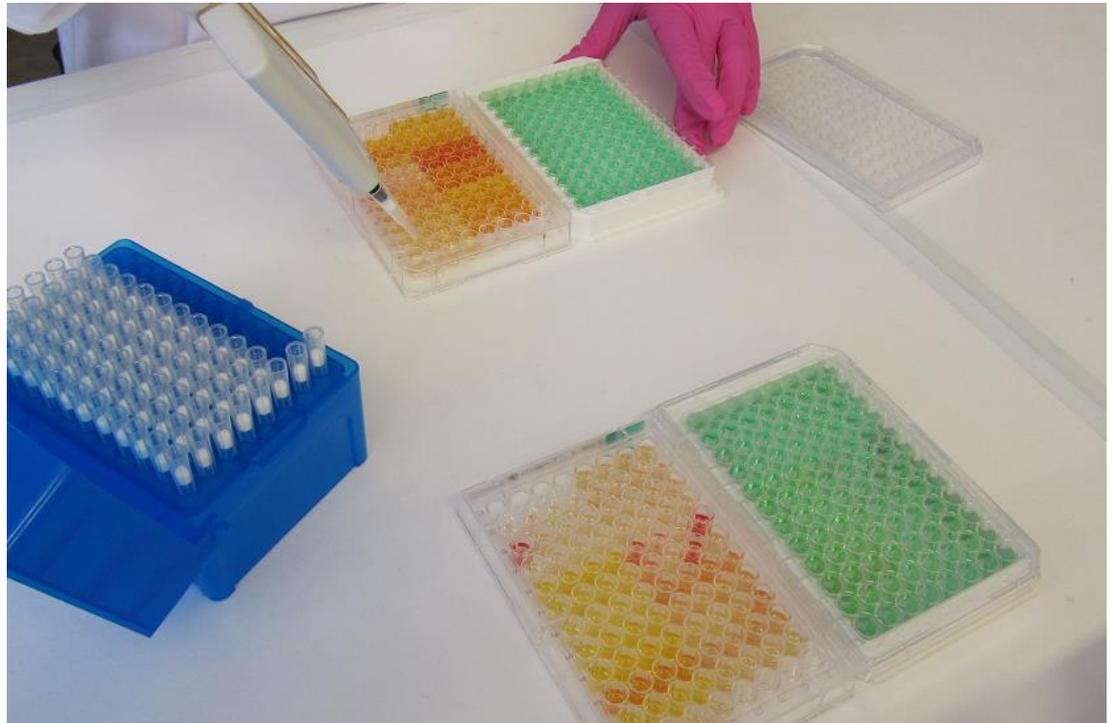


Interferon-gamma blood test

What is it and why do we use it?



Animal &
Plant Health
Agency

July 2021

- **What is it?**
- **How does it work?**
- **How accurate is it?**
- **Why do we use it?**
- **When do we use it?**



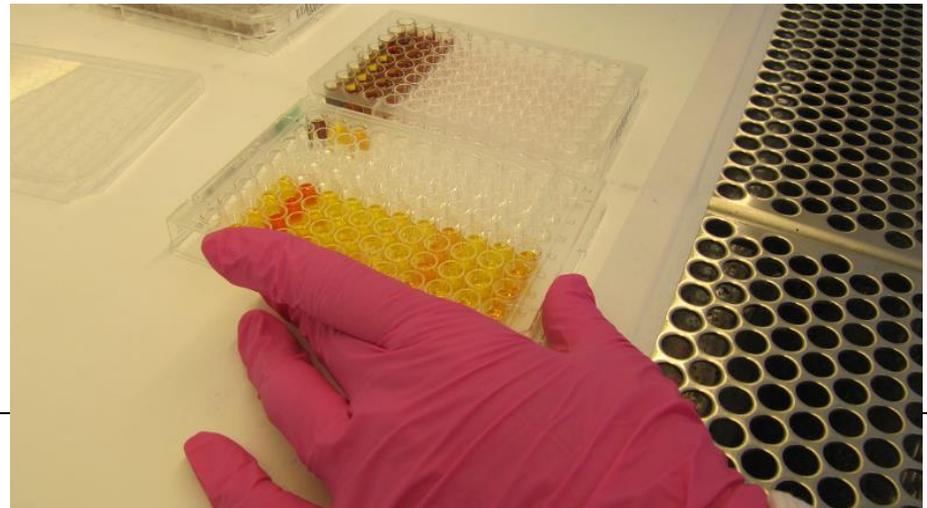
IFN γ test

- Supplementary blood test used in addition to the tuberculin skin test
- Approved for use in cattle under EU legislation and by the OIE (World Organisation for Animal Health)
- It's the only blood test approved in the UK and the EU to supplement the skin test for TB in cattle



Why do diagnostic tests for bovine TB need to be approved?

