Combined Camelid TB Antibody Test Package – comprising;

TC0867: IDEXX antibody ELISA (Idexx Laboratories, Inc., Westbrook, ME)
TC0611: DPP VetTB lateral flow rapid antibody test (Chembio Diagnostic Systems, Inc., Medford, NY, USA)
TC503: Enferplex-2-spot ELISA (Enfer Scientific, Co Kildare, Ireland)
TC603: Enferplex-4-spot ELISA (Enfer Scientific, Co Kildare, Ireland)
All are serological tests adapted to detect antibodies to *Mycobacterium bovis* in camelid serum.

Uses:
Mycobacterium bovis infection in camelids is recognised as a significant problem within the industry. Ante mortem TB testing of camelids is problematic since the tuberculin skin test is cumbersome and has very poor sensitivity. The Camelid Antibody Test Package comprises serological tests that detect antibodies to *Mycobacterium bovis* in serum samples from infected camelids. As single tests the IDEXX, DPPVetTB and Enferplex-2-spot have been shown to have similar and reasonable sensitivity. The Enferplex-4-spot has a higher specificity but a much reduced sensitivity compared to the other three tests.
By combining two tests we can;

(i) ***increase the sensitivity*** of detecting infected animals in TB breakdown herds using a ***Parallel Test* i**nterpretation - where a sample that is positive to ***either*** of two tests (IDEXX, DPPVetTB, enferplex-2-spot) is considered a reactor, or

(ii) ***increase the specificity*** of the test (for example in movement, routine or targeted testing of unrestricted herds) by using either a ***Serial Test*** interpretation of two tests (IDEXX, DPPVetTB) where a sample must be positive to ***both*** tests to be considered a reactor, or an Enferplex-4-spot test, which has *equivalent sensitivity and specificity to the Serial Test*..

The sensitivity and specificity values of combined antibody testing for camelids were initially described in AHVLA Project FT1477 (see also Rhodes et al., 2012). Test performances were reassessed during 2017/2018 and are shown in **Table 1 (High Sensitivity/Parallel test options)** and **Table 2 (High Specificity/Serial test options).**

Table 1 – High Sensitivity / Parallel test options:

	Sensitivity				Specificity			
	Positives	**Total**	**%**	**95% C.I.**	**Positives**	**Total**	**%**	**95% C.I.**
IDEXX /DPP VetTB	74	100	**74**	**65.4-82.6**	12	298	**96**	**93.8-98.2**
ENFER 2-SPOT/DPP VetTB	71	100	**71**	**62.1-79.9**	7	291	**96.7**	**95.8-99.4**
IDEXX/ENFER 2-SPOT	75	100	**75**	**66.5-83.5**	8	291	**97.3**	**95.4-99.1**

Table 1 shows the sensitivity and specificity of the combined antibody tests with parallel interpretation (positive to *either* test provides an overall test-positive result). Statistical comparison (using 95% confidence intervals, plus Fishers Exact [2-sided] test) showed **no significant difference in sensitivity or specificity between any of the parallel test combinations.**

Table 2 – High Specificity / Serial test options:

	Sensitivity				Specificity			
	Positives	**Total**	**%**	**95% C.I.**	**Positives**	**Total**	**%**	**95% C.I.**
ENFER 4-SPOT	60	100	60	50.4-69.9	1	291	99.66	98.9-100
IDEXX/DPP VetTB	56	100	56	46.3-65.7	0	298	100	

Table 1(c) shows the sensitivity and specificity of the combined IDEXX/DPP VetTB serial interpretation test (sample must be positive to *both* tests for an overall test-positive result), and the Enferplex-4-spot interpretation test. Statistical comparison (using 95% confidence intervals, plus Fishers Exact [2-sided] test) showed **no significant difference in test sensitivity or specificity between the Enferplex-4-spot test and the serial combined IDEXX/DPP VetTB test**.

Sensitivity data is based upon *M. bovis*-infected alpacas that had received a tuberculin skin test (known to induce an anamnestic specific antibody boost). The sensitivity levels shown, therefore, may be reliant upon a prior skin test. Specificity data is based upon TB-free alpacas that had not received a skin test.
A minimum of 1ml of serum is usually required – this is sufficient to cover all tests required in the Test Package.

