

# Supplementary TB blood tests for *Mycobacterium bovis* infection in South American Camelids

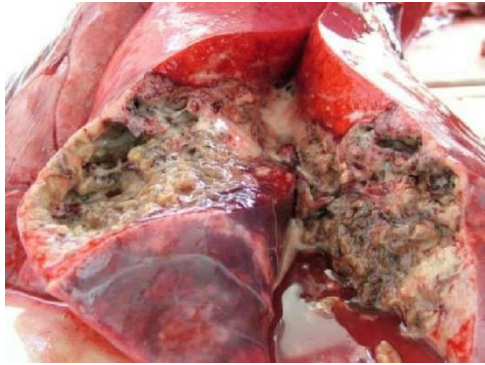
APHA presentation given at the  
Camelid Symposium of the British Society of Veterinary Pathology  
Bristol, 24<sup>th</sup> May 2023



**Camelids = “Spillover Hosts” of *Mycobacterium bovis*** (main hosts; cattle & badgers)  
“Clear TB from cattle & badgers and the problem in non-bovines will go away...”

Well it probably would, but what to do in the meantime?

## Pathology in infected camelids can be severe and extensive



**Skin test-negative alpaca with a cough & continuous hiccups.  
No other symptoms. Liquefactive flowing abscess in the Lung.**

**Lesions of the tracheal mucosa in an apparently healthy,  
skin-test-negative alpaca euthanized as a direct contact  
of a herd mate with a cough.**

*Photographs courtesy James Barnett, APHA.*



**Infection transmission risk** within herd and for neighbouring farms & wildlife

**Zoonotic** – potential risk to human health

**“Cutaneous TB caused by *Mycobacterium bovis* in a veterinary  
surgeon following exposure to a tuberculous alpaca”**

*Twomey et al., Vet Rec. 2010*



**Tuberculin skin test** - very poor sensitivity for camelids

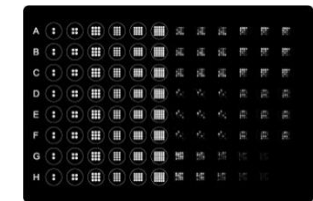
# Project FT1477 (2011-12) - Evaluate IFN- $\gamma$ and Antibody tests for bovine TB in alpacas

Funded by The British Alpaca Society, the British Llama Society, British Camelid Ltd. & Defra

Final report submitted to BAS and DEFRA March 2012  
*Clin. Vaccine Immunol.* 2012. 19(10: 1677-83)

## Main outcomes:

1. Antibody tests offered the best option (over IFN $\gamma$ ) for TB detection in alpacas
2. No significant difference between the different antibody tests (Sensitivity & Specificity)
3. Combining two antibody tests with flexible interpretation improved test performance
4. APHA rolled out statutory camelid tests in October 2014
5. Private antibody tests (Enferplex via SureFarm, IDEXX/DPP VetTB via APHA) available from 2015



**Enferplex ELISA**  
Enfer Scientific, Ireland)



**IDEXX M. bovis ELISA**  
(Idexx, USA)



**DPP VetTB**  
(Chembio, USA,  
replaced STAT-PAK)



**STAT-PAK**  
(Chembio, USA,  
obsolete 2015)

## High-Sensitivity antibody test combinations (e.g. for confirmed TB breakdowns): three options – same performance

Priority = to identify as many infected animals as possible

Positive outcome to either test gives overall positive result

	Sensitivity		Specificity	
	%	95% C.I.	%	95% C.I.
<b>IDEXX /DPP VetTB</b>	<b>74</b>	65.4-82.6	<b>96</b>	93.8-98.2
<b>ENFER 2-SPOT/DPP VetTB</b>	<b>71</b>	62.1-79.9	<b>96.7</b>	95.8-99.4
<b>IDEXX/ENFER 2-SPOT</b>	<b>75</b>	66.5-83.5	<b>97.3</b>	95.4-99.1

## High-Specificity antibody test combinations (e.g. for contiguous testing – assumption is one of “TB-free”): two options – same performance

Priority = to check that infection has not entered herd, using test with lowest risk of false-positives (highest Specificity)

Must be positive to both tests for overall positive result

	Sensitivity		Specificity	
	%	95% C.I.	%	95% C.I.
<b>ENFER 4-SPOT</b>	<b>60</b>	50.4-69.9	<b>99.66</b>	98.9-100
<b>IDEXX/DPP VetTB</b>	<b>56</b>	46.3-65.7	<b>100</b>	

# APHA antibody tests are “supplementary” to the tuberculin skin test

Skin test alone has poor sensitivity in camelids – BUT

**PPDB injection provides transient boost of specific antibody to enable its detection = “anamnestic boost”**

Result = increased sensitivity of antibody test (if blood sample taken within 30 days following a skin test)

