

# IDEXX *Mycobacterium bovis* Antibody Test Kit

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## Introduction

The IDEXX *Mycobacterium 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 laboratory-based format has been configured by coating a blend of *M. bovis* antigens in the wells of 96-well microtiter plates. It can be used as a supplemental test to increase sensitivity of existing testing programs, and a number of countries are also evaluating the antibody test as a potential new *Mycobacterium tuberculosis* (TB) surveillance tool.

## Materials and Methods

Characterized serum and plasma samples were obtained from worldwide sources and used to validate the *M. bovis* ELISA. Two temporal series were produced from animals exposed to *M. bovis* and followed over time. Positive samples from three countries were obtained from either culture-positive animals (n = 307) or animals from infected herds that presented visible lesions (VL) at slaughter (n = 50), or from SICCT or gamma interferon (GIFN) positive animals (n = 50) with no visible lesions (NVL) at slaughter. Sample sets (n = 100) with varying skin or gamma interferon results were evaluated to demonstrate subsets of positive animals within TB-infected herds (n = 45) and the power of combining tests to increase overall sensitivity. Negative samples (n = 1473) were obtained from four countries, with samples originating from negative herds, states or regions. In addition, to understand potential cross-reactivity with other mycobacteria, samples were obtained from animals exposed to large doses of *M. paratuberculosis*, *M. avium*, and *M. kansasii* or from a herd with high Johne's antibody levels.

All samples were evaluated on an *M. bovis* antibody kit manufactured at production scale, according to the standard kit protocol. Briefly, samples and kit controls were diluted 1:50 in a sample diluent and applied to microtiter plates. After a 60-minute incubation, the plates were washed, followed by the addition of an anti-bovine horseradish peroxidase conjugate (30 minute incubation). After another plate wash, tetramethylbenzidine (TMB) substrate was added. After color development of 15 minutes, plates were read on a spectrophotometer at 450nm. Sample optical densities were compared to those of the kit positive control to derive sample-to-positive (S/P) ratios. Samples with S/P ratios of  $\geq 0.30$  were considered positive for *M. bovis* antibodies.

## Results

Data from the *M. bovis* temporal series revealed that animals can develop antibody titers within weeks of exposure, and that an antibody response can be boosted after application of a skin test. The *M. bovis* ELISA detected 197/307 samples from culture-positive animals, resulting in a sensitivity of 64.2%. For NVL samples, the number of positives detected by GIFN, SICCT, and ELISA were 31/50 (62%), 30/50 (60%) and 22/50 (44%), respectively. For samples from VL animals, the number of positives detected by GIFN, SICCT, and ELISA were 47/50 (94%), 28/50 (56%), and 35/50 (70%), respectively. On a herd basis, the number of herds that would have been identified as positive by the *M. bovis* ELISA was 34/45 (75.6%), which was higher than the results of either the gamma interferon test (32/45 = 71.1%) or SICCT (33/45 = 73.3%). Using a single test method, between 71.1% and 75.6% of herds would have been detected. Combining tests resulted in an increase in herd sensitivity to between 86.7% (GIFN and ELISA) and 97.8% (GIFN, SICCT, and ELISA). On negative sample sets, the *M. bovis* ELISA demonstrated a specificity of 98%, with no cross reactivity observed on *M. paratuberculosis* (both experimental and field-infected) or *M. avium* samples. Transient, low-level reactivity was observed with animals inoculated with large doses of *M. kansasii*.

## Significance

Due to the complex nature of tuberculosis, the sensitivity of any one test is not sufficient. The data demonstrates the various subsets of infected animals within herds and the boosting effect that skin testing has on antibody response. Antibody detection is an easy and affordable method for TB testing, especially where there's an emphasis on cost-effective detection and removal of cattle from problem herds in endemic areas. IDEXX *M. bovis* test's high specificity also makes it useful as a potential new surveillance tool in other regions.

The strategic use of the IDEXX *M. bovis* antibody test represents a fast, easy, objective and cost-effective option for use in bTB programs, may increase overall diagnostic power by detecting subsets of infected animals missed by current methods,<sup>1,2,3,4</sup> and also has potential for use in surveillance programs.