APHA Camelid TB Serology Test: re-assessment – March 2018

1. Introduction

This document summarises a recent reassessment of all antibody tests that form part of the APHA camelid TB test package, and addresses the Validation Status Retention requirements of the OIE validation pathway for diagnostic tests (see Ch. 1.1.2. Principles and methods of validation of diagnostic assays for infectious diseases, p4, flow diagram), and the APHA validation process derived from that OIE document, in re-visiting validated tests to show fitness-for-purpose and to make any necessary adjustments to the test protocol.

This is pertinent particularly for the officially approved camelid TB tests available at APHA for which changes outside of APHA control have been made since the introduction of statutory testing in October 2014 as follows:

1.1. The initial combined camelid test package (2014) consisted of the STAT-PAK lateral flow (Chembio, USA) and IDEXX ELISA (Idexx Laboratories, USA) tests. The manufacture of the STAT-PAK test was replaced by the DPP VetTB lateral flow test by Chembio in 2015, with little notice to APHA. As this DPP VetTB test was very similar to a first generation DPP (also Chembio, USA) test previously evaluated by APHA within project FT1477[1] (*Ante mortem TB tests for camelids*; [2]*Rhodes et al., 2012, CVI, 19(10):1677*), a small comparative assessment was made to provide confidence for this substitution. This preliminary data suggested no difference between the new DPP VetTB test and any other antibody test evaluated in the FT1477 study (DPP, IDEXX, STAT-PAK and Enferplex multispot ELISA [at 2-spot interpretation]) - *however, this document recognised that, due to the lack of solid sensitivity data (limited sample availability at that time from animals with visible lesions of TB - VL) the test should be re-evaluated once sufficient samples could be collected.*

1.2. In November 2015 the Enferplex multispot ELISA test was introduced into APHA as an alternative camelid test option in both the combined parallel high sensitivity test (2-spot Enferplex interpretation with either DPP VetTB or IDEXX), and as a stand-alone alternative test (at 4-spot interpretation) to the high specificity serial combined DPP VetTB/IDEXX test. *Data specifically showing the performance of the 2-spot Enferplex in parallel with the new DPP VetTB test was therefore lacking, as indeed was updated parallel or serial test data for the DPP VetTB/IDEXX test combination.*

The accumulation of 100 serum samples from camelids with visible lesions from herds with confirmed *Mycobacterium bovis* infection, plus a larger cohort (305 in total) of TB-

free camelid serum samples from herds with no history of TB, presented the opportunity to (a) fully evaluate the DPP VetTB test, and (b) reassess the camelid IDEXX and Enferplex tests, thus overall allowing for any readjustments across the whole camelid TB test package.

The major collective result of this reassessment is that, for the first time since 2011, statistically-derived test sensitivity and specificity estimates for each of the individual and combined camelid TB tests can be described.

2. Samples

VL serum samples – 100 serum samples from camelids tested by APHA Starcross (SLSD) during and after the FT1477 study as a result of confirmed *M. bovis* in their herds. All had visible lesions typical of TB. These individuals were originally identified on the basis of a positive interferon-gamma (n=7) or STAT-PAK test (n=16), or by a positive combined parallel or serial test comprising two of the following tests; DPP VetTB, Enferplex or IDEXX.

TB-free serum samples – a total of 305 serum samples were available from presumed TB-free camelids: 226 from the previous FT1477 field study (comprising 50 samples provided courtesy of IDEXX Laboratories, USA, plus 176 samples from 17 premises in England, in areas of low TB incidence in cattle and with no history of TB) plus 79 samples from camelid herds tested due to being contiguous to a cattle herd with a new confirmed breakdown and (for the camelid herds) in which no evidence of TB was found (all 79 were Enferplex 2 [and 4-]-spot-negative).

3. Tests

Enferplex tests were carried out at the Enfer Scientific Laboratory, Ireland and at APHA Starcross. Results for 100 VL and 291 TB-free serum samples were compared between APHA-Starcross and the Enfer Scientific Laboratory (for which there was agreement between the results obtained in both laboratories as expected). ***IDEXX*** tests were carried out at APHA-Weybridge and results collated for 100 VL and 305 TB-free serum samples. ***DPP Vet TB*** tests were carried out at APHA-Weybridge and results collated for 100 VL and 298 TB-free serum samples.

The opportunity was taken by APHA and Enfer Scientific to make technical adjustments to the tests to ensure their continued optimal test performance. Test performance summary data are shown in Table 1a: single test performance, Table 1b: high sensitivity test performance, and Table 1c: high specificity test performance.

Table 1 - Summary of Camelid TB Antibody Test Performance

Table 1(a) – Single Test Sensitivity & Specificity:

	Sensitivity				Specificity			
	Positives	Total	%	95% C.I.	Positives	Total	%	95% C.I.
DPP VetTB	56	100	**56**	**46.3-65.7**	5	298	**98.3**	**96.8-99.8**
IDEXX	74	100	**74**	**65.4-82.6**	7	305	**97.7**	**95.3-99.1**
ENFER-2-SPOT	67	100	**67**	**57.8-76.2**	2	291	**99.3**	**98.4-100**

Table 1(a) shows the sensitivity and specificity of the individual antibody tests.

Statistical comparison (95% confidence intervals, plus Fishers Exact [2-sided] test) showed the DPP VetTB test to have a significantly lower sensitivity when compared to the IDEXX test ($p<0.05$), but not when compared to the Enferplex 2-spot test. There was no statistical difference in sensitivity between the IDEXX and Enferplex-2-spot tests. There was no significant difference in test specificities of the individual tests.

Table 1(b) – Parallel / high sensitivity 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(b) shows the sensitivity and specificity of the combined antibody tests with parallel interpretation; a positive readout for either of two tests provides a positive combined parallel test 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 1 (c) – Serial / high specificity 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 (both tests must be positive to generate positive combined serial test result) and the Enferplex-4-spot interpretation test.

Statistical comparison (95% confidence intervals shown, 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.

4. Summary

Reassessment of the camelid TB serology tests using expanded cohorts of serum samples from VL and TB-free camelids have allowed for the identification of technical improvements within each test and the incorporation of adjustments going forward. The resulting updated test performance data in this document provide continued confidence of the usefulness of all three tests and confirmed that there are no statistically significant differences in the diagnostic accuracies (sensitivity and specificity) of any of the three parallel testing combinations that are available to camelid owners. The same can be said of the sensitivity and specificity of the two serial testing options (i.e. Enferplex-4-spot test and the serial combined IDEXX/DPP VetTB test).

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[1] Rhodes, S. and Vordermeier, M. Validation of ante mortem TB tests in Camelids, Final Report commercial project FT1477, AHVLA TB Research Group, on behalf of The British Alpaca Society, the British Llama Society & British Camelid Ltd. 24th January 2012

[2] Rhodes, S., Holder, T., Clifford, D., Dexter I., Brewer, J., 1, Smith, N., Waring, L., Crawshaw, T., Gillgan, S., Lyashchenko, K., Lawrence, J., Clarke, J., de la Rua-Domenech, and H.M. Vordermeier. 2012. Evaluation of IFNγ and antibody TB tests in alpacas. Clin. Vaccine Immunol., 19(10): 1677-1683.