Enferplex Bovine TB

01I17 (460 Tests)

Test for the *in vitro* detection of *Mycobacterium bovis* antibodies in serum and provisionally milk

For *in vitro* veterinary diagnostic use

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1.0 General Information

Bovine tuberculosis is caused by the pathogen *Mycobacterium bovis (M. bovis)* and is a serious zoonotic disease. Bovine tuberculosis was first recognized in domesticated animals, although its host range is broad and includes most mammalian species. In addition to livestock and wild hoofed mammals, the disease has been reported in elephants, non-human primates, and many other species, (1). Apart from the pathogenic mechanisms, the ability of *Mycobacterium bovis* to infect such a wide variety of species can be attributed to the different routes of transmission by which *M. bovis* can be passed from animal to animal and therefore the disease has become endemic in many countries, (2, 3).

2.0 Intended Use

Certified by the OIE in May 2019 as fit for the detection of antibody to *Mycobacterium bovis* in cattle serum samples, to be used as an ancillary test in conjunction with other methods for serological prevalence surveys, or diagnosis and management of *M. bovis* infection within herds, for the following purposes;

1. To confirm, but not negate, diagnosis of suspect or clinical cases, including confirmation of positive screening tests in individual animals and in herds with infection prevalence ranging from very low to high, based on detection of antibodies in bovine serum.
2. To detect *Mycobacterium bovis* infected animals not positive by Single Intradermal Comparative Cervical Tuberculin or Interferon Gamma Release Assay (IFNɣ) tests, based on detection antibodies in bovine serum.
3. To confirm, but not negate, infection in animals giving inconclusive reactions in the SICCT, based on detection antibodies in bovine serum.
4. As a screening test to identify animals most likely to have visible lesions (VL) by scoring the number of *M. bovis* antigens recognised by seropositive animals with bovine tuberculosis.

Species and specimens:

This test has been validated and approved for testing serum samples from cattle, as noted above. Regarding intended use in point 4 above, during the first 5 years of registration, additional data will be required to better qualify and categorise the relationship between the number of *M. bovis* antigens and the likelihood of visible lesions. This test is also provisionally approved for testing milk samples from cattle as a herd screening test or as a supplemental confirmatory test for use in individual animals, when used in conjunction with other methods for diagnosing and managing *M. bovis* infection.

3.0 Principle of the Procedure

The Enferplex Bovine TB antibody test is a qualitative enzyme immunoassay based on the sequential addition of bovine serum or milk to a multiple antigen coated plate, antibody-enzyme conjugate and a chemiluminescent substrate.

Upon incubation of the test sample in the multiple antigen coated well, antibodies specific to bovine tuberculosis form complexes with the immobilized antigens.

This step is followed by a wash step with 1X Wash Buffer solution, and Sheep anti-bovine sera labelled with HRP (Horse radish peroxidase), forming an antigen-antibody-conjugate-peroxidase complex. Next, unbound conjugate is washed away and a chemiluminescent substrate is used to generate the signal and the image captured. The image is analysed and data reduced to determine sample status in the Enferplex Bovine TB Macro.

4.0 Reagents

Reagent pack 01I17 contains sufficient material for 460 tests. The reagent pack is stored at 2-8°C. Note the storage conditions for individual components.

Kit Contents	Quantity & Storage Conditions
Antibody Capture Plate Microtitre plates (96 well) coated with specific antigens for *Mycobacterium bovis*	5 x 96-well 2-8°C (in sealed in foil pouches)
20X Wash Buffer 20X Concentrate	1 x 500ml 2-8°C
BMD Sample Diluent Ready to use	1 x 500ml 2-8°C
BMD Conjugate Diluent Ready to use	1 x 500ml 2-8°C
Concentrate Conjugate Undiluted	1 x 0.1ml 2-8°C
Multi-Lite A Chemilumenescent substrate for peroxidase when combined with Multi-Lite B	1 x 15ml Room Temperature (+15 to 25°C)
Multi-Lite B Chemilumenescent substrate for peroxidase when combined with Multi-Lite A	1 x 15ml Room Temperature (+15 to 25°C)
BTB Negative Control Undiluted negative bovine serum containing preservative	1 x 0.1ml -20°C
BTB Positive Control Undiluted bovine serum containing preservative	1 x 0.1ml -20°C

5.0 Materials and Equipment required but not provided

- Microplate incubator/shaker thermostated at 37°C ± 2°C and capable of shaking at 900rpm
- Quansys Biosciences Q-View Imager or equivalent
- Device for the delivery and aspiration of wash solution
- High quality deionised, distilled or reverse osmosis water
- Microplate cover seals and reagent dispensing trays
- Precision single channel and multichannel micropipettes of appropriate volume and disposable tips
- Glass or polypropylene containers for dilution of the concentrate conjugate and other reagents
- Polypropylene tubes/plates for dilution of the sample

6.0 Warnings and Precautions

6.1 Follow the instructions and do not modify the test procedure or substitute reagents from other manufacturers. Do not use the reagents beyond the stated expiry date and do not intermix components from different kit lots.

