Mycoplasma bovis Antibody Test Kit (ELISA)

Bovichek® M. bovis

Product code: TRM-501 (2 plates)
TRM-502 (5 plates)

- ✓ **Highly sensitive ELISA kit**
- ✓ **A shelf life of 18 months**
- ✓ **Available in 2 different formats**
- ✓ **Approved by the Canadian Food Inspection Agency**

INTRODUCTION

Mycoplasma bovis is currently recognized as one of the most important and frequently isolated *Mycoplasma* species associated with disease in cattle worldwide. *Mycoplasma bovis* can cause several diseases in cattle including mastitis, arthritis, pneumonia, keratoconjunctivitis, otitis media and reproductive disorders. Clinical mycoplasma mastitis is often characterized by multiple affected quarters coupled with unresponsiveness to treatment. Adults and calves can also be affected by keratoconjunctivitis, arthritis and pneumonia, while otitis media is typically only observed in calves. *Mycoplasma bovis* has also been associated with reproductive disorders (vulvovaginitis, endometritis, and abortion) however these manifestations are less consistently reported.

The highly contagious nature of *Mycoplasma bovis*, their poor responsiveness to treatment and associated culling implications for affected stock make rapid and accurate diagnosis important for control and prevention of disease outbreaks. Laboratory confirmation is critically important for accurate clinical diagnosis of *M. bovis* as the clinical signs caused by this pathogen are not pathognomonic. Traditionally, the diagnosis of *Mycoplasma bovis* infections has been performed via microbial culture. During the last years, the polymerase chain reaction (PCR) is more and more used. PCR has a higher efficiency, specificity, and sensitivity when compared with culture-based methods. While culture and PCR diagnosis relies upon demonstrating the presence of the *Mycoplasma* organism, serological diagnosis using indirect ELISAs allows the detection of anti-*M. bovis* antibodies in plasma and/or serum samples. Their purpose is to identify animals that have been exposed to the pathogen and had mounted a humoral immunological response.

INTENDED USE

This kit is based on an immunoenzymatic assay for the detection of antibodies against *Mycoplasma bovis* in bovine serum.

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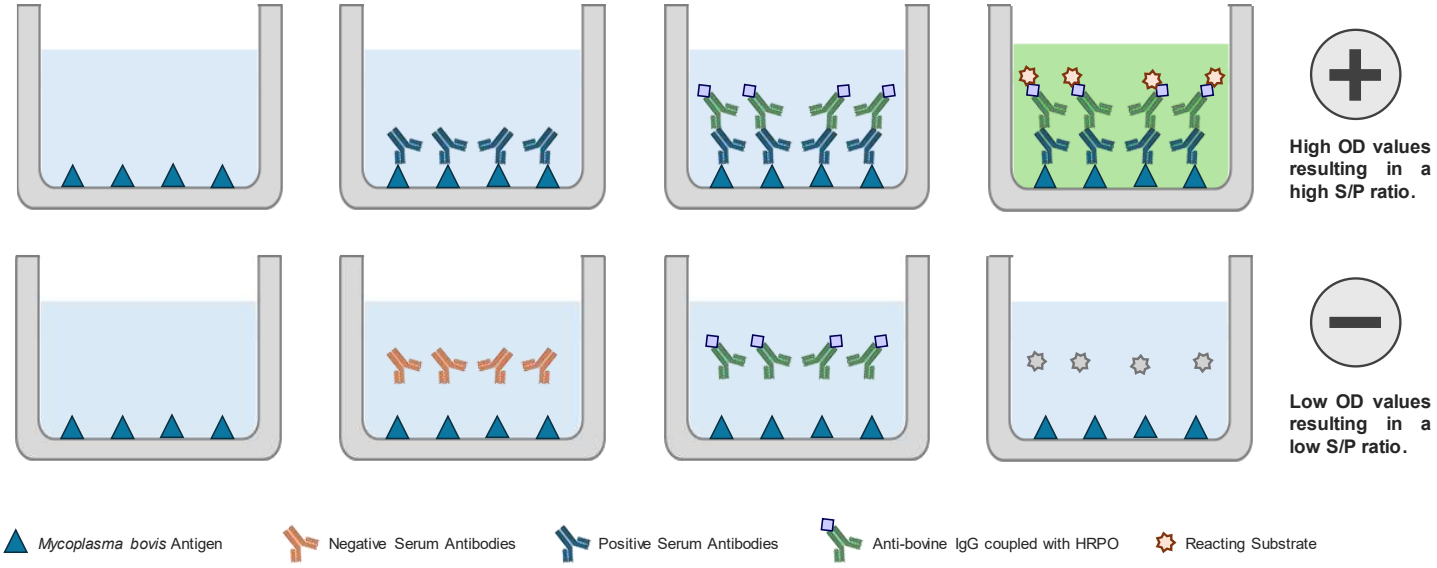
PRINCIPLE OF THE TEST

