INTERPRETATION

Calculation of the Inhibition Percentage

Calculate the mean of ODs obtained for the negative control (mOD_{neg}). Divide the OD obtained for each sample by the mOD_{neg}. Then, subtract this result from 1 and multiply by 100 to obtain the inhibition percentage (%).

$$\text{Inhibition percentage (\%)} = \left[1 - \left(\frac{OD_{sample}}{mOD_{neg}}\right)\right] \text{ X } 100$$

Validity Criteria

The following criteria must be met in order to validate the test:

- Mean OD value of the positive control has to be lower than 0.250.
- Mean OD value of the negative control has to be higher than 0.700.

Interpretation

- Sample with inhibition percentage greater or equal to 50% is considered BoHV-1-Ab positive.
- Sample with inhibition percentage greater or equal to 40 % and less than 50 % is considered as BoHV-1-Ab suspicious. A second serum sample should be collected 2 weeks later and retested.
- Sample with inhibition percentage less than 40 % is considered BoHV-1-Ab negative.

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Bovine Herpes Virus 1 Antibody Test Kit, ELISA
Bovichek® BoHV-1 gB
Insert

2013-03-11

This kit is based on an immunoenzymatic assay for the detection of antibodies against bovine herpes virus 1 (BoHV-1) in bovine serum using BoHV-1-gB specific monoclonal antibodies.

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease caused by BoHV-1. The virus which affects domestic and wild cattle is present worldwide but has been eradicated from several countries. The disease is characterized by clinical signs of the upper respiratory tract (rhinitis, conjunctivitis, tracheitis) accompanied by signs of general illness (fever, depression, inappetence, abortion). Moreover the virus can also infect the genital tract and cause pustular vulvovaginitis and balanoposthitis. Mortality is usually low and numerous infections are subclinical. Following infection the virus persists in a latent state for the entire life of the animals. It can be occasionally reactivated eventually resulting in virus shedding without clinical signs.

During the acute phase of infection the virus can be detected by virus isolation or polymerase chain reaction (PCR) from respiratory or genital samples. In the convalescent phase of infection, specific serum antibodies can be detected by techniques such as the virus neutralization test (VNT) or an enzyme immunosorbent assay (ELISA). The most efficient ELISA are pretty well correlated with the VNT but are more convenient than the VNT for large scale testing.

1. Anonymous. Infectious Bovine Rhinotracheitis/ Infectious Pustular Vulvovaginitis. OIE Terrestrial Manual 2010. www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.13_IBR_IPV.pdf
2. Kramps JA, et al. A simple, specific, and highly sensitive blocking enzyme-linked immunosorbent assay for detection of antibodies to bovine herpesvirus 1. J Clin Microbiol. 1994;32(9):2175-81.
3. Kramps JA, et al. Evaluation of tests for antibodies against bovine herpesvirus 1 performed in national reference laboratories in Europe. Vet Microbiol. 2004;102(3-4):169-81.

PRINCIPLE OF THE TEST

This kit is a blocking enzymatic immunoassay (blocking ELISA) based on the use of monoclonal antibodies against BoHV-1 viral antigens (Ag). It allows the detection of antibodies against BoHV-1 in bovine serum. Serum samples and controls are incubated in wells coated with BoHV-1 antigens. After several washes to eliminate unbound substances, a BoHV-1-gB specific monoclonal antibody (Mab) coupled to an enzyme (conjugate) is added. The Mab bind to the antigenic sites that have not been bound by the serum antibodies present in positive samples. After incubation, the excess of Mab is eliminated by a second wash and their attachment is revealed with a chromogenous substrate. Following this 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 inversely proportional to the quantity of Ab in serum samples.

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MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with BoHV-1 antigens	2
• Ready-to-use positive control	2.5 mL
• Ready-to-use negative control	2.5 mL
• Ready-to-use sample dilution buffer	30 mL
• Concentrated conjugate*	75 µL
• Ready-to-use conjugate dilution buffer	40 mL
• Concentrated wash solution (10X)**	2 X 100 mL
• Ready-to-use substrate	25 mL
• Ready-to-use stop solution	25 mL

*** Concentrated conjugate must be stored at -20°C in a manual-defrost freezer.**

** 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.

Material Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes (1 to 300 µL)
- Single-use micropipette tips
- Titer tubes or dilution plate for samples dilution.
- ELISA microplate washer (optional)
- Containers for preparation of solutions (from 0.5 to 250 mL)
- ELISA 96-well microplate reader equipped with 450 nm filter

PRECAUTIONS

- For *in vitro* veterinary use only.
- Store all kit components at 2-7°C except the concentrated conjugate.
- STORE THE CONCENTRATED CONJUGATE AT -20°C UPON ARRIVAL.
- Bring all components to room temperature before use except conjugate.
- Do not use the kit after the expiry date indicated on the package.
- Do not intermix instructions or components from different serials.
- 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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EXECUTION

A. Preparation of Wash Solution

After homogenizing the concentrated wash solution (no evidence of crystals), dilute at 1/10 with purified water (e.g., 100 mL of 10X concentrated wash solution in 900 mL purified water for each plate). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

B. 2-Step Conjugate Preparation

1. Pre-dilute the concentrated conjugate stored at -20°C at 1/100: 3µL in 297 µL of conjugate dilution buffer.
2. Dilute the pre-diluted conjugate obtained in 1. according to the dilution indicated on the Final Control Sheet. Example: if the FCS indicates a final dilution of 1/75, use 13 µL of the pre-diluted conjugate in 987 µL of conjugate dilution buffer for 1 strip of 8 wells.

Dilute conjugate a few minutes prior to its use and always prepare a fresh solution of both the pre-dilution and the final dilution.

C. Sample Preparation

Dilute bovine serum samples at 1/2 in the sample dilution buffer (e.g., 100 µL sample in 100 µL sample dilution buffer). Make sure to use a new tip for each sample. Also make sure that each solution is properly mixed before being distributed into the wells.

D. Test Procedure

Bring all reagents to room temperature and mix well manually before use. Once used, reagents have to be stored back at appropriate temperature as soon as possible.

1. Make a schematic representation of the distribution of samples and controls on the plate.
2. Dispense 100 µL of ready-to-use positive control, ready-to-use negative control or diluted samples (see section C) into appropriate wells (it is recommended to run control sera in duplicates). Cover with a plastic film or parafilm to decrease evaporation.
3. Incubate at 37 ± 1°C for 1 hour.
4. Wash each well 5 times with 300 µL of 1X wash solution (see section A). Throw away all liquid contained in the plate 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.
5. Dispense 100 µL of diluted conjugate (see section B) into each well. Cover with a plastic film or parafilm to decrease evaporation.
6. Incubate at 37 ± 1°C for 1 hour.
7. Wash each well 5 times following the procedure described at step 4.
8. Dispense 100 µL of ready-to-use substrate solution into each well.
9. Keep the plate for 15 minutes at 23 ± 2°C in the dark.
10. Dispense 100 µL of ready-to use stop solution into each well following the same order in which the substrate was added.
11. Measure optical densities (OD) at 450 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
12. Calculate the results.