Animal & Plant Health Agency

Cattle TB supplementary blood tests: Interferon-gamma & antibody

Shelley Rhodes

TB Test Consultant / TB Research Group
APHA Weybridge

Animal & Plant Health Agency

Cattle TB Control

Further information

http://www.tbhub.co.uk

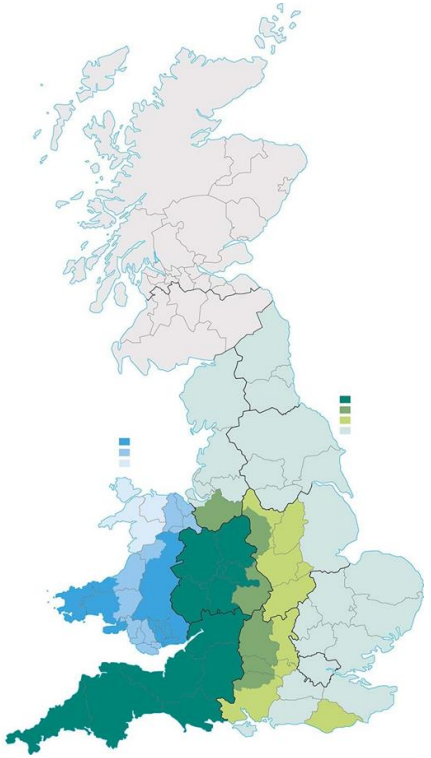
http://apha.defra.gov.uk/vet-gateway/ifng-testing/index.htm

Active surveillance - single intradermal comparative cervical tuberculin skin test (SICCT)

Supplementary interferon-gamma (IFNG, or “gamma”) testing since 2006 & IDEXX antibody testing since 2017

Private gamma test available at APHA since 2016

Both gamma and IDEXX antibody tests are OIE-registered and validated for use.



Animal & Plant Health Agency

Cattle gamma (IFNG) test

Animal & Plant Health Agency

Why is the gamma test useful?

It's more **sensitive** than the skin test

- 90% (IFNG) compared to 81% (SICCT)
- Detects infection earlier, before animals become skin test-positive
- Detects infected animals later, that never become skin test-positive

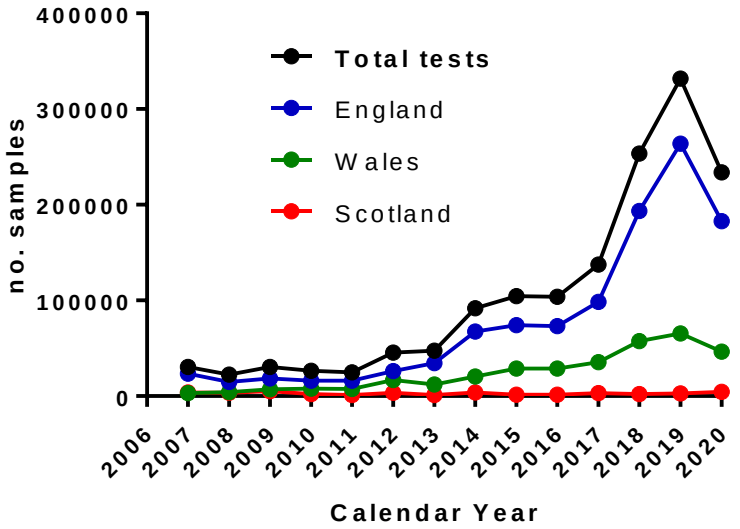
But it's less **specific** than the skin test

- 3.5% false-positives (IFNG) compared to 0.02% (1:5000 SICCT)
- So IFNG is applied mainly to confirmed infected herds (breakdowns with lesion and/or culture positive animals) where the priority of finding infected cattle is deemed higher than the risk of false-positives

International studies all show increased infection detection using skin and IFNG tests together in parallel (i.e. positives to both tests removed)