a. Assay description

1. The wells are coated with a highly purified recombinant protein of *M. bovis*.
2. Samples are added and incubated for 1 hour at 23 ± 2°C. Each well is then washed carefully to remove unbound material.
3. An anti-bovine IgG conjugated with HRPO is added and incubated for 30 min at 23 ± 2°C. Washing steps follow.
4. The substrate is added and incubated for 10 min at 23 ± 2°C. It will react with HRPO if present. The reaction is stopped, and OD values are measured.



b. Results interpretation

OD values are used to calculate sample / positive (S/P) ratio as follow: **S/P ratio = OD_{sample} / mean $OD_{pos.ctrl}$**

The status of a test sample is determined with the SP ratio.

Result	S/P ratio
Negative	< 0.40
Positive	≥ 0.40

The following criteria must be met in order to validate the test:

- Negative control ratio must be less than 0.35.
- Positive control's corrected OD must be greater or equal to 0.800.

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TECHNICAL DATA

a. Sensitivity & Specificity

The diagnostic accuracy was evaluated by calculating the relative sensitivity and the relative specificity (with 95% confidence limits) on an individual animal basis and using the ID Screen® Mycoplasma bovis Indirect ELISA kit (IDvet, Grabels, France) as reference test (A). Sensitivity was also assessed by observing the seroconversion of experimentally infected animals on different days post infection (B).

A	Reference test		
	+	-	Total
Biovet +	30	6	**36**
Biovet -	0	117	**117**
Total	**30**	**123**	**153**

B	Biovet test		Reference test	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
Positive	0	14	0	6
Negative	16	2	16	10
Total	**16**	**16**	**16**	**16**

The Bovichek® M. bovis kit showed an excellent sensitivity when compared with the reference test.

b. Stability

The stability of the kit was evaluated with a panel of 10 samples, including 4 negative, 4 positive, and 2 strong positive samples. Samples were tested with 3 serials of the Bovichek® M. bovis kit that had been stored at 4-8°C for up to 19 months. S/P ratios are presented in the following table.

Samples	Reactivity	Serial A		Serial B		Serial C	
		0 month	19 months	0 month	19 months	0 month	19 months
Pos Ctrl	**Positive**	1,00	1,00	1,00	1,00	1,00	1,00
Neg Ctrl	**Negative**	0,01	0,02	0,02	0,02	0,01	0,02
CQ # 451	**Negative**	0,05	0,04	0,04	0,05	0,05	0,04
CQ # 457	**Negative**	0,29	0,23	0,32	0,33	0,33	0,31
CQ # 472	**Negative**	0,14	0,11	0,14	0,13	0,15	0,13
CQ # 474	**Negative**	0,18	0,13	0,16	0,15	0,15	0,16
CQ # 193	**Weak Pos.**	0,69	0,57	0,67	0,65	0,69	0,66
CQ # 449	**Weak Pos.**	0,64	0,49	0,66	0,63	0,61	0,59
CQ # 188	**Positive**	1,03	1,01	1,02	1,14	1,07	1,17
CQ # 477	**Positive**	1,00	0,93	1,03	1,16	1,06	1,13
CQ # 492	**Positive**	1,04	0,97	1,03	1,22	1,01	1,17
CQ # 818	**Positive**	1,17	1,22	1,21	1,32	1,17	1,28

The ratios remained stable throughout the study, and no changes in result outcomes were observed highlighting the kit's great stability.

