV}} - \text{OD}_{\text{CONTROL}} = \text{OD}_{\text{corr}}
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Interpretation of the results
Criteria for test validity
To ensure validity the Positive Control Serum/Milk should have an OD\text{corr} greater than 0.5 and the Negative Control Serum/Milk should have an OD\text{corr} greater than 0.05%.