BoHV-4

ELISA kit for serodiagnosis of Bovine herpes virus type 4 (BoHV-4)
Indirect test for blood sera, plasma and milk
Diagnostic test for cattle
Monowell

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.
It is 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 entire surface of each microplate has been sensitised with purified BoHV-4.
The test blood sera and plasma are diluted in the dilution buffer. The milks samples are used undiluted. Sample are added to the plate which is then incubated and washed. 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 advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific BoHV-4 immunoglobulins are present in the test sera, plasma 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.

III - COMPOSITION OF THE KIT

- **Microplates**: 96-well microtitration plates (strips of 8 wells). The entire surface of each microplate has been sensitised BoHV-4.
- **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 50-ml bottle of 5x coloured and concentrated buffer for diluting the blood sera, plasma and the conjugate. 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**: Bottle of Protein G horseradish peroxidase-labelled.
- **Positive reference**: One bottle of positive serum. Store this reagent between +2°C and +8°C.
- **Negative reference**: One bottle of negative serum. Store this reagent between +2°C and +8°C.
- **Tracer:** One bottle of tracer. Reconstitute this reagent with 0.5 ml distilled or demineralised water. Once reconstituted, the reagent is stored at -20°C. Divide this reagent up into several portions before freezing it to avoid repeated freeze/thaw cycles. If these precautions are taken, the reagent can be kept for several months. The tracer is a reference sample that can be used to check the intra-laboratory reproducibility of the kit's batch. **Intra-laboratory reproducibility**: Degree of agreement between the results of reiterated tests on the same sample with an identical technical protocol in a given laboratory under variable working conditions.
- **Single component TMB** One 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 bottle of the 1 M phosphoric acid stop solution.

	BIO K 312/1	BIO K 312/2
Microplates	1	2
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Dilution buffer	1 X 50 ml (5 X)	1 X 50 ml (5 X)
Conjugate	1 X 0,3 ml (50 X)	1 X 0,5 ml (50 X)
Positive serum	1 X 0,5 ml (1 X)	1 X 0,5 ml (1 X)
Negative serum	1 X 0,5 ml (1 X)	1 X 0,5 ml (1 X)
Tracer	1 X 0,5 ml (freeze-dried)	1 X 0,5 ml (freeze-dried)
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stop solution	1 X 6 ml (1 X)	1 X 15 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, microplates for dilution, 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 components to 21°C +/- 3°C before use. Remove the microplate from its wrapper.

2- DILUTION OF SAMPLES

2.1- Blood sera and plasma preparation
The blood serum and plasma samples must be diluted 1:100. Avoid using haemolysed samples or those containing coagula.

2.1.1- Dilution in tubes
Distribute 990 µl aliquots of dilution buffer, prepared as instructed in the section "Composition of the Kit", to 5 or 10 ml tubes. Add 10 µl aliquots of the samples to each of these tubes and mix briefly on a mechanical stirrer (final dilution: 1:100).

2.1.2- Dilution on a microplate
Distribute 20 µl aliquots of each of the samples to the microwells of a dilution plate. Add 180 µl of dilution buffer. Mix five times by pumping and surging or orbital agitation (dilution: 1:10). Distribute 90 µl aliquots of dilution buffer to the wells of the kit's microplate. Transfer 10 µl of the 1:10 prediluted samples. Mix five times by pumping and surging or orbital agitation (final dilution: 1:100).

2.2- Dilution of the kit's reference sera (positive and negative controls) and tracer
The positive and negative sera and the tracer must be diluted 1:100 in dilution buffer. Do these dilutions in one step in a tube (see Point 2.1.1.) or in two steps on a dilution microplate (see Point 2.1.2.).

2.3-Milk preparation
Centrifuge at 4000 g for 20 minutes. Take up the middle layer of liquid by means of a glass Pasteur pipette inserted through the upper layer of cream, taking care not to touch the underlying cell sediment.
Use undiluted skimmed milk samples in the wells.

3- Distribute the samples (blood serum, plasma, or milk) at the rate of 100 µl per well. One well per sample. For example, the following pattern may be followed: Positive serum in well A1, Negative serum in well A2, tracer in well A3, Sample 1 in well A4, and so on. Cover the plate with a lid and incubate the plate at 21°± 3°C for one hour.

4- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, eliminate the microplate's contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation two more times, taking particular care to avoid bubble formation in the wells. After these three rinses, go on to the next step.
Using a plate washer (whether automatic or manual) is also recommended. However, the depth of the needles' immersion must be set so as not to disturb the layer of reagents adsorbed to the bottom of each well.

5- Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.250 ml of diluent). Add 100 µl of the diluted conjugate solution to each well. Cover the plate with a lid and incubate for 1 hour at 21°C +/- 3°C.

6- Wash the plate as described in step 4 above.

7- 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.

8- 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.

9- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.

10- Read the optical densities in the microwells using a plate reader and a 450 nm filtre. Results must be read fairly soon after the stopping solution has been added since the chromogen may crystallize in wells with strong signals and thereby distort the data.

VII – INTERPRETING THE RESULTS

The test can be **validated** only if the difference between the optical density readings of the positive control serum and negative control serum (OD positive serum - OD negative serum) at ten minutes is greater than 0,700 and the negative serum yields an optical density that is lower than 0,300.

Calculate each serum's coefficient by means of the following formula:

$$\text{Sample's Coeff.} = \frac{\text{OD sample - OD negative serum}}{\text{OD positive serum - OD negative serum}} \times 100$$

Using the following table, determine each serum's, plasma's or milk's degree of positivity.

0		+		++		+++		++++		+++++
Val <=	30 %	< Val <=	60 %	< Val <=	90 %	< Val <=	120 %	< Val <=	150 %	< Val

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

Monoscreen AbELISA BoHV-4:	1x96 tests	BIO K 312/1
	2x96 tests	BIO K 312/2