Enferplex Camelid TB

(465 Tests)

Test for the *in vitro* detection of *Mycobacterium bovis* antibodies in Camelid serum

For *in vitro* veterinary diagnostic use only

Manufactured & Distributed by:
Enfer Scientific,
Unit T, M7 Business Park,
Newhall, Naas,
Co. Kildare.
Ireland
Tel.: +353 459 83800

Contents Page

1.0 General Information

Tuberculosis is a chronic, contagious, granulomatous disease caused by mycobacterial species belonging to the mycobacterium tuberculosis complex, (1). Camelids were generally not considered highly susceptible to tuberculosis (TB), (2), but in recent years serious concern has arisen about TB in New World Camelids (NWC) particularly Llamas and Alpacas in some countries where they are reared not just their native South America. Tuberculosis also affects old world camelids including dromedaries and Bactrian Camels. Mycobacteria are generally not species-specific pathogens (1). Inter-species transmission may therefore occur and there are many potential sources of infection for camelids. *M. bovis* strains isolated from NWCs are often the same molecular types that are isolated from tuberculous cattle and badgers in the same geographical area, suggesting spill over of infection from non-camelid reservoirs (3).

The clinical signs in Camelids include wasting, anorexia, and respiratory distress, enlargement of the superficial lymph nodes, recumbency and eventually death (4). Clinical signs are often associated with extensive respiratory pathology, and it is surprising that overt respiratory distress is sometimes not observed in animals with serve lung lesions (5). Animals are occasionally found dead with no previous clinical observations.

2.0 Intended Use

The Enferplex Camelid TB assay is a qualitative luminescent (emission) immunological method for the detection of *Mycobacterium bovis* antibodies in camelid serum. The Enferplex Camelid TB kit is intended for in vitro veterinary diagnostic use and research purposes only.

3.0 Principle of the Procedure

The Enferplex Camelid TB assay is a qualitative enzyme immunoassay based on the sequential addition of camelid serum to a multiple antigen coated plate followed by and 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 Protein G labelled with HRPO (Horseradish peroxidase) is added forming an antigen-antibody-conjugate-peroxidase complex. Next, unbound conjugate is washed away and a chemiluminescent substrate is used to generate the light signal and the image captured. The image is analysed and data reduced to determine sample status in the Enferplex Camelid TB Macro.

4.0 Reagents

Kit pack 01F15 contains sufficient material for 465 tests. The kit 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
CMDTB Sample Diluent Ready to use	3 x 500ml 2-8°C

CMDTB Conjugate Diluent Ready to use	1 x 500ml 2-8°C
Camelid 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)
CMDTB Negative Control Undiluted Camelid serum containing preservative	1 x 0.1ml -20°C
CMDTB Positive Control Camelid serum containing preservative	1 x 0.1ml -20°C

5.0 Materials and Equipment required but not provided

- Microplate incubator/shaker thermostated at 25°C ± 2°C and capable of shaking at 900rpm
- Quansys Biosciences Q-View Imager
- 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), (+18°C to 30°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, CMDTB Positive and Negative Controls and the CMDTB Conjugate Diluent must be handled with care. Please note hazard identified on individual container label. The 20X Wash Buffer, CMDTB Positive and Negative Controls and the CMDTB Conjugate Diluent 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.

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 Camelid Concentrate Conjugate in CMDTB Conjugate Diluent to 1:40000. Mix by inversion. **3.** For example, make a 1:5000 pre-dilution by adding 5µl of Camelid Conjugate concentrate to 25ml of CMDTB Conjugate diluent. For 1 plate add 1ml of the 1:5000 pre-dilution to 7ml of CMDTB 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 samples to room temperature prior to testing. All samples and controls must be added to the 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 capture plate.

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

9.2 Ensure the serum sample is mixed thoroughly before addition to the CMDTB Sample Diluent.

9.3 The CMDTB Negative Control, CMDTB Positive Control and samples are prepared to a 1:500 dilution by adding for example 5µl of the serum to 2.5ml of the CMDTB Sample Diluent and mixed by inversion.

9.4 Add 50µl of CMDTB Sample Diluent to A1 of the test plate, CMDTB Negative Control to B1 and CMDTB Positive Control to C1.

10.0 Test Protocol

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

10.2 Buffer, CMDTB Negative Control and the CMDTB Positive Control are dispensed into one well 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 25±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 30 minutes at 25±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 CMDTB Buffer, CMDTB Negative Control, and CMDTB Positive Control are all contained within the 'Enferplex Camelid 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

2 Antigen Rule & 4 Antigen Negative Result

Samples giving a 'Negative' result in the macro are considered non-reactive in the Enferplex Camelid TB assay.

2 Antigen Rule & 4 Antigen Positive Result

Samples giving a 'Positive' result in the macro are considered reactive in the Enferplex Camelid TB assay and must be put forward for appropriate action to the appropriate authority.

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. A test 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 References

1. Theon C.O., LoBue P.A., & de Kantor I., (2006). The importance of Mycobacterium bovis as a soonosis. *Vet. Microbiol*., 112 (2-4), 339-345
2. Twomey D.F., Crawshaw T.R., Anscombe J.E., Barnett J.E.F., Farrant L., Evans L.J., McElligott W.S., Higgins R.J., Dean G.S., Vordermeier H.M., & de la Rua-Domenech R. (2010). Assessment of antemortem tests used in the control of an outbreak of tuberculosis in Llamas *(Lama glama)*. *Vet. Rec*., 167 (13), 475-480

3. Connolly D.J., Dwyer P.J., Fagan J., Hayes M., Ryan E.G., Costello E., Kilroy A., & More S.J., (2008). Tuberculosis in alpaca *(Lama pacos)* on a farm in Ireland. 2. Results of an epidemiological investigation. *Irish vet. J.*, 61 (8), 533-537.
4. Abdurahman O.S., & Bornstein S. (1991). Diseases of camels *(Camelus dromedarius)* in Somalia and prospects for better health. *Nomadic Peoples*, 29, 104-112.
5. Barlow A.M., Mitchell K.A., & Vusram K.H. (1999). Bovine tuberculosis in Llama *(Llama glama)* in the UK. *Vet Rec*., 145 (22), 639-640.

14.0 Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Buffer**	**S6**	**S14**	**S22**	**S30**	**S38**	**S46**	**S54**	**S62**	**S70**	**S78**	**S86**
B	**NC**	**S7**	**S15**	**S23**	**S31**	**S39**	**S47**	**S55**	**S63**	**S71**	**S79**	**S87**
C	**PC**	**S8**	**S16**	**S24**	**S32**	**S40**	**S48**	**S56**	**S64**	**S72**	**S80**	**S88**
D	**S1**	**S9**	**S17**	**S25**	**S33**	**S41**	**S49**	**S57**	**S65**	**S73**	**S81**	**S89**
E	**S2**	**S10**	**S18**	**S26**	**S34**	**S42**	**S50**	**S58**	**S66**	**S74**	**S82**	**S90**
F	**S3**	**S11**	**S19**	**S27**	**S35**	**S43**	**S51**	**S59**	**S67**	**S75**	**S83**	**S91**
G	**S4**	**S12**	**S20**	**S28**	**S36**	**S44**	**S52**	**S60**	**S68**	**S76**	**S84**	**S92**
H	**S5**	**S13**	**S21**	**S29**	**S37**	**S45**	**S53**	**S61**	**S69**	**S77**	**S85**	**S93**

Buffer = Sample Diluent
NC = Negative Control
PC = Positive Control
S = Test Samples in singlicate

C0701F15GB November 2019