- Diagnostic tests need to be fully **validated** as per OIE guidelines, so that we can interpret the results that they generate
- Validation involves multiple steps to assess the diagnostic performance characteristics of the test
- The test must also be shown to be repeatable and standardised



- Tests for bovine TB need to be approved under EU legislation for trade purposes and to establish the official disease status of herds
- The IFN γ test, IDEXX antibody test and Enferplex antibody test are the only blood tests currently listed by the OIE as validated for TB in cattle

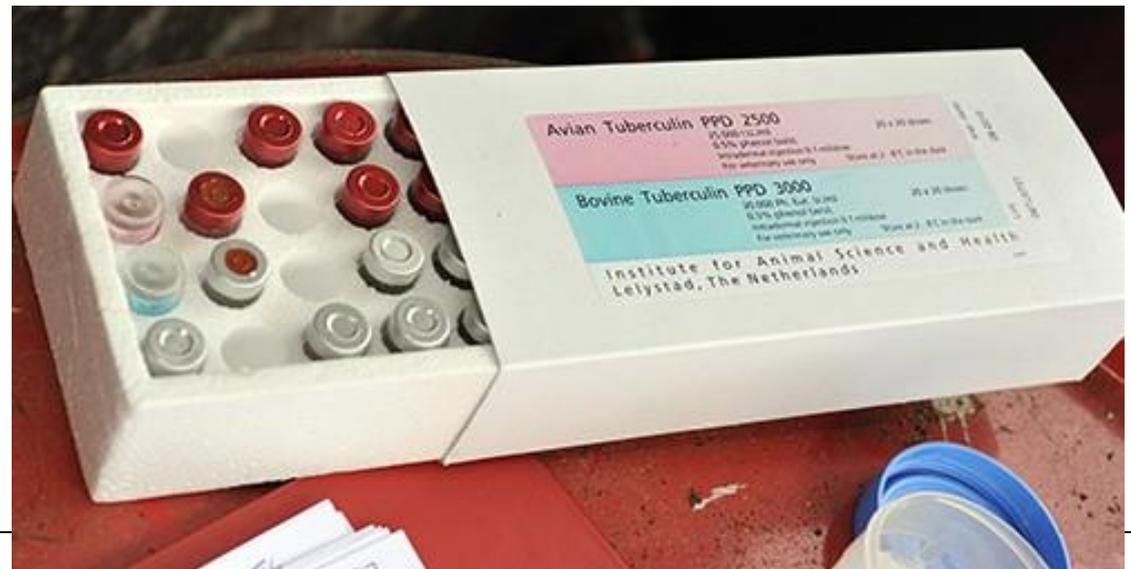


How does the IFN γ test work?