Animal & Plant Health Agency

APHA has been increasing gamma testing



Reduction 2020 due to Covid related effects across lab and field

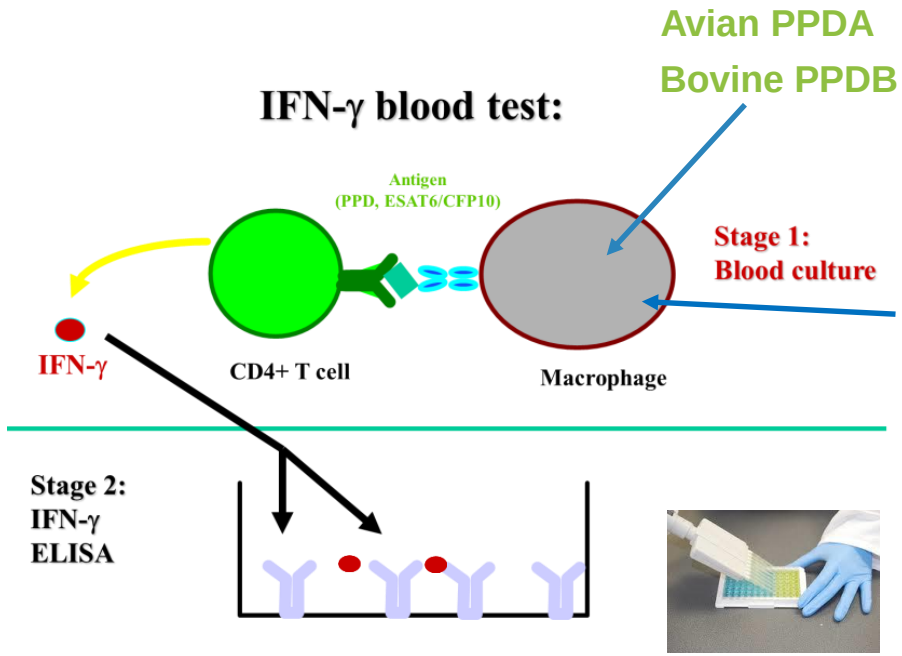
Pre-covid test numbers were increasing across England & Wales in line with respective eradication programmes (Defra - Strategy for achieving Official Bovine TB-free Status for England [2014], and Wales TB Eradication Programme Delivery Plan [2017])

Animal & Plant Health Agency

How does the gamma test work?

“SICCT in a dish”

- It’s a comparative test using the same tuberculin products used for the skin test
- These are used to challenge the T cells in the whole blood sample



Avian PPDA

Bovine PPDB

Extended test format using additional specific PEPTIDE

Its readouts are:

Positive

Negative

Resample

Rejected

Animal & Plant Health Agency

How does the gamma test work?

- The amount of IFNG produced by the sample will depend upon the presence of memory T cells that recognise and respond to *M. bovis*-specific antigens within the PPDB as a result of the animal being infected with M*. bovis*
- The test subtracts the PPDA response from the PPDB response
- This gives the *M. bovis*-specific IFNG response (PPDA being used, as in the SICCT as a measure of background responses to environmental mycobacteria).
- If the PPDB-PPDA (M. bovis-specific) response is above the test cut-off (which is determined during test validation) then the result will be "positive"
- If it is below the test cut-off the result will be "negative"

Animal & Plant Health Agency

Gamma test quality control

- Every sample has its own positive and negative QC. This is because the IFNG is a biologically active test that requires viable cells in the sample that are capable of performing in the test.
- Both sample QC must be satisfied for the sample to provide a valid test result. Should one or other sample QC fail, then the results report will show “resample”. This means that sampling the animal again is required.
- The ELISA test kit (BOVIGAM) that measures the levels of IFNG produced by the sample also has its own kit positive and negative QC, which must be satisfied on every BOVIGAM plate which tests up to 22 samples (animals).
- Rejected samples are those not tested due to for example mislabelled or replicate tubes, extensive clotting in the sample, and submissions going astray in the delivery system arriving far too late for the sample to have good viability. These are all quite rare but do happen occasionally, usually < 1% reject rate.