6.2 Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

6.3 Use a new pipette tip for each sample.

6.4 Allow the reagents to adjust to room temperature (RT), (+15°C to 25°C). Immediately after use, return all reagents to their appropriate storage conditions.

6.5 Avoid cross contamination of the Multi-Lite solution with the diluted conjugate solution. Do not pour unused Multi-Lite solution back into the Multi-Lite bottles.

6.6 Do not allow plates to sit for more than 3 minutes between wash steps and the addition of reagents.

6.7 Do not expose the substrate solution to strong light or oxidizing agents.

6.8 All reagents must be prepared in either clean glass, or polypropylene bottles. Care must be taken to avoid cross contamination of reagents. Use separate dispensing trays for each reagent.

6.9 All unused biological materials should be disposed according to the local, regional and national regulations.

7.0 Health and Safety Information

The 20X Wash Buffer must be handled with care. Please note hazard identified on individual container label. 20X Wash Buffer contains 2-methyl-2H-isothiazol-3-one, which is classified as per EC Directive EC 1272/2008 Skin. Sens 1 – H317. The following are the appropriate Hazard (H) and Precautionary (P) Statements.

H317 May cause an allergic skin reaction
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P272 Contaminated work clothing should not be allowed out of the workplace
P302 & P352 IF ON SKIN: Wash with plenty of soap and water
P321 Specific treatment (see on this label)
P333 & P313 If skin irritation or rash occurs: Get medical advice/attention.
P363 Wash contaminated clothing before reuse.

The BMD Sample Diluent must be handled with care. Please note hazard identified on individual container label. The BMD Sample Diluent contains Donor Goat serum which is classified as per EC Directive No. 1272/2008 [CLP] Resp. Sens. 1 H334. The following are the appropriate Hazard (H) and Precautionary (P) Statements.

H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements (CLP)
P261 - Avoid breathing vapours, mist, or spray.
P272 - Contaminated work clothing should not be allowed out of the workplace.
P280 - Wear protective gloves, protective clothing, and eye protection.
P284 - [In case of inadequate ventilation] wear respiratory protection.
P302+P352 - IF ON SKIN: Wash with plenty of water.
P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P321 - Specific treatment (see section 4 on this SDS).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P342+P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor.
P362+P364 - Take off contaminated clothing and wash it before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Safety Data Sheets are available upon request.

8.0 Preparation of Reagents

Component	Storage of Prepared Component
Working Strength Wash Buffer (1X) **1.** Dilute the 20X Wash Buffer, 1/20 in deionised water. Prepare 500ml per plate by adding 25ml of 20X Wash Buffer to 475ml deionised water and mix thoroughly.	1 month at RT (+15 to 25°C) or at 2-8°C

Working Strength Conjugate **1.** Prepare only the required volume for the number of tests to be carried out. **2.** Dilute the Concentrate Conjugate in BMD Conjugate Diluent to 1:20000. Mix by inversion. **3.** For example, make a 1:5000 pre-dilution by adding 5µl Conjugate concentrate to 25ml of BTB Conjugate diluent. For 1 plate add 2.5ml of the 1:5000 pre-dilution to 7.5ml of BMD Conjugate diluent.	Use within 2hrs of preparation
Multi-Lite Working Solution **1.** Prepare only the required volume for the number of tests to be carried out. **2.** Add 1-part of Multi-Lite A to 1-part Multi-Lite B in either a clean glass or plastic vessel. Mix by inversion. **3.** For example, add 1ml of Multi-Lite A to 1ml of Multi-Lite B. Mix by inversion.	Store Multi-Lite solution at +15 to 25°C in the dark and use within 30 minutes of preparation.

9.0 Sample and Control Preparation

Bring all specimens to room temperature prior to testing. All samples and controls must be added to the BTB antibody capture plate at approximately the same time, therefore use of a transfer/master plate is recommended to add samples and controls to first, and then transfer to the BTB antibody capture plate.

9.1 Controls

9.1.1 Ensure the negative and positive controls are mixed thoroughly before addition to the BMD Sample Diluent.

9.1.2 The controls are prepared to a 1:200 dilution ratio by adding for example 5µl of the control to 1ml of the BMD Sample Diluent.

9.1.3 Mix the prepared control by inversion or by pipetting up and down a minimum of 2 times.

9.1.4 Add 50µl of BTB Negative Control to A1 & B1 of the test plate.

9.1.5 Add 50µl of BTB Positive Control to C1 & D1 of the test plate.

9.2 Serum Samples

9.2.1 Fresh, refrigerated, or previously frozen serum can be tested. Icteric, lipemic, haemolysed, heat treated and contaminated sera may cause erroneous results.

9.2.2 If specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than 24 hours, freeze the serum at -20°C or below.

9.2.3 Specimens containing precipitate may yield inconsistent test results and such specimens must be clarified prior to testing.