**Most APHA-funded camelid testing for two reasons:**

- 1. Confirmed infected herds (culture- and/or PCR-positive); High Sensitivity** testing to increase the overall sensitivity of infection detection & confidence of TB clearance
- 2. Contiguous herd testing (assumption is “TB-free”) – High Specificity** testing to get a good look inside the herd to provide confidence that *transmission into the herd has not happened*

Transmission may be direct (nose-to-nose) or indirect (wildlife);





# Statutory camelid antibody tests are carried out at APHA-Starcross



Vets should notify Starcross before submitting samples

APHA Statutory Test Submission form TN183:

Tick reason for the test

Tick whether a prior skin test has been performed (APHA strongly recommend this)

Owner/PVS may choose tests applied to their animals

Now need to sign form

Test turnaround time = 10 working days

## TN183:

Department for Environment, Food and Rural Affairs  
Scottish Government  
Welsh Government

Animal & Plant Health Agency

### Camelid TB Serology Package - Statutory Sample Submission Form

**Please submit clotted blood or serum ONLY**

Local APHA office submitting samples:  Manage Sample WSA ID:

Number of samples:  Number of boxes:  Date of Sampling:

APHA Regional Laboratory: **APHA Starcross** Case Vet or other APHA office contact name:

Samples packed by:  Herd owner/keeper's name:

CPHH:  Species:

Owner/keeper address:

Postcode:

Practice clinician:

Practice address:

Postcode:

Your reference number:

Has a tuberculin injection been administered to the animal(s) 10 - 30 days before blood sampling? Yes  No

**Reason for Submission (please tick the applicable reason)**

High Sensitivity (Parallel) Test	
TB breakdown camelid herd in which <i>M. bovis</i> has been isolated on culture. In Wales, this also includes herds considered high risk on epidemiological grounds	PAR-TB <input type="checkbox"/>
Forward (spread) tracing(s) from an <i>M. bovis</i> infected camelid herd.	PAR-TRC <input type="checkbox"/>

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**(Wales Only)** Camelids under restrictions by virtue of sharing a holding (co-located) with an Officially Tuberculosis Free Status Withdrawn (OTFW) cattle herd or with other non-bovine animals affected by a culture-confirmed TB incident. **PAR-COL-W**

**England and Scotland only: Combined parallel test Selection - choose two or tick No Preference:**

- IDEXX
- Enferplex 2-spot interpretation
- DPP VetTB
- No Preference

**High Specificity (Serial) Test**

TB strongly suspected (e.g. characteristic TB lesions detected in one or more animals at Post Mortem Examination) pending laboratory confirmation of <i>M. bovis</i> infection in a herd under TB restrictions. If infection is confirmed, the initial High Specificity (serial) test results can be reinterpreted at High Sensitivity on request from the APHA case vet	SER-SUS <input type="checkbox"/>
Camelids not under TB restrictions, but contiguous to an OTFW cattle herd or to non-bovine animals affected by a culture-confirmed TB incident	SER-CONT <input type="checkbox"/>
<b>(England/Scotland only)</b> Camelids under TB restrictions by virtue of sharing a holding (co-located) with an OTFW cattle herd or with other non-bovine animals affected by a culture-confirmed TB incident	SER-COL <input type="checkbox"/>
Camelids not under TB restrictions, but undergoing TB testing due to an epidemiological link with a known infected herd (e.g. back or source-tracing)	SER-EPI <input type="checkbox"/>
Check test of camelids with two consecutive positive results on a private Enferplex test read at the 2-spot interpretation	SER-CHK <input type="checkbox"/>

**England and Scotland only: Serial test Selection - choose one or tick No Preference:**

- Enferplex 4-spot interpretation
- DPPVetTB and IDEXX ELISA combined serial test
- No Preference

**Please Note - Blood Test Options:**

The DPP test is reserved for submissions consisting of ≤40 samples from the same holding. Larger sample submissions will be tested using the IDEXX/Enferplex options (England and Scotland only). An Enferplex test run requires a minimum of ten samples. The laboratory will inform you if this is likely to cause a delay in test reporting, and may offer an alternative, equivalent test option (using IDEXX/DPP) if this is the case.

**England and Scotland only:**

I have discussed and agreed the selection of the blood tests with the owner/keeper.

Signature  Tel. no.   
Official Veterinarian (OV)

Name in BLOCK LETTERS  Date

I have reviewed the section of blood tests

Signature   
Name in BLOCK LETTERS   
Owner/keeper

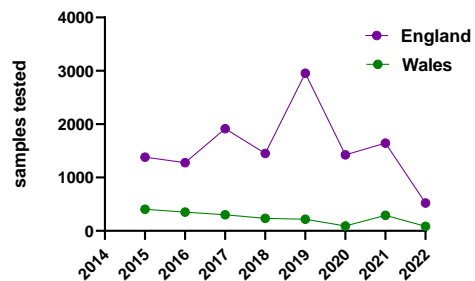
**Data Protection**  
For information on how we handle personal data please go to [www.gov.uk](http://www.gov.uk) and search Animal and Plant Health Agency Personal Information Charter.

