Bovine respiratory

ELISA kit for antigenic diagnosis of
BoHV-1, BVDV, BRSV and BPI3
Sandwich test for tissue lysates
Diagnostic test for cattle

I - INTRODUCTION

Respiratory disorders are of major concern for bovidae, given the frequency of such infections and the high number of animals affected. These infections occur in all countries that practice intensive livestock farming in which large numbers of animals are confined to small areas. Treatment and diagnosis are both complicated due to the multifactorial character of this diseases etiology. Viruses and bacteria combined with stress due either to transport in overcrowded vans or dirty or poorly-ventilated stabling, for instance, play a key role in triggering acute respiratory infections. These infections are particularly common among young animals, although they also affect adult animals.
In most cases the animals that show signs of respiratory ailments harbour several pathogens, some of which may act synergistically. So, it is generally recognised that viruses are the first pathogens to intervene, whereas bacteria act as second invaders to worsen the animal's condition. Shipping fever is a good example of the synergism that can exist between a virus (PI3) and a bacterium, such as *Manheimia haemolytica*, in the respiratory tract.
The respiratory kit can be used to obtain a diagnosis from a minced lung tissue sample taken from a corpse.

II - PRINCIPLE OF THE TEST

Specific antibodies produced against pathogens responsible of respiratory diseases have been immobilized on alternate rows of 12 x 8-well microtitre plates. These antibodies allow the specific capture of the corresponding pathogens in the samples. Rows A, C, E, G have been sensitised with these antibodies and rows B, D, F, H contain non-specific antibodies. These rows allow the differentiation between specific immunological reactions and non-specific binding so as to eliminate false positives.
The samples are diluted in lysis solution and incubated on the microplate for 1 hour at 21°C +/- 3°C.
After this first incubation step, the plate is washed and incubated for 1 hour with conjugates, peroxidase labelled anti-virus specific monoclonal antibodies. After this 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 pathogens are present in the tested samples, conjugates remain bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titres of the specific pathogens in the sample. The enzymatic reaction can be stopped by acidification and the resulting optical density at 450 nm can be recorded

using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells optical densities.
Positive control is provided with the kit so as to validate the test results.

III – COMPOSITION OF THE KIT

- **Microplates**: 96-well microtitration plates. The rows A, C, E, G are sensitised by specific antibodies, the rows B, D, F, H by non specific antibodies.
  Row A: anti-BoHV-1 Row B: control
  Row C: anti-BVDV Row D: control
  Row E: anti-BRSV Row F: control
  Row G: anti-Parainfluenza 3 virus Row H: control
- **Washing solution**: 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 disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water. Store the diluted solution between +2°C and +8°C.
- **Lysis buffer**: bottle of 5x concentrated buffer. Dilute this concentrated solution 1:5 with distilled or demineralised water. Store the diluted solution between +2°C and +8°C.
- **Conjugates**: vials of coloured conjugate. These solutions are ready to use.
  BoHV-1 (red), BVDV (yellow), BRSV (blue) and Parainfluenza 3 (green).
  Specificity of conjugates is indicated on vials.
- **Positive Control:** The reagent is ready to use.
- **Single component TMB:** bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use.
- **Stopping solution:** bottle of the 1 M phosphoric acid stop solution.

	BIO K 340/2	BIO K 340/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Lysis buffer	1 X 50 ml (5 X)	1 X 50 ml (5 X)
Conjugates	4 X 6 ml (1 X)	4 X 15 ml (1 X)
Positive control	1 X 4 ml (1 X)	1 X 10 ml (1 X)
Single component TMB	1 X 25 ml (1 X)	1 X 55 ml (1 X)
Stopping solution	1 X 15 ml (1 X)	1 X 30 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 lysis 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

A. SAMPLE PREPARATION.

1. **Homogenised tissue**. Collect an approximately 1 gram sample of lung tissue. Take this sample from areas in the apical lobes that show gross lesions. Place the lung tissue fragment in a Petri dish with 2 ml of lysis solution and snip it into tiny pieces with a pair of scissors. Homogenize, transfer the dish's contents to a test tube, and centrifuge at 500 g for 10 minutes to separate out the insoluble fragments on the bottom of the tube. Collect the supernatant for the ELISA test.
2. **Cell culture.** The Respiratory kit may be used to test for viral growth in likely host cells (primary lines, VERO, MDBK and HEP2). In this case you may deposit the culture medium directly on the microplate.

B. ELISA.

1- All components must be brought to 21°C +/- 3°C before use.
2- Remove the microplate from its wrapper.
3- Add 100-µl aliquots of the diluted samples to the wells as follows: sample 1 in wells of column 2, sample 2 in wells of column 3, etc. Do not forget positive control: 8 wells of column 1 (100 µl/well)
4- Cover with a lid and incubate the plate at 21°± 3°C for one hour.
5- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, dispose of 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 twice, taking special care to avoid the formation of bubbles in the wells. Upon completing these three washes, go on to the next step.
6- Add 100 µl of the conjugate solutions per well. Cover with a lid and incubate the plate at 21°± 3°C for one hour.

Anti - BoHV-1 conjugate (red)	rows A and B
Anti - BVDV conjugate (yellow)	rows C and D
Anti - BRSV conjugate (blue)	rows E and F
Anti - PI3 conjugate (green)	rows G and H

7- Wash the plate as described in Step 5.
8- 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. Incubate at 21°C +/- 3°C and away from light for 10 minutes. Do not cover. This time interval 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 to each well. The blue colour will change into a yellow colour.
10- Read the optical densities by means of a microplate spectrophotometer with a 450 nm filter. The results must be read as quickly as possible after the stop solution has been applied, for in the case of a strong signal the chromogen can crystallise and lead to incorrect measurements

VII – INTERPRETING THE RESULTS

Subtract from each reading in the odd rows (A, C, E, and G) the signal of the corresponding negative control well (B, D, F, and H) and write down the result (calculation of the difference in optical density, delta OD). To calculate this value allow for the possible existence of negative values. Proceed in the same way for the positive controls. The test can be validated only if the positive references give 10 minutes optical density differences that are greater than the values for the quality control appended to the package insert.

Divide each resulting value by the corresponding value obtained for the corresponding positive control and multiply this result by 100 to express it as a percentage.

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

Using the first table in the quality control procedure, determine each sample's status (positive, negative).

VIII – ORDERING INFORMATION

Multiscreen AgELISA Bovine respiratory:	2 X 12 samples	BIO K 340/2
	5 X 12 samples	BIO K 340/5