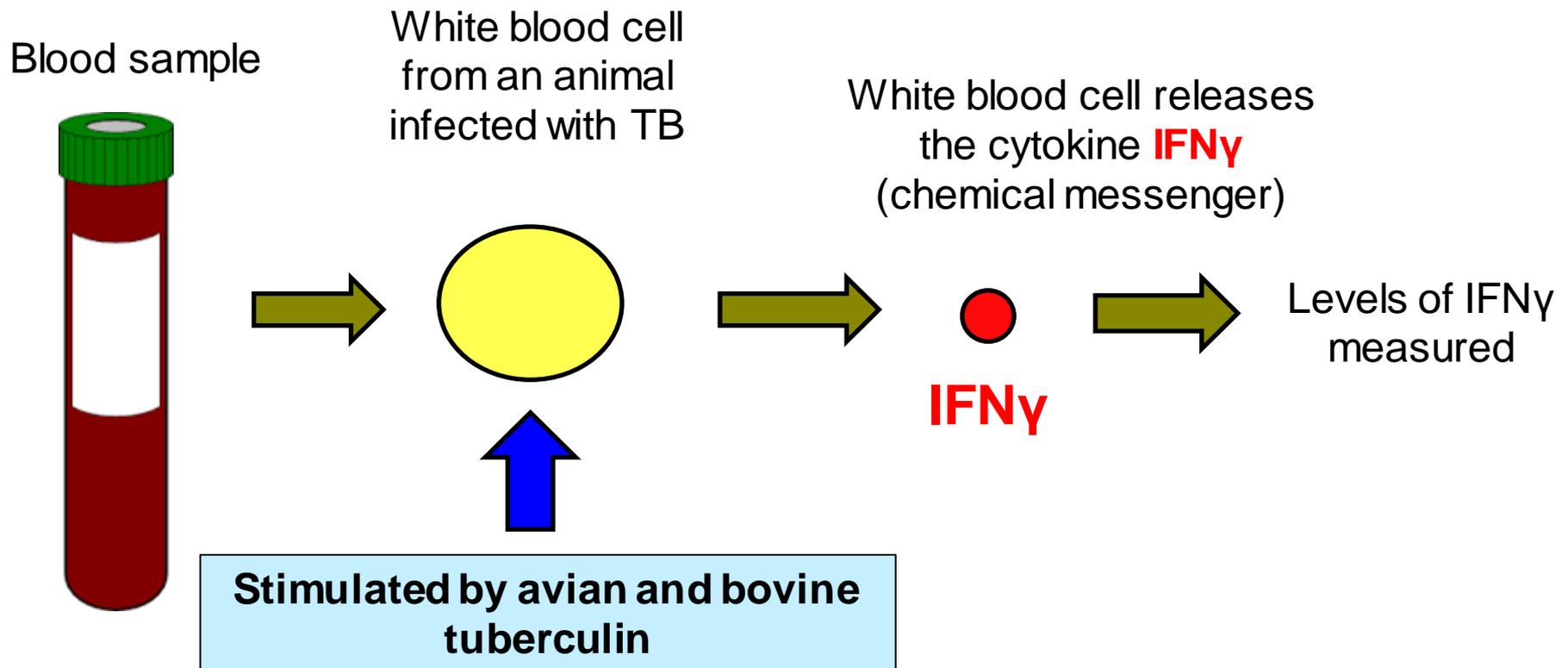
The tuberculin skin test and IFN γ test both measure the **host's immune response** to the TB bacterium (*Mycobacterium bovis*), rather than detect the bacterium itself



- The IFN γ test is a **comparative** test like the skin test
- It measures the animal's immune response to avian and bovine tuberculins (same as used in the skin test)
- Tuberculins are mixtures of proteins extracted from cultures of *M. bovis* bacteria grown in liquid media in the laboratory, and then killed by heat



Instead of injecting tuberculin into the skin, the animal's blood is stimulated with avian and bovine tuberculin in the laboratory



- Blood samples from TB-infected animals will release greater amounts of IFN γ in response to bovine tuberculin compared to avian tuberculin
- Being a laboratory-based test, it is subject to **strict quality controls**, its readout is more objective and its protocol is easier to standardise compared to the skin test
- Samples need to be transported to the lab quickly in temperature-controlled packaging systems as the white blood cells must be kept alive



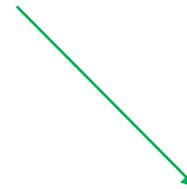
How accurate is it?

No diagnostic test is perfect!

