MONOSCREEN Ab ELISA

Instruction manual
BIOK432-MYCB_NO_(EN)_V02
26/09/2024

Monoscreen AbELISA *Mycoplasma bovis HS*

Reference : BIO K 432

ELISA test for serodiagnosis of *Mycoplasma bovis*
Monowell, indirect test

For veterinary *in vitro* use only

Sample / dilution	Bovine
Serum – plasma* / 100X	✓
Skimmed and non-skimmed milk / 1X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 432/2	BIO K 432/5
Format	2 plates, strips of 8 wells	5 plates, strips of 8 wells
Reactions	192 tests	480 tests

Kit composition

Provided material	BIO K 432/2	BIO K 432/5
Microplates	2	5
Washing solution (20X)	1 x 100 mL	1 x 250 mL
Colored dilution solution (5X)	1 x 50 mL	2 x 100 mL
Single component TMB (1X)	1 x 25 mL	1 x 55 mL
Stopping solution (1X)	1 x 15 mL	1 x 30 mL
Conjugate (50X)	1 x 0,5 mL	1 x 1,4 mL
Positive control	1 x 0,5 mL	1 x 0,5 mL
Negative control	1 x 0,5 mL	1 x 0,5 mL
Tracer	1 x 0,5 mL	1 x 0,5 mL

Revision history

Date	Version	Modifications
26/09/2024	V02	Layout and simplification of the entire manual. Addition of milk matrix.

Note : minor typographical, grammar and formatting changes are not included in the revision history.

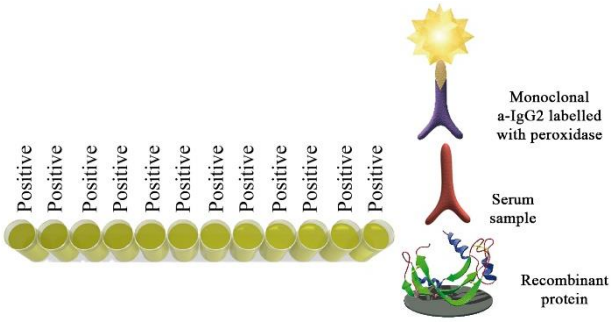
A. Introduction

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 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.

B. Test principle

The test uses 96-well microtitration plates sensitised by a recombinant protein from *Mycoplasma bovis* expressed by *E. coli*. The entire surface of each microplate has been sensitised with the recombinant protein from *Mycoplasma bovis*.

The test blood sera and plasma are diluted in the dilution solution. The milk samples are used undiluted. Samples are added to the plate which is then incubated and washed. The conjugate, anti-bovine IgG2 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* IgG2 immunoglobulins are present in the test sample, the conjugate remains bound to the microwell that contains the recombinant *Mycoplasma bovis* protein 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.



C. Material required but not provided

- Distilled/demineralized water.
- Dilution microplates.
- Graduated mono or multichannel pipettes (2-20µL, 20-200µL and 10-1000µL range) and single-use tips.
- Microplate reader (450nm filter).
- Microplate washer.
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...

D. Warnings and precautions of use

- The reagents must be kept between +2 and +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Do not use reagents from other kits.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of the solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution is to be diluted 5 times in distilled/demineralized water. The dilution solution is colored in yellow. It is used for dilution of samples, kit controls, and conjugate.
- The conjugate must be diluted 50-fold in the dilution solution.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.
- The tracer is a reference sample that can be used to check the intra-laboratory reproducibility of the kit's batch. It must be diluted 100 times in the dilution solution.

F. Preparation of the samples

- **Serum samples** and kit controls (positive and negative control, and tracer) are to be diluted **100 times** in the dilution solution, and homogenized.
  Avoid using hemolyzed or coagulated samples.

  Recommended dilution:
  *10µL of sample + 990µL of dilution solution.**

- To skim the **milk, samples** are to be centrifuged **20 min at 4000g**.
  Take up the middle layer of liquid, taking care not to touch the underlying cell sediment.
  Use undiluted milk samples in the wells.

G. Procedure

- Bring all the reagents to 21±3°C before use.
- Carefully read through the previous points.

