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RESPIRATORY ELISA KIT
PENTAKIT (IBRPA)

ELISA kit for serodiagnosis of the main bovine respiratory infections

Indirect test for blood sera

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 *Mannheimia haemolytica*, in the respiratory tract.

The BIO-X RESPIRATORY ELISA kit consequently enables one to evaluate the humoral immune response of cattle to five pathogens commonly implicated in bovine respiratory infections. These are the BHV-1 virus causing infectious bovine rhinotracheitis (IBR), bovine virus diarrhoea virus (BVDV), which is also responsible for mucosal disease, bovine respiratory syncytial virus (BRSV), parainfluenza 3 virus (PI3) and adenovirus type 3.

II – PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by monoclonal antibodies specific to the five pathogens listed above. These antibodies are used to trap the pathogens as well as to purify them from lysates of the cells in which the viruses were grown . The distribution of these pathogens on the microtitration plate is as follows:

Columns 1 & 7: BoHV-1
Columns 2 & 8: BVDV
Columns 3 & 9: BRSV
Columns 4 & 10: PI3
Columns 5 & 11: Adenovirus 3
Columns 6 & 12: negative control antigens for the viruses

Columns 6 & 12 contain a lysate of the bovine kidney cell line that was used as a substrate to propagate the viruses. We thus have a genuine negative control to differentiate the virus-specific antibodies from those directed

against the antigenic determinants of the kidney cells used for their replication. Using such a control reduces the number of false positives considerably. The test sera are diluted 1:100 in an appropriate buffer and incubated on the plate for one hour at 21°C +/- 3°C. The plate is washed and the conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, is added to the wells. The plate is reincubated at 21°C +/- 3°C for 1 hour. After this second incubation, the preparation is washed 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 immunoglobulins are present in the test sera the conjugate remains bound to the corresponding microwell 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 titre of specific antibody in the sample. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells. . It is possible to quantify the reactivity of an unknown serum on a scale ranging from 0 to +++++.

III - COMPOSITION OF THE KIT

- Microplates: 96-well microtitration plates. The distribution of the different valencies is indicated on the aluminium wrapper.
- Washing solution: One 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.
- Dilution buffer: One bottle of 5x concentrated buffer for diluting the blood sera and conjugate. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- Conjugate: One bottle of anti-bovine immunoglobulin-peroxidase conjugate (horseradish peroxidase-labelled anti-bovine IgG1 monoclonal antibody).
- 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.
- Single component TMB: One bottle of the chromogen tetramethylbenzidine (TMB).
  Store between +2°C and +8°C protected from the light.
- Stopping solution: One bottle of the 1 M phosphoric acid stop solution.

	BIO K 028/2	BIO K 028/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Dilution buffer	1 X 50 ml (5 X)	1 X 100 ml (5 X)
Conjugate	1 X 0,5 ml (50 X)	1 X 1,4 ml (50 X)
Positive serum	1 X 0,5 ml (1 X) freeze-dried	1 X 0,5 ml (1 X) freeze-dried
Monocomponent TMB	1 X 25 ml (1 X)	1 X 55 ml (1 X)
Stop 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 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- 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- Dilute positive serum 1/100 in dilution buffer (see point 2).
4- Distribute samples and the positive serum (100 µl/well) as follows: positive serum in wells H1 to H6, sample 1 in wells A1 to A6, sample 2 in wells B1 to B6 etc... Incubate the plate at 21°C +/- 3°C for one hour. Use a lid.
5- 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.
6- Dilute the conjugate 1:50 in the buffer for dilution (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.25 ml of diluent). Add 100 µl of the dilute conjugate solution to each well. Incubate for 1 hour at 21°C +/- 3°C. Use a lid.
7- Wash the plate as described in step 5 above.
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.
9- 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.
10- Add 50 µl of stop solution to each microwell.
11- 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 in columns 1, 2, 3, 4, 5 the signal of the corresponding negative control well 6 and write down the result (calculation of Î OD. for the viral valencies). Allow for any negative values that may exist in performing this calculation. Carry out the same operations for the row corresponding to the positive control (wells H1 to H6).
The test can be validated only if the positive serum yields a difference in optical density at 10 minutes that is greater for each valence than the values given on the QC data sheet:

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 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 (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 RESPIRATORY ELISA KIT (IBRPA):	2 X 48 tests	BIO K 028/2
	5 X 48 tests	BIO K 028/5

Li StarFish distribuisce:
Jackson Immuno Research Laboratories, Inc.
Acris
WAK - CHEMIE MEDICAL GMBH