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# Statutory APHA camelid antibody tests 2015-2022 – what they show

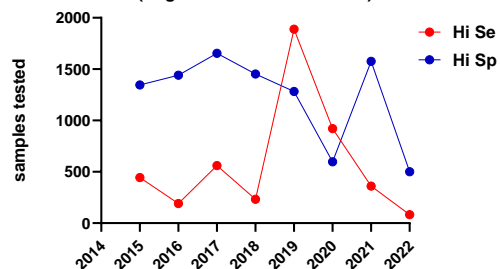
(Data source: Camelid Quarterly Reports – GOV.UK)

England & Wales Camelid TB antibody test use



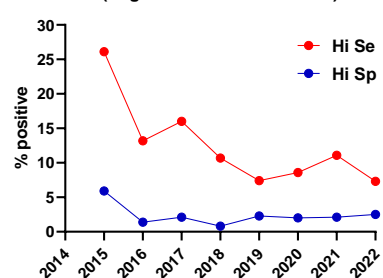
More tests carried out in **England** compared to **Wales**  
(more camelid herds in England)

High Sensitivity v High Specificity Camelid test use  
(England & Wales combined)



More **High Specificity tests** (e.g. contiguous testing) than **High Sensitivity tests**  
(confirmed breakdown) performed  
Exceptions (e.g. 2019) where a large confirmed breakdown may be occurring

% Test-positivity  
(England & Wales combined)



**Test-positivity depends on level of infection**

**Higher positivity for High Sensitivity tests** in confirmed breakdowns  
Lower positivity for High Specificity tests e.g. contiguous, where assumption is "TB-free"

**Overall from 2015 to 2022 – 615 test-positives out of 13,907 tested (4.4%)**  
- all will have received a prior skin test  
- i.e. skin test is not generating antibody test false-positives

# Private camelid antibody tests also carried out at APHA-Starcross



Vets should notify Starcross before submitting samples

APHA Private Test Submission form TN186:

Tick reason for the test

High Specificity test; IDEXX/DPP VetTB test only available at APHA (private Enferplex available via Sure Farm)

Tick whether a prior skin test has been performed (APHA strongly recommends this)

Test turnaround time = 10 working days

Remember TN184 form (Request for Permission to Test)

TN186:

Department for Environment, Food and Rural Affairs  
Scottish Government  
Welsh Government

Animal & Plant Health Agency

For APHA Use  
 APHA Ref. No.   
 Date received.

**Camelid TB Serology Package - Private Sample Submission Form**

Submit samples to Animal and Plant Health Agency (APHA) Starcross, Staplake Mount, Starcross, Exeter, Devon, EX8 8PE.

This form is only for use for private testing of animals not subject to tuberculosis movement restrictions and/or awaiting mandatory tuberculosis blood testing by Animal and Plant Health Agency (APHA).

Please submit clotted blood or serum ONLY

Clients name and Farm address  Veterinary Practice

Postcode:  Clinician:   
 CPHH No.  Your reference:

Private Veterinary Surgeons are required to confirm that they have secured written agreement to undertake this test and the sampled animals were not under TB related movement restrictions or awaiting testing by APHA at the point of sampling. Please tick to confirm

**Animal Details - enter on sampling sheet**  
**Purpose/Housing - enter the main enterprise under which the affected animals are kept**

Organic? Yes  No  Unknown  In Transition   
 Purpose Fibre  Pet  Zoo  Open farm  N/A   
 Housing Housed  Outdoors  Mixed  Unknown

**Reason for Submission**

Pre or post-movement test SER-MVT <input type="checkbox"/>	Pre-export test SER-EXP <input type="checkbox"/>
Diagnostic to exclude TB from differential diagnoses in a herd not restricted (TB not strongly suspected) SER-DIAG <input type="checkbox"/>	Monitoring - voluntary routine testing (Herd not restricted) SER-RHT <input type="checkbox"/>

Tick if a tuberculin injection has been administered to the animals 10 - 30 days before blood sampling

**Clinical history (only if diagnostic)**

Total No. in herd	No. in affected group	No. affected including dead	No. dead	Duration of clinical signs (in affected case)		
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	0-3 days <input type="checkbox"/>	4 days - 2 weeks <input type="checkbox"/>	>2 weeks <input type="checkbox"/>
					Unknown <input type="checkbox"/>	

**Clinical Signs** Please rank in order of importance e.g. 1 = main clinical sign

Wasting/poor condition  Malaise  Respiratory  Recumbent   
 Skin  Diarrhoea  Other

**Clinical history/provisional diagnosis if any**

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TN184:

Department for Environment, Food and Rural Affairs  
Scottish Government  
Welsh Government

Animal & Plant Health Agency

**Request to Undertake Private TB Testing of Non-bovine Species**

1. Application to Test Section (to be completed by the Private Veterinary Surgeon (PVS))  
 I am writing to seek the agreement of the Secretary of State/Scottish Ministers/Welsh Ministers\* to carry out a TB test on the following non-bovine animals:

(Insert Name and Address of the keeper where the test is to be undertaken)

Species:  No. of animals:   
 Date of test:  CPH/Customer No.