Methodology:

TC0867: **IDEXX ELISA**

The IDEXX ELISA for bovine TB has been modified to detect camelid antibodies. The ELISA plates are pre-coated with mycobacterial antigens (MPB83 and MPB70). For the test wells the supplied anti-bovine secondary antibody is replaced with a goat anti-llama secondary antibody to detect specific camelid antibodies in the serum samples. Serum samples are diluted in kit sample diluent and then added to the ELISA plate together with plate positive and negative controls. Camelid positive and Negative control samples are also added. The plates are incubated for one hour, then washed and drained. The secondary antibody reagent is then added to the plate which is incubated for a further 30 minutes. Plates are washed and a substrate added for 15 minutes. This will result in colour formation in those wells containing specific antibodies to *M. bovis*. The reaction is stopped and the plates are read at 450 nm optical density (OD) on an ELISA reader. IDEXX sample results are determined (positive/negative) by comparing individual sample OD readouts with the OD cut-off determined by statistical ROC (Receiver Operator Curve) analysis.

TC0611: DPP VetTB

The DPP VetTB lateral flow cassette test uses mycobacterial antigens immobilised onto 2 separate lines/bands; T1 (MPB83) and T2 (ESAT6 and CFP10) on a nitrocellulose strip and a Protein A/G colloidal gold signal detection system. One cassette is used per animal. Thirty microliters of serum is dispensed into the sample well of the cassette followed by 2 drops of sample buffer. The cassette is incubated for 5 minutes and then a further 4 drops of sample buffer are added to the buffer well and the cassette incubated for a further 15 minutes. For a valid test a complete line/band must appear across the "Control" window of the cassette. Test results are obtained by inserting the cassette into the Chembio Optricon Reader which measures each antigen band as reflective light units. A readout for either of the T1 or T2 antigen bands above its cut-off (each determined by statistical ROC analysis) results in a positive DPPVetTB test readout.

Enferplex ELISA

The Enferplex ELISA for bovine TB has been modified for the detection of antibodies in camelids. Serum samples are diluted and added to the ELISA plate, each well of which has been printed with 7 separate antigen spots (PPDB, ESAT6, CFP10, MPB83, MPB70, MPB70 peptide and Rv3616c) plus an uncoated control spot. Positive and Negative Controls are also added to the plate for each test run. Plates are incubated, shaking for 90 minutes, washed and the secondary detection reagent added. Plates are incubated for a further 30 minutes, washed and substrate added. Signals from each spot (as Relative Light Units, RLU) are then captured using a Quansys ELISA photo-reader camera system and the data extracted from the image using the Q-View software. For each sample the blank spot is subtracted from each of the 7 antigen spots, and the reading for each modified spot assessed against its own cut-off

(Cut-offs determined for each kit batch by the manufacturer, Enfer Scientific, Ireland).

TC503 Enferplex-2-spot test:
A positive result is recorded if a sample is positive to any 2 out of 7 antigens.
TC603: Enferplex-4-spot test:
A positive result is recorded if a sample is positive to any 4 out of 7 antigens.

Results Reporting:
Where 2 tests are combined in a test package – for High Sensitivity (*Parallel Test*) or High Specificity (*Serial Test*) - the results from the two individual tests will be combined to provide the overall "positive" or "negative" reported test result.

Where the Enferplex-4-spot test is used for statutory High Specificity testing the result from this one test will be reported as "positive" or "negative". **N.B. APHA are not permitted to use the Enferplex-4-spot test for private testing.**

References:
Rhodes, S., Holder, T., Clifford, D., Dexter, I., Brewer, J., Smith, N., Waring, L., Crawshaw, T., Gillgan, S., Lyashchenko, K., Lawrence, J., Clarke, J., de la Rua-Domenech, R., Vordermeier, M., 2012. Evaluation of gamma interferon and antibody tuberculosis tests in alpacas. Clin Vaccine Immunol 19, 1677-1683.

Rhodes and Vordermeier. Validation of ante mortem TB tests in camelids. Project FT1477 reported to The British Alpaca Society, the British Llama Society & British Camelids Ltd. and DEFRA on 23rd March 2012.

Whelan, C., Shuraley, E., O'Keefe, G., Hyland, P., Kwok, H.F., Snoddy, P., O'Brien, A., Connolly, M., Quinn, P., Groll, M., Watterson, T., Call, S., Kenny, K., Duigan, A., Hamilton, M.J., Buddle, B.M., Johnston, J.A., Davis, W.C., Olwill, S.A. and J. Clarke. 2008. Multiplex immunoassay for serological diagnosis of *Mycobacterium bovis* infection in cattle. Clin. Vaccine Immunol., 15)12): 1834-1838.