1. Distribute the **diluted serum samples** or **undiluted milk samples** and the **diluted kit controls** at a rate of **100µL per well**.
   Cover with a lid and incubate the plate at **21±3°C** for **60±5min**.
2. Remove the content of the microplate. **Wash the microplate 3 times** with **300µL of washing solution per well.** Avoid the formation of bubbles in the wells between each wash.
3. Distribute the **diluted conjugate** at a rate of **100µL per well**. Cover with a lid and incubate at **21±3°C** for **60±5min**.
4. Remove the content of the microplate. **Wash the microplate 3 times** with **300µL of washing solution per well.** Avoid the formation of bubbles in the wells between each wash.

5. Distribute **100µL** of **TMB solution** per well. Incubate at **21±3°C** for **10±1min** away from the light, without covering.
6. Distribute the **stopping solution** at a rate of **50µL per well**. Color changes from blue to yellow.
7. Record optical densities using a plate spectrophotometer with a **450nm filter** within **5 minutes** after adding the stopping solution.

H. Validation of results

The test can only be **validated** if:

- The difference between the optical density (OD) readings of the odd and even wells of the positive control is greater than 0,700.

  Positive control: $OD_{\text{odd well}} - OD_{\text{even well}} > 0{,}700$

- The difference between the optical density (OD) readings of the odd and even wells of the negative control is less than 0,400.

  Negative control: $OD_{\text{odd well}} - OD_{\text{even well}} < 0{,}400$

I. Interpretation of results

- Calculate for each sample its coefficient by means of the following formula.

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

Use the table below to interpret the sample's results.

Sample type	Serum	Milk
Cut-off (%)	80	40

A sample is **negative** if its coefficient is less than the corresponding cut-off.
A sample is **positive** if its coefficient is greater than or equal to the corresponding cut-off.

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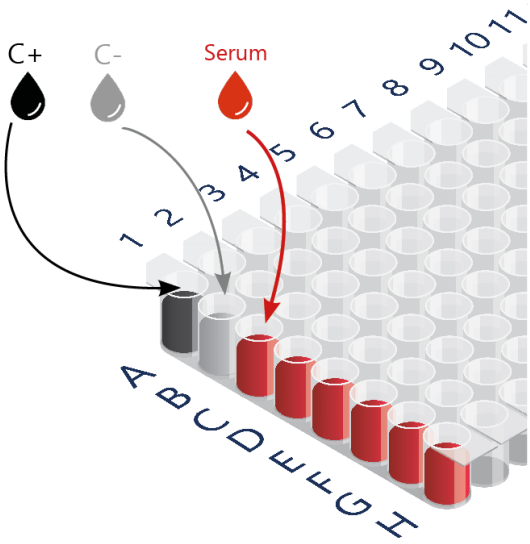
Notes*

Serum protocol

1 Dilution of samples 1/100
Dilution of the kit controls (positive and negative control and tracer) 1/100



Kit microplate

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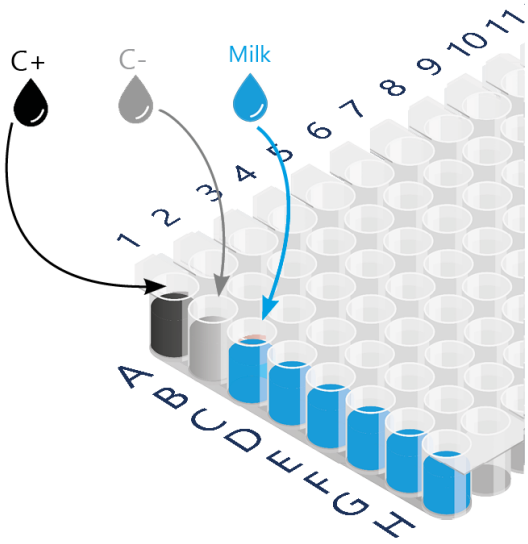
1 Distribute the centrifuged samples
Dilution of the kit controls (positive and negative control and tracer) 1/100



Kit microplate

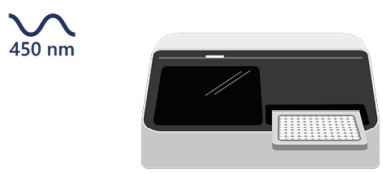
Joint protocol

2 Add 100 µL of diluted conjugate (1/50)

3 Add 100 µL of TMB

4 Add 50 µL of stopping solution

5 Record the optical densities



* Notes do not replace the instructions for use of which they are a synthesis.

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