**There is always a trade off between
sensitivity and specificity**



Find all infected animals



Don't remove uninfected
animals

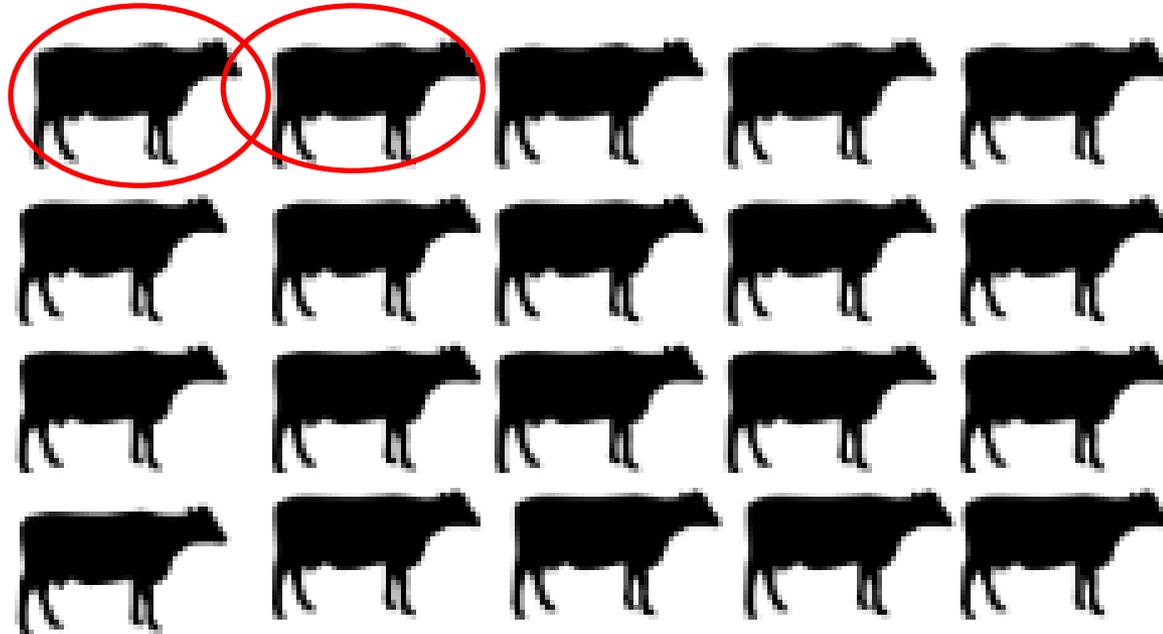
90%

Sensitivity

The probability that a test will correctly identify an infected animal as positive

The higher the sensitivity of the test, the lower the probability of incorrectly classifying an infected animal as uninfected (a false negative result)

The IFN γ test is **90% sensitive** and will miss, on average, 10% of infected animals in a herd

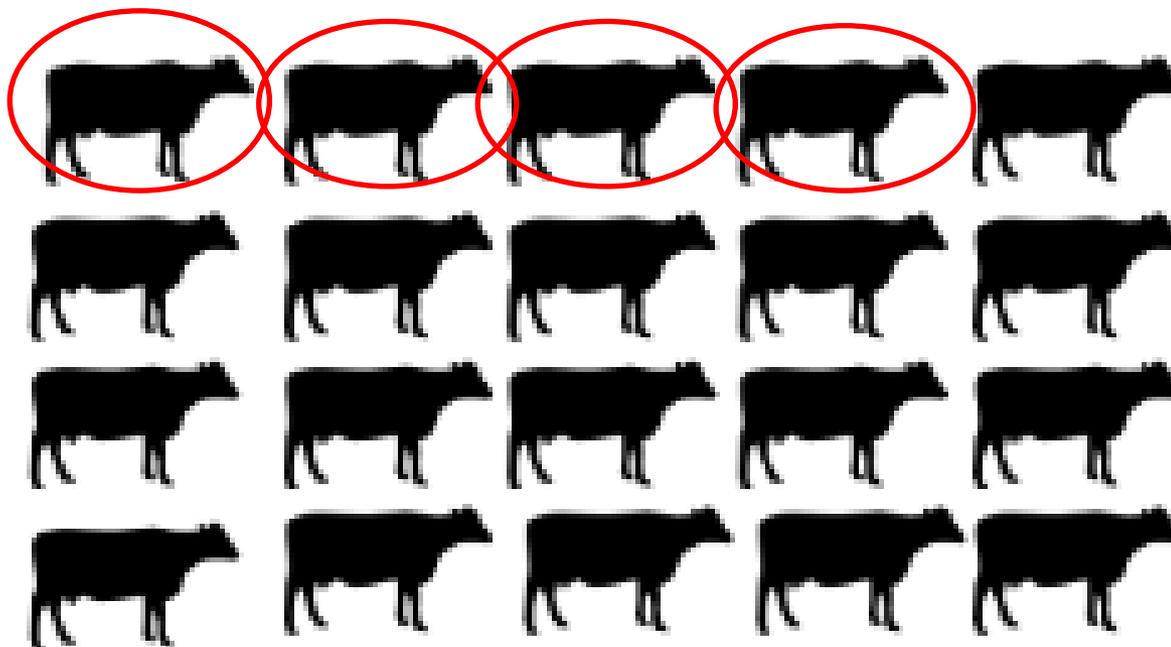


20 truly infected animals – 2 missed by the IFN γ test

How does this compare with the skin test?

