Bio-X Diagnostics

BOHV-4 ELISA KIT

ELISA kit for serodiagnosis of Bovine Herpes Virus 4
Indirect test for blood sera and milk

Diagnostic test for cattle

I - INTRODUCTION

The BoHV-4 (Bovine herpesvirus-4) is considered as a probable pathogen of cattle. This virus was isolated from animals showing various clinical signs. It is often associated with pathologies affecting the genital tract (orchitis or metritis). It has also been isolated from cattle suffering from ocular, respiratory, or digestive disorders or even skin lesions. Its presence was also demonstrated in animals apparently healthy.
In order to diagnose an infection caused by BoHV-4, it is necessary to subject coupled blood samples to an ELISA test. In the event of a clear seroconversion between the sample collected during the acute phase of infection and that collected during the convalescent phase (i.e. 2 to 3 weeks after the first sample), one can conclude to a contamination by BoHV-4. It is also possible to diagnose an infection caused by BoHV-4 by isolating the virus on a susceptible cell line. The growth of the virus in this cell line can be shown by the use of a specific antibody coupled to a flurochrome.

II – PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by purified BoHV-4. The plate's odd columns (1, 3, 5, 7, 9 and 11) contain the virus, whereas the even columns (2, 4, 6, 8, 10 and 12) contain a control antigen.
The test blood sera are diluted in the dilution buffer. The milks samples are used undiluted. The plate is incubated and washed, then the conjugate, protein G peroxidase-labelled, is added to the wells. The plate is incubated a second time at 21°C +/- 3°C. After the second incubation, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific BoHV-4 immunoglobulins are present in the test sera or milk the conjugate remains bound to the microwell that contains the viral antigen and the enzyme catalyses the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitised by the viral antigen.
It is possible to quantify the reactivity of an unknown serum on a scale ranging from 0 to +++++.

III - COMPOSITION OF THE KIT

- **Microplates**: Two 96-well microtitration plates (6 strips of 16 wells). The odd columns (1, 3, 5, 7, 9 and 11) are sensitised by BoHV-4 antigen and the even columns (2, 4, 6, 8, 10 and 12) by the control.
- **Washing solution**: One 100-ml bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until all crystals have disappeared. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.

- **Dilution buffer**: One 30-ml bottle of 5x coloured and concentrated buffer for diluting the blood sera. The bottle's content is to be diluted with distilled or demineralised water. If a deposit forms at the bottom of the receptacle filter the solution on Whatman filter paper.
- **Conjugate**: 1 bottle of anti-bovine immunoglobulin-peroxidase conjugate (Protein G horseradish peroxidase-labelled). **The reagent is ready to use.**
- **Positive serum**: One bottle of positive serum. Reconstitute this serum with 0.5 ml of distilled or demineralised water. The reconstituted serum may be kept at -20°C. Divide the reconstituted serum into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Negative serum**: 1 bottle containing the negative serum. Reconstitute this serum with 0.5 ml distilled or demineralised water. The reconstituted serum must be kept at -20°C. Divide this reagent into several portions before freezing it to avoid repeated freeze-thaw cycles. If these precautions are taken, the reagent may be kept for several months.
- **Single component TMB** One 25-ml bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. **This solution is ready to use.**
- **Stop solution**: One 15-ml bottle of the 1 M phosphoric acid stop solution.

	BIO K 263/2
Microplates	2
Washing solution	1 X 100 ml (20 X)
Dilution buffer	1 X 30 ml (5 X)
Conjugate	1 X 25 ml (1 X)
Positive serum	1 X 0,5 ml (1 X) freeze-dried
Negative serum	1 X 0,5 ml (1 X) freeze-dried
Single component TMB	1 X 25 ml (1 X)
Stop solution	1 X 15 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

V - PRECAUTIONS FOR USE

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

1- Bring all the reagents at 21°C +/- 3°C. Remove the microplate from its wrapper.

2- For sera, place 1-ml aliquots of the dilution buffer, prepared as instructed in the "Composition of the Kit" section, in 5- or 10-ml hemolysis tubes. Add 10 µl of the serum samples to each of these tubes (dilution 1/100) and shake briefly on a mechanical agitator.
3- Prepare the milk samples as follows: Centrifuge 20 minutes at 4000 g. Using a glass Pasteur pipette, cross through the upper layer of cream and take up the intermediate liquid layer, taking care not to touch the underlying cell sediment. Use undiluted skimmed milk samples in the wells.
4- Dilute positive and negative sera 1/100 in dilution buffer (see point 2).
5- Distribute samples (sera and/or milks) and the positive and negative sera (100 µl/well) as follows: positive serum in wells A1 and A2, negative serum in wells B1 and B2, sample 1 in wells C1 and C2, sample 2 in wells D1 and D2 etc... Incubate the plate at 21°C +/- 3°C for one hour. Use a lid.
6- Rinse the plate with the washing solution, prepared as instructed in the "Composition of the Kit" section, as follows: empty the microplate of its contents by flipping it over sharply above a sink. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill the used wells with the washing solution using a squeeze bottle or by plunging the plate in a vessel of the right dimensions, then empty the wells once more by turning the plate over above a sink. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times go on to the next step. An automatic plate washer may also be used, but in this case take care that the needles do not get too close to the bottoms of the wells to prevent damaging the reagent layer.
7- Add 100 µl of the conjugate solution to each well. Incubate for 1 hour at 21°C +/ -3°C. Use a lid.
8- Wash the plate as described in step 6 above.
9- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.
10- Incubate for 10 minutes at 21°C +/- 3°C protected from the light and uncovered. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
11- Add 50 µl of stop solution to each microwell.
12- Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may cristallize in wells with strong signals and thereby distort the data.

VII – INTERPRETING THE RESULTS

Subtract from each value recorded for the odd columns the signal of the corresponding negative control well and write down the result. In performing this calculation, allow for any negative values that may exist. Carry out the same operations for the column corresponding to the positive and negative controls
The test can be **validated** only if the positive serum yields a difference in optical density at 10 minutes that is greater than the value given in the QC data sheet: **(validation: ...).**

Divide the signal read for each sample well by the corresponding positive control serum signal and multiply this result by 100 to express it as a percentage.

$$\text{Val} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the first table in the quality control procedure, determine each serum's or milk's degree of positivity.

A reliable diagnosis can be made only if frank seroconversion can be documented using two coupled serum samples taken at 2- to 3-week intervals. The first sample must be taken during the acute phase of the infection. A frank seroconversion is considered to have occurred if the signal increases by two orders of magnitude (two plusses; for example, ++ -> ++++ or + -> +++). A sample must be considered positive if it yields a result that is **greater than or equal to one plus sign (+).**

VIII – ORDERING INFORMATION

BIO-X BoHV-4 ELISA KIT:	2x48 tests	BIO K 263/2