Animal & Plant Health Agency

Gamma test resample reason (% of total tests)

Sample QC	2014	2015	2016	2017	2018	2019
Total resamples	4.13	4.18	4.99	4.8	5.7	5.3
Positive QC fail	0.83	0.94	1.8	1.8	2.2	0.85
Negative QC fail	3.3	3.24	3.1	3.0	3.5	3.9

Overall resample rates are low and have been steady over the years

~3-4% negative QC fails

~1-2% positive QC fails

Animal & Plant Health Agency

Why do resamples occur?

- Resamples due to sample QC fails can vary between herds and samplings. But overall the percentage of resamples remains low and steady over the years
- Negative sample QC measures the background release of IFNG in the absence of any stimulation of the sample in the laboratory. If the cut-off for this QC is exceeded (i.e. background is too high) then there is a risk that any *M. bovis*-specific response by the sample will not be identified
- Positive sample QC measures the IFNG response of the sample to a general T cell stimulus (a mitogen). A poor response to this general stimulus suggests a low viability of the cells, or that the individual animal just may have viable but low-responding cells in this test. In the case of a poor sample viability, extremes of temperature (cold & hot) during sampling and transit are known to be important. That is why temperature-controlled delivery boxes are used (to mitigate against the cold) and instructions are in place also for hot weather sampling
- Only one “resampling” is recommended per animal, since if the animal fails the same QC again, this reduces the likelihood of it ever passing (law of diminishing returns)

Animal & Plant Health Agency

Flexible test interpretation

- The gamma test can be tailored for certain circumstances, by the inclusion of a specific peptide cocktail and a different test interpretation (“extended test” format)
- A positive response to the peptide cocktail is determined by subtracting the sample’s own background IFNG release from the response to the peptide cocktail. If this value exceeds the cut-off (also determined during test validation) then a positive response to the peptide cocktail is recorded.
- Using the tuberculin and peptide components of the test in different test interpretations can provide the following:
  - Higher specificity test – positive responses to both the tuberculin (PPDB>PPDA) and the peptide components are required
  - Higher sensitivity test – positive response to either the tuberculin or the peptide component is required

Animal & Plant Health Agency

Three gamma test interpretations depending on test reason:

	Gamma test format	Positive test criteria	% Se	% Sp
1. **~98% of tests***	Parallel (New / persistent)	PPDB > PPDA	90	96.5
2. **~0.01% of tests***	Serial extended (NSR / anomalous)	PEPTIDE & PPDB > PPDA	74	99.2
3. **~1.98% of tests***	Flexible extended (Johne's co-infected)	PEPTIDE OR PPDB > PPDA	82 - 90	96.5

- These three interpretations of two formats (with and without peptide) capitalise on whether a test for higher sensitivity or higher specificity is required.
- Sensitivity and specificity are linked in assessing test performance, so if you increase one, the other will fall – you can't have it both ways

* % of total tests is taken from 2019 data

Animal & Plant Health Agency

Gamma test formats

Parallel test

- Most gamma testing uses the basic comparative tuberculin test denoted “parallel” i.e. parallel with the SICCT; remove the SICCT-positives then gamma test the rest and remove any gamma test-positives.

Serial extended test (i.e. “extended” due to the addition of peptide)

- A higher specificity test option used since 2006 on a small number of cases of individual non-specific skin reactor (NSR) animals or where fraudulent tampering with the skin test is suspected.
- In these cases, a positive result from this higher specificity test can offer confidence that the skin reaction observed is due to *M. bovis* infection. The higher specificity is achieved by including the peptide cocktail, to which a positive response must also be achieved as well as a positive comparative PPD response.
- The higher specificity test (99.2%) comes with a lower sensitivity (74%) compared to the parallel test (90%) – which is why this test is not used in breakdown testing (for which the higher sensitivity parallel test is required).

