



MONOSCREEN[®] Ab ELISA

Mycoplasma bovis

ELISA Kit for serodiagnosis of *Mycoplasma bovis*

Indirect test for blood sera, plasma and milk

Diagnostic test for cattle

Monowell

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 and plasma are diluted in the dilution buffer. The milk samples are used undiluted. Samples 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 anti-*Mycoplasma bovis* immunoglobulins are present in the test sera or milk the conjugate remains bound to the microwell that contains the recombinant *M. bovis* protein 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 by the recombinant from *Mycoplasma bovis* antigen.
 - **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:** Bottle of 5x colored and concentrated buffer for diluting the blood sera, plasma 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 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. Store this reagent between +2°C and +8°C.
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 302/2	BIO K 302/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Colored 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)	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 (1 X)	1 X 0,5 ml (1 X)
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, 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. 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) using 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 with a lid and incubate the plate at 21°C ± 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, 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 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 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 filter. 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 0,400.

Calculate each serum's, plasma's or milk's coefficient by means of the following formula:

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

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

Monoscreen AbELISA *Mycoplasma bovis*:

2x96 tests BIO K 302/2

5x96 tests BIO K 302/5