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c. Repeatability

The intra-lot repeatability (A) was evaluated using 10 samples with a reactivity ranging from negative to strongly positive. Each sample was tested 3 times in one serial of the kit. The inter-lot repeatability (B) was conducted the same way with 3 different serials and a different panel of samples. Mean and standard deviation of S/P ratios were used to calculate the Coefficient of Variation (CV).

A

Sample ID	Well 1	Well 2	Well 3	Mean ± SD	CV (%)
Pos Ctrl.	1,000	1,000	1,000	1,000 ± 0,000	N/A
Neg Ctrl.	0,015	0,015	0,016	0,015 ± 0,001	N/A
Sample A	0,311	0,295	0,305	0,303 ± 0,008	2,5%
Sample B	0,926	0,917	0,887	0,910 ± 0,020	2,2%
Sample C	0,383	0,392	0,389	0,388 ± 0,005	1,2%
Sample D	0,798	0,825	0,814	0,813 ± 0,014	1,7%
Sample E	0,748	0,777	0,728	0,751 ± 0,024	3,3%
Sample F	1,064	1,085	1,104	1,084 ± 0,020	1,9%
Sample G	0,977	0,960	0,930	0,956 ± 0,024	2,5%
Sample H	0,586	0,575	0,580	0,580 ± 0,006	1,0%
Sample I	0,943	0,953	0,899	0,932 ± 0,028	3,1%
Sample K	0,512	0,496	0,512	0,507 ± 0,009	1,8%

B

Sample ID	Serial A	Serial B	Serial C	Mean ± SD	CV (%)
Pos Ctrl.	1,000	1,000	1,000	1,000 ± 0,000	N/A
Neg Ctrl.	0,013	0,016	0,011	0,013 ± 0,002	N/A
Sample 1	0,046	0,042	0,046	0,045 ± 0,002	4,6%
Sample 2	0,294	0,322	0,331	0,316 ± 0,019	6,0%
Sample 3	0,137	0,141	0,145	0,141 ± 0,004	2,9%
Sample 4	0,185	0,163	0,148	0,165 ± 0,019	11,2%
Sample 5	0,691	0,674	0,686	0,683 ± 0,009	1,3%
Sample 6	0,636	0,657	0,609	0,634 ± 0,024	3,8%
Sample 7	1,033	1,023	1,066	1,041 ± 0,023	2,2%
Sample 8	0,998	1,033	1,058	1,030 ± 0,030	2,9%
Sample 9	1,038	1,032	1,009	1,026 ± 0,015	1,5%
Sample 10	1,175	1,213	1,173	1,187 ± 0,023	1,9%

The results demonstrate the Bovichek® M. bovis kit's high repeatability. No sample had a CV greater than 15% in both intra- and inter-lot studies.

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CONCLUSION

The "*Mycoplasma bovis* Antibody Test Kit, ELISA – Bovichek M. bovis" demonstrates satisfactory diagnostic performances (sensitivity, specificity, repeatability) and has a shelf life of 18 months at 2-8°C. It is available in 2-plates and in 5-plates format. This kit is licensed by the Canadian Centre for Veterinary Biologics for export only.

KIT COMPOSITION

Components	Quantity TRM-501	Quantity TRM-502
12 strips of 8 wells coated with *M. bovis* Ag	2	5
Ready-to-use Positive Control	4 mL	8 mL
Ready-to-use Negative Control	4 mL	8 mL
Ready-to-use Dilution Buffer	130 mL	3 x 110 mL
Concentrated Conjugate (300X)	125 µL	325 µL
Concentrated Wash Solution (10X)	2 x 125 mL	4 x 125 mL
Ready-to-use Substrate	30 mL	60 mL
Ready-to-use Stop Solution	30 mL	60 mL

CFIA-licensed FEO; 710DR/M3.0/D10

For more info, contact us at: 1-888-824-6838, option 3
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