Animal & Plant Health Agency

Gamma test formats

Flexible extended test

- An increasing number of flexible extended gamma tests are being used where there is evidence that Johne's (*M. avium paratuberculosis*, or MAP) may be interfering with the skin or gamma tests, usually as part of persistent breakdown herd testing.
- MAP infection (or vaccination) can increase the response to PPDA, potentially masking any *M. bovis*-specific response that might otherwise be detected.
- The antigens in the peptide cocktail are not present in MAP, so MAP co-infection/vaccination will not affect an *M. bovis*-driven peptide response, if present. A positive test result is obtained if there is EITHER a positive comparative PPD response OR a positive response to the peptide. Using the peptide in this flexible way offers a "second chance" to identify *M. bovis* infection in the face of Johne's.
- The sensitivity of the flexible extended test will vary depending on how much the presence of MAP is interfering with testing. MAP coinfection seems to affect some herds more than others. But we can estimate the range of sensitivity (82-90%) using the two components of the test.

Animal & Plant Health Agency

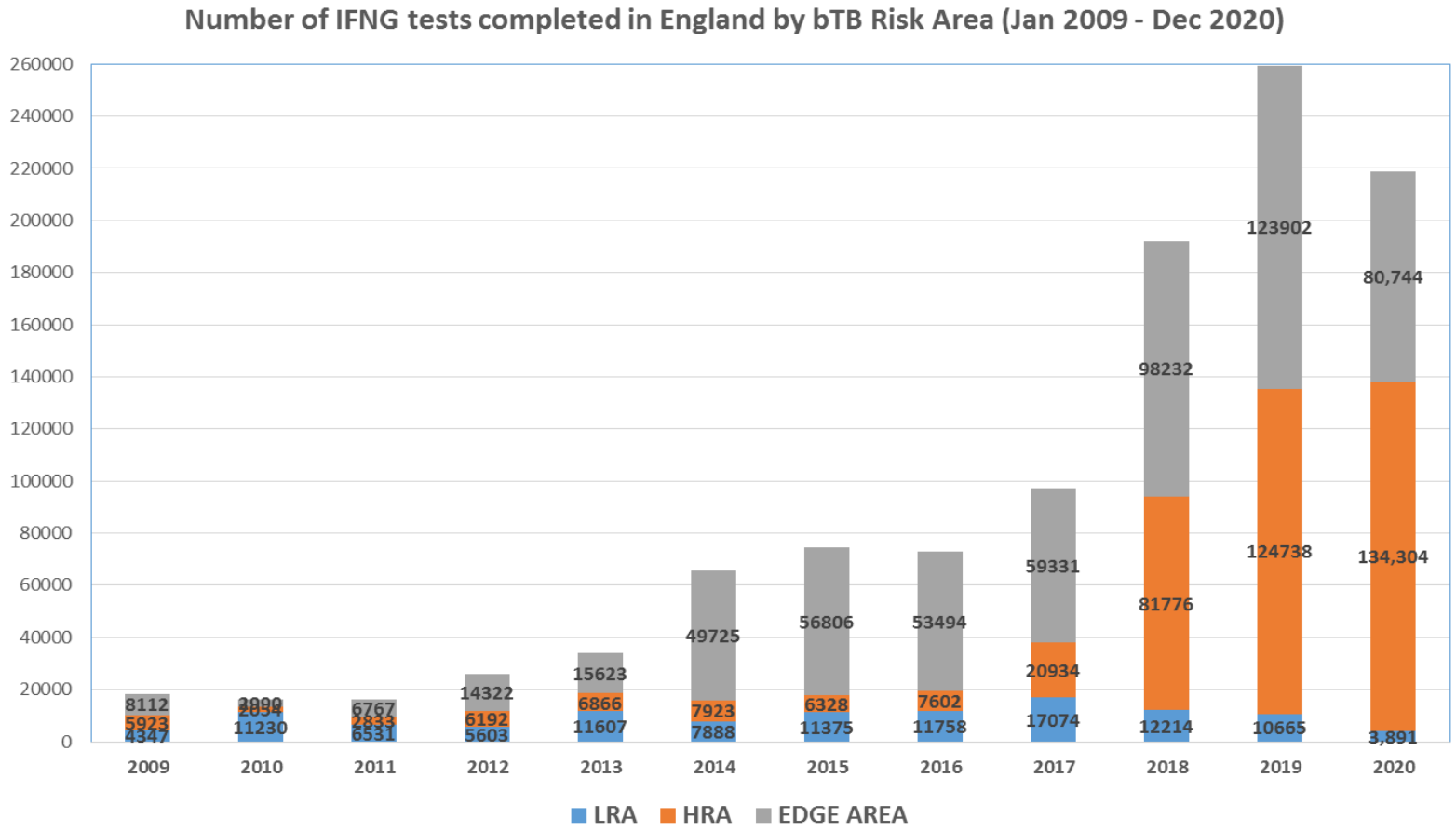
England gamma use 2020

Number of samples tested under each test reason as % of total samples tested and with % test-positive within each test reason

	High Risk Area				Edge Area				Low Risk Area			