9.2.4 Ensure the serum sample is mixed thoroughly before addition to the BMD Sample Diluent.

9.2.5 The samples are prepared to a 1:200 dilution ratio by adding for example 5µl of the serum to 1ml of the BMD Sample Diluent.

9.2.6 Mix the prepared sample by inversion or by pipetting up and down a minimum of 2 times.

9.3 Individual Milk Samples

9.3.1 Whole milk samples can be used after centrifugation for 15 minutes at 2000 x g or left to stand if refrigerated (2-8°C) to remove the fat. No pre-treatment is needed for defatted milk.

9.3.2 If specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than 24 hours, freeze the milk at -20°C or below.

9.3.3 Individual milk samples are prepared to a 1:5 dilution ratio by adding for example 20µl of the individual milk to 100µl of the BMD Sample Diluent.

9.3.4 Mix the prepared sample by inversion or by pipetting up and down a minimum of 2 times.

10.0 Test Protocol

10.1 Prepare the samples and controls as described above (use of transfer/master plate is recommended).

10.2 The BTB Negative Control and the BTB Positive Control are dispensed into 2 wells each.

10.3 All samples are tested in singlicate.

10.4 Transfer 50µl of the controls and samples into the appropriate wells of the coated plate and cover the plate with a cover seal.

10.5 Incubate the plate, shaking, for 60 minutes at 37±2°C.

10.6 Wash the wells 6 times with 250-300µl of 1X wash buffer. Ensure that all wells are completely filled, then completely emptied. Do not adjust the recommended washing steps. Inadequate washing can give incorrect results.

10.7 Dry by inversion on absorbent paper.

10.8 Add 50µl of working strength conjugate to each well. Seal the plate and incubate the plate, shaking, for 60 minutes at 37±2°C.

10.9 Wash the wells 6 times with 250-300µl of 1X wash buffer and dry by inversion on absorbent paper.

10.10 Add 50µl of the substrate solution to each well of the microplate. Immediately read the plate on the Quansys Biosciences Q-View Imager set at 220 seconds.

11.0 Results

11.1 Validation of Test Performance

Each plate must be considered separately when calculating and interpreting results of the assay. The control results must be validated before the sample results can be interpreted. The criteria for the BTB Negative Control and BTB Positive Control are all contained within the 'Enferplex Bovine TB Macro' provided and the results are calculated automatically.

11.2 Acceptable Range of Control Results

If the criteria for the controls are not met, the assay is invalid and must be repeated.

11.3 Interpretation of Results

The Enferplex Bovine TB Macro has a high specificity and a high sensitivity settings for the test.

2 Antigen Rule

Samples giving a 'Negative' result in the macro are considered non-reactive in the Enferplex Bovine TB antibody test, i.e. no sample positive against 2 or more antigens in both the high specificity and high sensitivity settings.

2 Antigen Rule

Samples giving a 'Positive' result in the macro are considered reactive in the Enferplex Bovine TB antibody test i.e. sample positive against 2 or more antigens both the high specificity and high sensitivity settings.

12.0 Limitations of the Procedure

As with any biological test, this test may give a false positive or a false negative result owing to local conditions. As with all tuberculosis test results (TT, Gamma Interferon etc.), the potential exposure and response to environmental *Mycobacteria,* should be interpreted in the context of all available clinical, historical, and epidemiological information relevant to the animal(s) under test. Any change or modification of the procedure might affect the results. *Enfer Scientific accepts no responsibility for any loss or damage, howsoever caused, arising out of the interpretation of test results.*

13.0 Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	**NC**	**S05**	**S13**	**S21**	**S29**	**S37**	**S45**	**S53**	**S61**	**S69**	**S77**	**S85**
B	**NC**	**S06**	**S14**	**S22**	**S30**	**S38**	**S46**	**S54**	**S62**	**S70**	**S78**	**S86**
C	**PC**	**S07**	**S15**	**S23**	**S31**	**S39**	**S47**	**S55**	**S63**	**S71**	**S79**	**S87**
D	**PC**	**S08**	**S16**	**S24**	**S32**	**S40**	**S48**	**S56**	**S64**	**S72**	**S80**	**S88**
E	**S01**	**S09**	**S17**	**S25**	**S33**	**S41**	**S49**	**S57**	**S65**	**S73**	**S81**	**S89**
F	**S02**	**S10**	**S18**	**S26**	**S34**	**S42**	**S50**	**S58**	**S66**	**S74**	**S82**	**S90**
G	**S03**	**S11**	**S19**	**S27**	**S35**	**S43**	**S51**	**S59**	**S67**	**S75**	**S83**	**S91**
H	**S04**	**S12**	**S20**	**S28**	**S36**	**S44**	**S52**	**S60**	**S68**	**S76**	**S84**	**S92**

NC = BTB Negative Control
PC = BTB Positive Control
S = Test Samples in singlicate

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References

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