OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: IDEXX M. bovis Antibody Test Kit
Manufacturer: IDEXX Laboratories
OIE Approval number: 20120107
Date of Registration: May 2012

Disease: Bovine tuberculosis

Pathogen Agent: *Mycobacterium bovis (M.bovis)*

Type of Assay: Indirect ELISA

Purpose of Assay: Certified by the OIE in May 2012 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.

Species and Specimen: cattle serum and plasma samples.

1. Information on the kit

General information on the kit can be found on the IDEXX website at www.idexx.com. Queries on the kit can be sent to lpdtechservices.com.

2. Summary of validation studies

Analytical characteristics

Precision: Plate CVs (Optical Density of Negative Sample) = 14.5% to 17.7%
Plate CVs (Optical Density of Weak Positive) = 8.7% to 11.9%
Plate CVs (Optical Density of Moderate Positive) = 5.7% to 10.3%

Repeatability: Plate to Plate (S/P value of Equivocal Sample) = 18% to 27%
Plate to Plate (S/P value of Positive Samples) = 12% to 21%

Analytical specificity:

The data indicate that the kit is very specific with respect to other environmental Mycobacteria (e.g. *M. avium*, *M. paratuberculosis*, *M. kansasii*). *M. kansasii* isolations are rare, but infection with (high doses) of *M. kansasii* may result in false positive results, underscoring limitations of screening tests based on proteins conserved among Mycobacterial species.

Analytical sensitivity: Not provided

Diagnostic Characteristics

Test Cut-off Determination

The test S/P cut-off (Sample to Positive ratio) of 0.30 was determined by targeting overall test specificity at 97% to 98% during development. This level of specificity was based on customer feedback, limiting the extent of false positives and subsequent tracebacks, and is similar to the specificity to both the Skin and Gamma Interferon tests[1, 2]. Most ELISA positive samples exhibit a very strong response in the test and there are relatively few samples at or around the test cutoff. Lowering the test cut-off only reduces specificity with no appreciable increase in test sensitivity.

Diagnostic sensitivity (DSn) and specificity (DSp) estimates

The performance levels indicated below are based on 3 different ELISA lots manufactured with significant biological diversity with respects to kit components (ascites, purified antibody, recombinant proteins, negative and positive serum).

M. bovis ELISA results compared to culture positive status and samples from designated TB-free herds or regions.

Test method under evaluation	**IDEXX M. bovis Antibody ELISA**	**Target Species (Cattle)**
Diagnostic sensitivity (using *M. bovis* culture positives)	N DSn CI	**307** **(64.6%)** **(59.7% - 69.5%)**
Diagnostic specificity (samples from TB-free areas)	N DSp CI	**1473** **(98%)** **(97.5% - 98.4%)**

Comparative performance

The **sensitivity** level indicated below is based on 3 different ELISA lots manufactured with significant biological diversity with respects to kit components (ascites, purified antibody, recombinant proteins, negative and positive serum). The **specificity** data are representative of only one ELISA lot.

***M. bovis* ELISA results compared to single intradermal comparative cervical tuberculin test (SICCT) positives and negatives.**

Test method of comparison	**IDEXX M. bovis Antibody ELISA**	**Target Species (Cattle)**
Diagnostic sensitivity (vs SICCT)	N DSn CI	**344** **(69.5%)** **(64.4% - 74.1%)**
Diagnostic specificity (vs SICCT)	N DSp CI	**144** **(97.2%)** **(92.8% – 99.1%)**

***M. bovis* ELISA results compared to Gamma Interferon (GIFN) positives.**

Test method of comparison	**IDEXX M. bovis Antibody ELISA**	**Target Species - Cattle**
Diagnostic sensitivity (vs Gamma IFN)	N DSn CI	**166** **(62.7%)** **(64.4% - 74.1%)**
Diagnostic specificity (vs Gamma IFN)	N DSp CI	**No direct comparison performed on Gamma negatives from negative herds**

Agreement and discrepancies

As mentioned previously, the nature of TB testing (classification based on visual slaughter inspection, relative success with culture and differences between cell-mediated [GIFN and SICCT] and humoral responses) generated, as expected, a large number of discrepancies. These discrepancies illustrate the complex nature of bovine TB and highlight the importance of applying multiple diagnostic tools in order to understand a more complete picture of the infection. Even though there were significant discrepancies between methods, the data show the increased sensitivity that can be attained by the strategic use of the antibody test – detecting subsets of true positives missed by other methods.

Reproducibility

As part of the USDA biological licensing process, a panel of characterised samples (n=30) and 3 distinct lots of kits were provided to 3 laboratories in order to determine reproducibility at the end-user level. At this stage in the licensing process, data on 2 sites are available (plus IDEXX data).

Kits and sample panel were submitted to the University of Minnesota and Texas Animal Health Commission animal disease diagnostic laboratories. Each laboratory tested the panel, in duplicate, unsupervised, on each of the 3 kit lots provided and the data was forwarded back to IDEXX for analysis.

The test panel was comprised of 30 samples in total (10 negatives and 20 positives). There was 100% agreement between kit lots at each of the 3 sites. Both the Texas and Minnesota

laboratories generated sample results consistent with those obtained by IDEXX Research & Development.

Applications

The test is being used as a supplemental test on skin test-negative cohorts within TB problem herds in order to increase overall sensitivity (Ireland).

The test is being evaluated as a logistically simple method for understanding TB prevalence and risk in known infected herds - management (Chile).

Other studies are underway in France, Holland and Belgium to understand performance on archived sample sets and the potential use in various applications.

References

1. de la Rua-Domenech, R., A. T. Goodchild, H. M. Vordermeier, R. G. Hewinson, K. H. Christiansen, and R. S. Clifton-Hadley. 2006. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. *Res. Vet. Sci*. 81:190-210.

2. Schiller, I., et al. 2010. Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transbound. Emerg. Dis*. 57:205–220.

3. Waters W. R., Buddle B. M., Vordermeier H. M., Gormley E., Palmer M. V., Thacker T. C., Bannantine J. P., Stabel J. R., Linscott R., Martel E., Milian F., Foshaug W., and Lawrence J. C. Development and evaluation of an enzyme-linked immunosorbent assay for use in the detection bovine tuberculosis in cattle. Clinical and Vaccine Immunology, November 2011 18:1882-1888.

4. Poster: Casal, C. et. Al. Evaluation of a serological assay for the detection of bovine tuberculosis. Poster presented at the Animal Health and Veterinary Laboratories Agency (AHVLA) Animal Disease Conference held at AHVLA from 13 to 15 September 2011.