Test Reason	No. samples	% of tests	Positive samples	% pos	No. samples	% of tests	Positive samples	% pos	No. samples	% of tests	Positive samples	% pos
Flexi/extended	2201	1.6	96	4.4								
New breakdown	9622	7.1	439	4.6	80,268	97.4	2476	3.1	4,148	98.4	103	2.5
New breakdown - cull area	104,838	77.6	4596	4.4	1332	1.6	29	2.2				
Parallel - Other	489	0.4	80	16.4								
Persistent breakdown	8552	6.3	415	4.9	816	1.0	22	2.7	67	1.6	1	1.5
Persistent breakdown - cull area	9410	7.0	599	6.4								
Serial - Anom Reaction	25	0.02	0	0								
Totals	135,135	100	6,225	4.6	82,416	100	2,533	3.1	4,215	100	104	2.5

Monthly reports available on GOV.UK

Animal & Plant Health Agency



2020 HRA tests up by ~8%

LRA tests down by ~60%

Edge Area tests down by 40% in counties previously part HRA and by 18% in counties entirely Edge

Animal & Plant Health Agency

Wales gamma use 2020

Number of samples tested under each test reason as % of total samples tested and with % test-positive within each test reason

	High TB				Intermed TB				Low TB			
Test Reason	No. samples	%	Positive samples	% pos	No. samples	%	Positive samples	% pos	No. samples	%	Positive samples	% pos
Flexi/extended	1891	5.7	160	8.5	520	3.0	39	7.5				
Herd-S	1300	3.9	75	5.8	30	0.2	5	16.7	155	2.4	1	0.6
New breakdown	164	0.5	7	4.3	12,385	72.5	373	3.0	6100	96.0	112	1.8
Persistent breakdown	22,169	67.2	1169	5.3	3182	18.6	200	6.3	40	0.6	0	0
IR	1477	4.5	287	19.4	167	1.0	43	25.7	34	0.5	1	2.9
Parallel-Other	5972	18.1	586	9.8	793	4.6	58	7.3	23	0.4	1	4.3
Totals	**32,973**	100	2,284	6.9	**17,077**	100	718	4.2	**6,352**	100	115	1.8

Monthly reports available on GOV.UK

Animal & Plant Health Agency

Scotland gamma use 2020

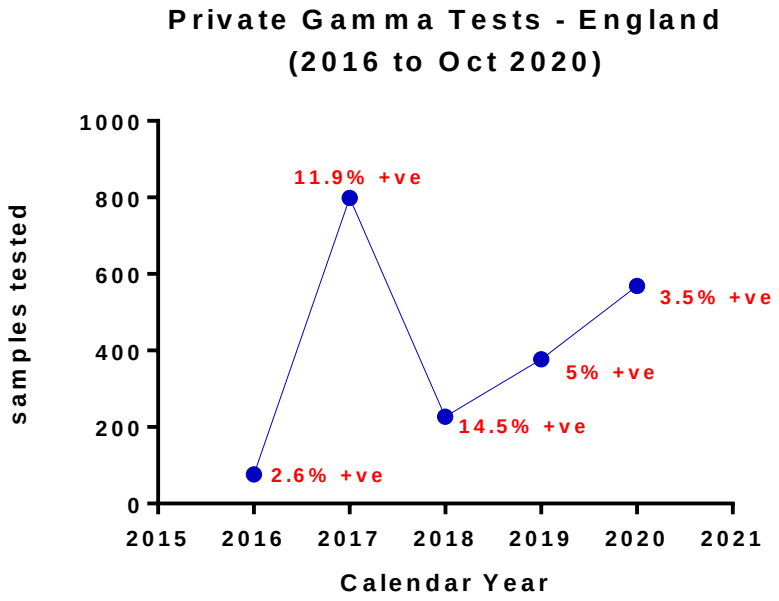
Number of samples tested under each test reason as % of total samples tested and with % test-positive within each test reason

Test Reason	No. samples	%	Positive samples	% pos
New breakdown	5221	83.2	115	2.2
Persistent breakdown	249	4.0	27	10.8
Parallel Other	782	12.5	15	1.9
Serial NSR	27	0.4	4	14.8
Totals	**6,279**	**100**	**161**	**2.6**

Scotland is officially TB-free, but has occasional new breakdowns, most due to inward movement of cattle.

Monthly reports available on GOV.UK

Private gamma testing also available at APHA (England only)



Permission to test has strict criteria – see http://apha.defra.gov.uk/vet-gateway/ifng-testing/index.htm

Small number tests carried out, but test-positivity shows that infected cattle are being identified (test-positivity well above the 3.5% false-positive level of the test)
Some of these are **sales movement tests**, **IR**s, or additional individuals not eligible for statutory testing

Animal & Plant Health Agency

Cattle IDEXX antibody test

Animal & Plant Health Agency

Cattle – Serum Antibody Testing

Measures *M. bovis*-specific antibody
Antibody levels can be low
Skin test boosts specific antibody to detectable level (sample within 10-30 days). Without a prior skin test, antibody detection (test sensitivity) can be poor

Antibody tests for bovine TB are;

- Generally less sensitive than the gamma test
- Dependent on skin test status – SICCT positive infected cattle are more likely to be seropositive (gamma test works equally well in SICCT positive or SICCT- infected cattle)
- Not a marker of advanced disease; seropositives usually NVL

BUT we recognise that antibody tests can have use in chronic/persistent breakdown herds to identify small numbers of skin- and gamma-negative infected individuals. APHA generally use IDEXX as a **third-line test** (i.e. after skin & gamma) on a case-by-case basis

Animal & Plant Health Agency

IDEXX M. bovis ELISA Test – OIE-registered [2012]

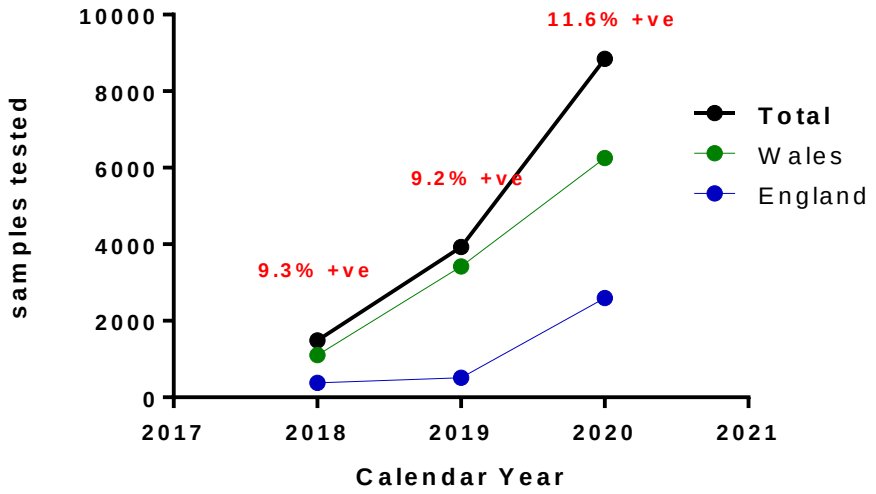
Se: 64.65% [59.7-69.5], **Sp: 98%** [97.5-98.4]
Measures antibodies to two immuno-dominant antigens **MPB83** & **MPB70**

Year	Samples tested	Samples positive	% positive
2018	1489	139	9.3
2019	3931	360	9.2
2020	8845	1030	11.6

Test-positivity well above test specificity [2% false positive] - IDEXX proving useful at identifying additional infected skin- and gamma-negative cattle

Animal & Plant Health Agency

APHA Cattle IDEXX antibody testing is increasing



Wales uses more IDEXX tests than England following policy changes for application to certain groups of high risk cattle (e.g. IRs).

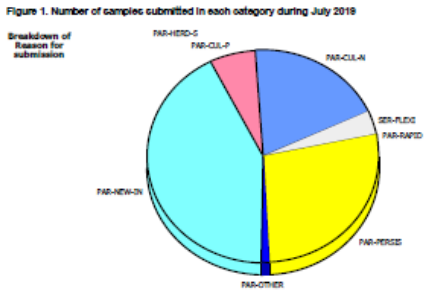
England use remains *ad hoc* and focussed usually on small groups e.g. older milkers within selected persistent breakdown herds, and occasionally suspect youngstock (<6m old, for which gamma test is unsuitable)

Test-positivity is well above the specificity of the test, identifying cattle that might otherwise be missed

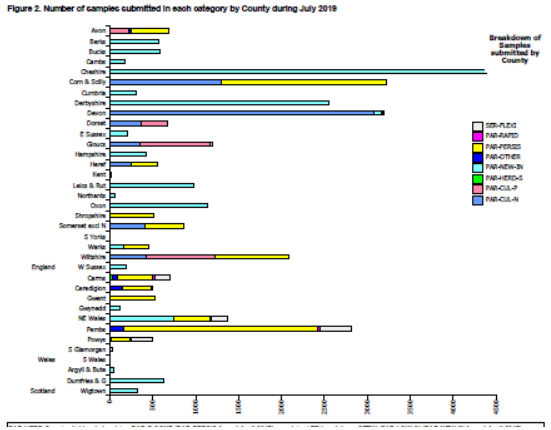
Animal & Plant Health Agency

Summary

- Diagnostic TB blood tests used by APHA are validated for use in GB
- Their performance and their limitations (Se & Sp) are well described and are applied in a way that gets the best out of them
- They are effective in detecting infection that would otherwise be missed by the skin test alone
- They make a valuable contribution to TB eradication



PAR-CUL-N	6170	19.0 %
PAR-CUL-P	2137	6.6 %
PAR-HERD-S	33	0.1 %
PAR-NEW-IN	13695	42.0 %
PAR-OTHER	396	1.2 %
PAR-PERSIS	8996	27.5 %
PAR-RAPID	83	0.3 %
SER-FLEXI	1079	3.3 %
Total	32549	100.00 %



Monthly Report: Surveillance Project SB4008 IFNg tests for bovine tuberculosis

(TC0651 and TC0751)

Number 155

Report Period 1st - 31st July 2019

Issued 27 August 2019