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MYCOPLASMA BOVIS ELISA KIT

ELISA kit for serodiagnosis of *Mycoplasma bovis*
Indirect test for blood sera and milk
Diagnostic test for cattle

I - INTRODUCTION

Mycoplasma bovis is associated with many cattle diseases, including arthritis, pneumonia in calves and young stock, mastitis, and genital infections. The infectious pneumonias that affect intensively-raised calves are responsible for sizable economic losses due to the mortality, treatment costs, and growth delays that they cause. These respiratory infections often involve multiple factors and are caused by interactions among viruses, mycoplasmas, and bacteria. Several species of Mycoplasma have been isolated from the respiratory tracts of calves. Some of them are most probably simple commensals or opportunistic species that merely worsen the lung damage caused by other agents. *Mycoplasma bovis* has been isolated from the lungs of calves with pneumonia. It is probably the most pathogenic species affecting the Bovidae after *Mycoplasma mycoides* subsp *mycoides. Mycoplasma bovis* can induce the development of pneumonia in gnotobiotic calves. *Mycoplasma bovis* is frequently found in association with *Mannheimia haemolytica* in pneumonia in calves.

II – PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by a recombinant protein from *Mycoplasma bovis* expressed by *E. coli*. A gene from *Mycoplasma bovis* is expressed by this recombinant *E.coli* culture. The entire surface of each microplate has been sensitised with recombinant from *Mycoplasma bovis*.
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 ant 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 *Mycoplasma bovis* immunoglobulins are present in the test sera or milk the conjugate remains bound to the microwell that contains the bacterial recombinant 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 (12 strips of 8 wells). The entire surface of each microplate has been sensitised with recombinant from *Mycoplasma bovis*.
- **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 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 bottle of 5x concentrated buffer for diluting the blood sera and 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**: One bottle of anti-mammalian IgG peroxidase conjugate (Protein G horseradish peroxidase-labelled).
- **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**: One 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 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 302/2	BIO K 302/5
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)	2 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
Negative serum	1 X 0,5 ml (1 X) freeze-dried	1 X 0,5 ml (1 X) freeze-dried
Single component 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. before use. 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). One well per sample. 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- 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 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

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 0,400.

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

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

A sample is negative if its coefficient is less than 37%.
A sample is positive if its coefficient is greater than or equal to 37%.

VIII – ORDERING INFORMATION

BIO-X *Mycoplasma bovis* ELISA KIT:	2x96 tests	BIO K 302/2
	5x96 tests	BIO K 302/5

Li StarFish distribuisce: