Mycobacterium Bovis Antibody Test Kit

For veterinary use only.

Name and Intended Use

IDEXX *M. bovis* Antibody Test Kit is intended for the detection of antibody to *Mycobacterium bovis* (*M. bovis*) in cattle serum and plasma samples. The test is designed to be used in conjunction with other methods for diagnosing and managing tuberculosis infection, however it is not suitable to diagnose individual animals or herds to be free of disease.

OIE Statement

The validation data for the IDEXX *Mycobacterium bovis* Antibody Test Kit have been certified in May 2012 by the OIE, based on expert review, as fit for the detection of antibody to *M. bovis* in cattle serum and plasma samples to be used as a supplemental test, in conjunction with other methods, for diagnosing and managing *M. bovis* infection.

The test also has utility when performing sero-surveys to understand prevalence and risk of *M. bovis* infection at a herd management level.

General Information

Bovine tuberculosis, caused by *Mycobacterium bovis* (*M. bovis*), continues to be an important livestock disease in many countries and its control and eradication is complicated by the lack of sensitive tests as well as the presence of significant wildlife reservoirs. Although tuberculosis tests based on cell-mediated responses (skin and gamma interferon) can detect animals in the early stages of infection, they still fail to detect up to 20% of truly infected animals.[1] The strategic supplemental use of an *M. bovis* antibody test may increase overall diagnostic power by detecting subsets of infected animals missed by current methods.[2,3,4]

Descriptions and Principles

The IDEXX *M. bovis* antibody test kit is an enzyme immunoassay designed to detect the presence of antibody to *M. bovis* in bovine serum and plasma samples. A microtiter format has been configured by coating *M. bovis* recombinant antigens onto the wells of 96-well microtiter plates. Upon incubation of the test sample in the coated well, antibody to *M. bovis* forms a complex with the coated antigens. After washing away unbound material from the wells, an antibovine: horseradish peroxidase conjugate is added that binds to any bovine antibody attached in the wells. Unbound conjugate is washed away and TMB substrate is added. Color development is related to the amount of bound antibody against *M. bovis*.

Reagents		Volume
1	*M. bovis* Antigen Coated Plate	5
2	Positive Control — preserved with sodium azide	1 x 0.05 mL
3	Negative Control — preserved with sodium azide	1 x 0.05 mL
4	Conjugate — anti-bovine: IgG HRPO Conjugate	1 x 60 mL
5	Sample Diluent — preserved with sodium azide	1 x 120 mL
A	TMB Substrate	1 x 60 mL
B	Stop Solution	1 x 60 mL
C	Wash Concentrate (10X) — phosphate/tween wash; preserved with gentamicin	1 x 235 mL
Other Components: Zip lock bag		1

Note: See table at the end of the insert for a description of symbols used on the insert and labels of this kit.

Storage

Store the reagents at 2–8°C. Reagents are stable until expiration date, provided they have been stored properly.

Materials Required but Not Provided

- Precision micropipettes or multi-dispensing micropipettes
- Disposable pipette tips
- Graduated cylinder for wash solution
- 96-well microplate reader (equipped with 450 nm filter)
- Microplate washer (manual, semi-automatic or automatic system)
- Use only distilled or deionized water for preparation of the reagents used in the test
- Vortex or equivalent
- Tubes for diluting samples
- Microplate covers (lid, aluminium foil or adhesive)

Precautions and Warnings

- Handle all biological material as potentially infectious.
- Wear protective gloves / protective clothing / eye or face protection when handling samples and reagents.
- Refer to the product Material Safety Data Sheet for additional information.
- See the end of this insert for reagent hazard and precaution warnings.

Laboratory Practices

- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting, timing, and washing throughout this procedure are necessary to maintain precision and accuracy. Use a separate pipette tip for each sample and control.
- Do not expose TMB solution to strong light or any oxidizing agents. Handle TMB solution with clean glass or plastic ware.
- All wastes should be properly decontaminated prior to disposal. Dispose of contents in accordance with local, regional, and national regulations.
- Care should be taken to prevent contamination of kit components. Do not pour unused reagents back into containers.
- Do not use kit past expiration date.
- The use of bleach based cleaning solutions for laboratory surfaces, equipment or vessels near the testing location may interfere with the assay performance and should be avoided.

Sample Collection

Either fresh or previously frozen serum or plasma samples may be tested. All thawed samples must be thoroughly mixed prior to dilution.

Preparation of Reagents

Wash Solution

The Wash Concentrate (10X) may form precipitates upon 2–8°C storage; make certain that the Wash Concentrate (10X) is fully in solution prior to diluting. To prepare the Wash Solution, transfer 50 mL of the Wash Concentrate (10X) to a 500 mL volumetric flask or graduated cylinder; add 450 mL distilled/deionized water and mix thoroughly. The diluted solution can be stored at 18–26°C for up to 3 days or at 2–8°C for up to 2 weeks.

Samples and Controls

Dilute samples and kit controls 1/50 (1 part sample, 49 parts diluent) in Sample Diluent. Kit controls must be tested in duplicate for each test series.

Test Procedure

All reagents must be allowed to come to 18–26°C before use. Mix reagents by gentle inverting or swirling.

1 Obtain antigen-coated plate(s) from foil bag and record the sample position. If using partial plates, remove only those wells sufficient for samples to be tested. Place the remaining wells, along with desiccant, in the extra ziplock bag provided and return to 2–8°C.

2 Dispense 100 µL of DILUTED Negative Control (NC) into duplicate wells.

3 Dispense 100 µL of DILUTED Positive Control (PC) into duplicate wells.

4 Dispense 100 µL of DILUTED sample into appropriate wells. Samples may be tested in duplicate but a single well is acceptable.

5 Cover the wells and incubate at 18–26°C for 60 minutes (±5 minutes).

6 Remove the solution and wash each well with approximately 300 µL of Wash Solution 3–5 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tap each plate onto absorbent material after the final wash to remove any residual wash fluid.

7 Dispense 100 µL of Conjugate into each well.

8 Cover the wells and incubate at 18–26°C for 30 minutes (±2 minutes).

9 Repeat Step 6.

10 Dispense 100 µL of TMB Substrate into each well.

11 Cover the wells and incubate at 18–26°C for 15 minutes (±1 minute).

12 Dispense 100 µL of Stop Solution into each well.

13 Measure and record absorbance values at 450nm, A(450).

14 Calculations:

Controls

$$\overline{NC} = \frac{NC1\ A(450) + NC2\ A(450)}{2} \qquad \overline{PC} = \frac{PC1\ A(450) + PC2\ A(450)}{2}$$

Validity criteria

$\overline{NC} \leq 0.200$ $\overline{PC} \geq 0.300$

For invalid assays, technique may be suspect and the assay should be repeated following a thorough review of the package insert.

Samples

$$S/P = \frac{Sample\ A(450) - \overline{NC}}{\overline{PC} - \overline{NC}}$$

The presence or absence of antibody to *M. bovis* is determined by calculating the sample to Positive (S/P) ratio for each sample.

15 Interpretation:

Negative	Positive
S/P < 0.30	S/P ≥ 0.30

Note: IDEXX has instrument and software systems available which calculate results and provide data summaries.

Limitations of use

A negative result does not exclude the possibility of *M. bovis* infection. Antibody to *M. bovis* can be transient, can develop later in infection and not all infected animals will seroconvert. A positive test result suggests the presence of *M. bovis* antibodies in the subject animal. Due to potential exposure and response to environmental *Mycobacteria* (*M. kansasii*, for example), all tuberculosis test results (skin, gamma interferon and antibody ELISA), in conjunction with herd histories, should be considered when determining animal or herd classification. The test is not suitable to diagnose individual animals or herds to be free of disease.