Combined Camelid TB Antibody Test Package – comprising;

TC0867: IDEXX ELISA (Idexx Laboratories, USA) test to detect antibodies to *Mycobacterium bovis* in camelid serum, and
TC0615: DPP VetTB lateral flow rapid antibody test (Chembio, USA) 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. This Camelid Antibody Test Package comprises two tests that detect antibodies to *Mycobacterium bovis* in serum samples from infected camelids. As single tests they have both been shown to have similar and reasonable sensitivity. However by combining the two tests in an Antibody Test Package we can (i) *increase the sensitivity* of detecting infected animals in TB breakdown herds using a *Parallel Test* interpretation - where a sample that is positive to *either* test is considered a reactor, or (ii) *increase the specificity* of the test (for example in movement, routine or targeted testing of unrestricted herds) using a *Serial Test* interpretation - where a sample must be positive to *both* tests to be considered a reactor.

The sensitivity and specificity values of combined antibody testing for camelids were described under AHVLA Project FT1477 as follows (see also Rhodes et al., 2012);

Parallel Test (TB breakdown herd):
Sensitivity = **81.3%** [71.9-91.6]
Specificity = 95.8% [93.3-98.2]

Serial Test (e.g. movement test):
Sensitivity = 55.8% [42.3-69.3]
Specificity = **99.7% [**99.1-100]

[95% confidence intervals]

N.B. The above combined test used by APHA from 1st October 2014 comprised the IDEXX ELISA and the Chembio STAT-PAK lateral flow tests. Replacement of the STAT-PAK with the DPP VetTB lateral flow test by Chembio led to the exchange of the STAT-PAK in the APHA camelid combined test with the new DPP VetTB test. APHA validation data showed the DPP VetTB to have similar test specificities when used in the above combined tests (Parallel 92.9% [89.6-96.2]; Serial 99.6% [98.7-100]). Preliminary data also suggested that the sensitivity of the DPP VetTB is equivalent to that of the STAT-PAK.

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.

Methodology

TC0867: ELISA test to detect antibodies to *Mycobacterium bovis* in camelid serum
A minimum of 1ml of serum is usually required – this is sufficient to cover both tests in the Test Package. The IDEXX ELISA for bovine TB (IDEXX Laboratories, Inc., Westbrook, ME) has been modified to detect camelid (not bovine) 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. The plates are incubated for one hour, then washed and drained. The secondary antibodies are 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 on an ELISA reader.

IDEXX sample results are determined (positive/negative) by comparing individual sample optical density (O.D.) with the O.D. cut-off determined by statistical ROC (Receiver Operator Curve) analysis of sensitivity and specificity data during the AHVLA FT1477 Camelid ante mortem test validation study.

TC0615: DPP VetTB lateral flow rapid antibody test to detect antibodies to *Mycobacterium bovis* in camelid serum (Chembio Diagnostic Systems, Inc., Medford, NY, USA) This 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. Five microliters of serum is dispensed into the sample well of the cassette followed by 3 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 (RLU). A readout for either of the T1 or T2 antigen bands above its threshold (specificity determined by Validation) results in a positive DPPVetTB test readout.

The readouts of the two antibody tests (IDEXX and DPP VetTB) are then combined in a *Parallel* or *Serial* Test interpretation for the antibody test package, depending upon the submission request and test requirement. Reactors are reported as "positive" or "negative" to the combined test package.

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.