MONOSCREEN Ab ELISA

Instruction manual
BIOK139-MANH_NO_(EN)_V04
29/06/2026

Monoscreen AbELISA *Mannheimia haemolytica*

Reference : BIO K 139

ELISA test for serodiagnosis of *Mannheimia haemolytica*

Monowell, indirect test

For veterinary *in vitro* use only

Sample / dilution	Bovine
Serum – plasma* / 100X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 139/2
Format	2 plates strips of 8 wells
Reactions	192 tests

Kit composition

Provided material		Code	Type*	BIO K 139/2
Microplate	Microplates	D01028	1	2
Washing solution (20X)	Washing solution (20X)	D00695	A	1 x 100 mL
Dilution solution (1X)	Colored dilution solution (1X)	D01511	A	3 x 125 mL
TMB solution (1X)	Single component TMB Solution (1X)	D01585	A	1 x 30 mL
Stop solution (1X)	Stopping solution (1X)	D00680	A	1 x 30 mL
Conjugate (50X)	Conjugate (50X)	D01596	1	1 x 0,6 mL
CTL POS	Positive control	D01574	a	1 x 0,5 mL
CTL NEG	Negative control	D01030	a	1 x 0,5 mL

*: (1): dependent on kit and batch / (a): dependent on kit / (A): substitutable with components A / (B): substitutable with components B.

Revision history

Date	Version	Modifications
29/06/2026	V04	Layout and simplification of the entire manual. Adjustment of component volume. Distribution of stop solution modified from 50 µL to 100 µL.

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

A. Introduction

Mannheimia haemolytica is the cause of epizootic pneumonia in cattle known as Shipping Fever or pneumonic *pasteurellosis*. 90% is caused by *Mannheimia haemolytica* Biotype A, serotype 1 but also *Pasteurella multocida*. It is usually secondary to viral infections such as parainfluenza-3 or IBR, bacterial infections such as *Mycoplasma* or environmental stress.

Mannheimia haemolytica may contribute to Enzootic pneumonia of calves; Enzootic pneumonia of lambs and peritonitis in sheep. It also causes gangrenous mastitis in sheep.

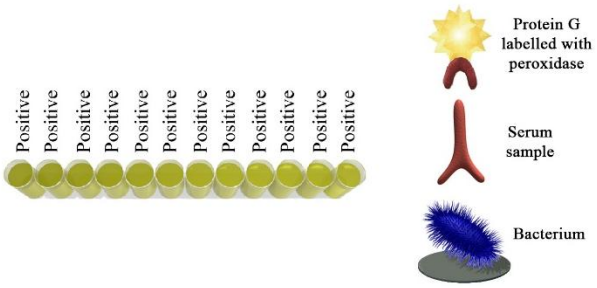
Mannheimia strains often produce a cytotoxin, known as leukotoxin, which kills leukocytes of ruminants. Leukotoxin is a member of the RTX group toxins and is probably largely responsible for the pathogenicity of the bacteria in septicemia and pneumonia.

B. Test principle

The test uses 96-well microtitration plates sensitized by purified LipoPolySaccharide (LPS) from Mannheimia haemolytica. The entire surface of each microplate has been sensitized with LPS.

The test blood sera and plasma are diluted in the dilution solution. 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 Mannheimia haemolytica immunoglobulins are present in the test sera or plasma the conjugate remains bound to the microwell that contains the bacterial recombinant antigen, and the enzyme catalyzes 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.



C. Material required but not provided

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

Additional kit

- **Tracer *Mannheimia haemolytica* (Ref.: BDE K 139):** Internal reference material for *Mannheimia haemolytica* serology by ELISA.

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.
- 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 ready to use. The dilution is colored in yellow. It is used for dilution of samples, kit controls (positive and negative control), 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.

F. Preparation of the samples

- **Serum samples** and kit controls (positive and negative control) 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.

G. Procedure

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

N.B: To avoid differences in incubation time between samples, sample dilutions and reference dilutions can be prepared in a dilution microplate before transfer (200 µL) into the test microplate using a multi-channel pipette.

1. Distribute the **diluted samples** and **diluted kit controls** at a rate of **100µL per well**. Cover 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 **100µL 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 readings of the positive control and negative control (OD positive control – OD negative control) at ten minutes is greater than 0,400 and the negative control yields an optical density that is lower than 0,300.

I. Interpretation of results

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

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

Using the following table, determine each sample's degree of positivity.

	Results	Status
Sample	Val ≤ 23%	0
	23% < Val ≤ 87%	+
	87% < Val ≤ 151%	++
	151% < Val ≤ 216%	+++
	216% < Val ≤ 280%	++++
	Val > 280%	+++++

A reliable diagnostic can be made only if frank seroconversion can be documented using two coupled serum samples taken at 2 to 3 weeks 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 (two crosses; for example, ++ → ++++ or + → +++).

A sample must be considered **positive** if it yields a result that is **greater than or equal to one plus sign**.

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ANALYSISCREEN

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- Very easy to use

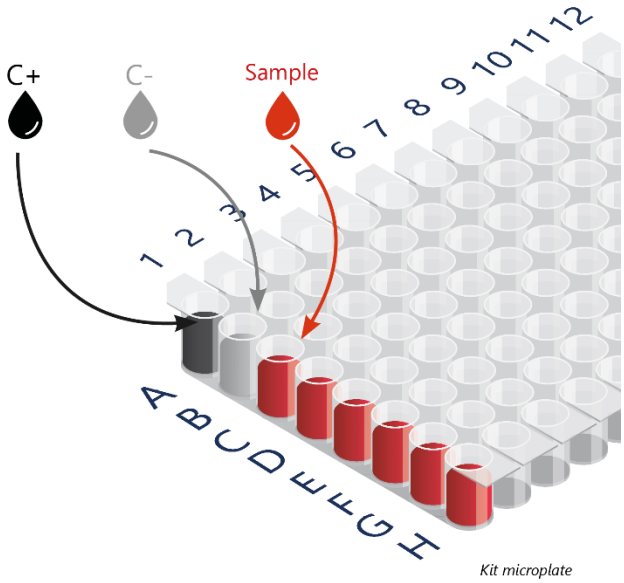
SCAN ME

Symbols

Symbol	Meaning
REF	Catalog number
	Manufacturer
	Temperature limit
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	Keep away from light
	Keep dry
	Corrosive substance
	Hazardous/irritating product

Notes*

1 Distribute 100µL of the diluted samples (1/100) and diluted controls (1/100)



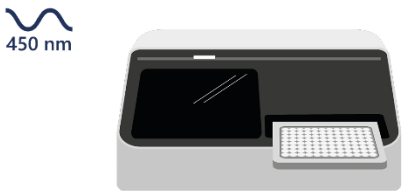
Kit microplate

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

3 Distribute 100 µL of TMB solution

4 Add 100 µL of stopping solution

5 Record optical densities



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

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