Postcode

Is this test for export purposes? Yes  No

If no, what is the reason for the test? (i.e. pre or post-domestic movement testing, voluntary testing scheme, etc.)

Do you have experience of skin testing the species to which this request applies? Yes  No

If No, you must arrange for this test to be conducted under the supervision of a veterinary surgeon, who is experienced in testing the species to which this request applies and has the OCQ(V)-TT qualification.

If Yes, what experience do you have that demonstrates your competency to carry out the skin test, in a to holding the OCQ(V)-TT qualification?

Is this test: Skin Test  Blood Test

Signature  Official Veterinarian Tel. no.   
 Name in block letters  Date   
 Email to:  Veterinary Practice  Tel. No.

**All completed forms should be emailed to:**

- In England: [CSC\\_TBOS@apha.gov.uk](mailto:CSC_TBOS@apha.gov.uk)
- In Scotland: [ScotlandEndemics@apha.gov.uk](mailto:ScotlandEndemics@apha.gov.uk)
- In Wales: [APHAWalesTBCaseManagementTeam@apha.gov.uk](mailto:APHAWalesTBCaseManagementTeam@apha.gov.uk)

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## Private APHA camelid antibody tests 2015-2022 – what they show

*(Data source: Camelid Quarterly Reports – GOV.UK)*

**High Specificity** testing of individuals from unrestricted herds for reason of e.g. export, movement/sales, monitoring

**(assumption is “TB-free”)**

Serial IDEXX / DPP VetTB test

**GB total private tests = 411; Positives = 5 (1.2%)**

**Non-GB total tests = 195; Positives 9 (4.6%) – all 9 within one large herd in 2015, no positives since**

**Almost all will have received prior skin test**

**i.e. skin test is not generating antibody test false-positives**

# Post Mortem findings for APHA statutory test-positive camelids

PM is recognised as a low sensitivity method of confirming infection status

It is carried out mainly for purpose of identifying the *M. bovis* genotype for epidemiological tracing

	High Sensitivity test-positives					High Specificity test-positives			
	% VL	% AL	% NVL	Total lesioned (VL & AL)		% VL	% AL	% NVL	Total lesioned (VL & AL)
<b>2015</b>	44.2	3.2	52.6	47.4		56.9	7.8	35.3	64.7
<b>2016</b>	4.5	27.3	68.2	31.8		90	10	0	100
<b>2017</b>	48.2	27.6	24.2	75.8		78.6	0	21.4	78.6
<b>2018</b>	20	0	80	20		46.1	30.8	23.1	76.9
<b>2019</b>	60	30	10	90		70.4	11.1	18.5	81.5
<b>2020</b>	31.3	6.3	62.5	37.6		0	0	100	0
<b>TOTALS</b>	<b>37.4</b>	<b>10.8</b>	<b>51.7</b>	<b>48.2</b>		<b>65.4</b>	<b>9.2</b>	<b>25.4</b>	<b>74.6</b>

(VL = visible lesions typical of TB; AL = atypical TB lesions; NVL = no visible lesions)

**PM findings are as expected;**

**Lower lesion rate in positives to Hi Se test** - identifies more animals at earlier stage of infection where lesions will be few

**Higher lesion rate in positives to Hi Sp test** – identifies more animals at later stages where lesions more likely

# SUMMARY

Supplementary antibody tests for TB in camelids rolled out in October 2014 at APHA

Tests are validated for use in camelids (Sensitivity & Specificity defined)

Private tests are available (APHA: IDEXX/DPP VetTB, or Sure Farm: Enferplex)

Antibody tests currently offer best option for TB diagnostics in camelids;

**Higher Sensitivity** (but lower specificity) tests for clearing infection from confirmed breakdowns &  
**Higher Specificity** (but lower sensitivity) tests for checking herds where the assumption is TB-free

**Test Sensitivity:** to achieve levels shown requires a prior skin test – without which test sensitivity will be lower and could give a false sense of security following a negative test result

**Test Specificity:** is not affected by skin test - it does not generate antibody test false-positives

**Test-positivity generally reflects level of infection in the herd**

Non-perfect test specificity (0-4% false-positives) means veterinary discretion is important to decide when repeat antibody testing (e.g. in TB breakdown) is no longer adding value to infection detection.