The IFN γ test is **more sensitive** than the skin test

The skin test is 80% sensitive at standard interpretation and will miss, on average, 20% of infected animals



20 truly infected animals – 4 missed by the skin test

96.5% (minimum)

Specificity

The probability that a test will correctly identify an animal that is free from infection as negative

The higher the specificity, the lower the probability of incorrectly classifying an uninfected animal as infected (a false positive result)

How does it compare to the skin test?

- With the IFN γ test you expect to get, on average, 3-4 false positives per 100 disease-free animals tested (false positive rate of 3.5%)
- This means that the IFN γ test is **less specific** than the skin test
- The skin test has a higher specificity of 99.98%, which means you only get one false positive per 5,000 disease-free animals tested (false positive rate of 0.02%)

So why use IFN γ if there is more chance of false positives?

- Due to a higher probability of false positive results, the IFN γ test is not used for testing of officially TB-free (OTF) herds
- In most cases it is only applied to TB breakdown herds with lesion and/or culture positive animals
- In these herds, the probability that a test-positive animal indicates a true infection is higher than in OTF herds, and the risk of taking out low numbers of false positives is outweighed by the much higher risk of false negatives and the need to identify and remove all infected cattle



So why use IFN γ if there is more chance of false positives?

- The overall proportion of IFN γ test-positive results in England in 2020 was 4.0%*
- The IFN γ test is used in a targeted, proportionate way in TB breakdown herds to maximise the sensitivity of testing
- The IFN γ test policy must balance costs and benefits in the context of the strategy for achieving officially TB free status for England

*Interferon-Gamma Testing for Bovine Tuberculosis - Monthly Reports from April 2016

<https://data.gov.uk/dataset/f48e8d0a-f527-4f69-8aff-b07db7c26f09/interferon-gamma-testing-for-bovine-tuberculosis-monthly-reports-from-april-2016>



What are the benefits?

The skin and IFN γ tests are used together to **maximise the sensitivity** of testing and increase the chances of finding all infected animals in the herd



- The IFN γ test can identify cattle at an **earlier stage of infection** than the skin test (experimental cattle infection shows 3-4 weeks vs. 7 weeks)
- Studies in GB, NI & RoI consistently show that IFN γ -positive but skin test-negative cattle have a higher risk of becoming skin test reactors and develop visible lesions if not removed (compared with IFN γ and skin test-negative animals in the same herds)



- Using the IFN γ test at the beginning of a new TB breakdown can shorten the duration of movement restrictions by increasing detection of infected animals
- It can also reduce the likelihood of residual cattle infection in herds that regain OTF status after a breakdown
- In 2019, 57.7% of infected herds in the High Risk Area (HRA) had at least one other breakdown in the past three years*



*Bovine tuberculosis in England in 2019 - Epidemiological analysis of the 2019 data and historical trends

<https://www.gov.uk/government/publications/bovine-tb-epidemiology-and-surveillance-in-great-britain-2019>



What about 'no visible lesion' (NVL) IFN γ and skin-test positive animals?

If a skin test or IFN γ positive animal does not have visible lesions at slaughter, it does not mean that it wasn't infected



- Post mortem meat inspection is a relatively insensitive method of confirming TB infection
- Test positive (but NVL) animals may have been in the early stages of infection when TB lesions were too small to see with the naked eye or not yet developed in the organs
- Small lesions can be missed at the slaughterhouse
- Can get genuine false positives (around 3.5%), i.e. the animal was not infected but tested positive

What about further IFN γ positive results after a skin test?

- Residual infection, or the reintroduction of infection can cause more test-positives downstream
- The skin & IFN γ tests identify slightly different populations of infected individuals
- Overall in a confirmed infected group, while most infected animals will be identified by both tests, there will always be animals picked up by one test but not the other
- Infected individuals in a herd will not all be at the same stage of infection. So while removing those that have reacted to the skin or IFN γ test, there may still be others that have only just been infected

When do we use the IFN γ test?

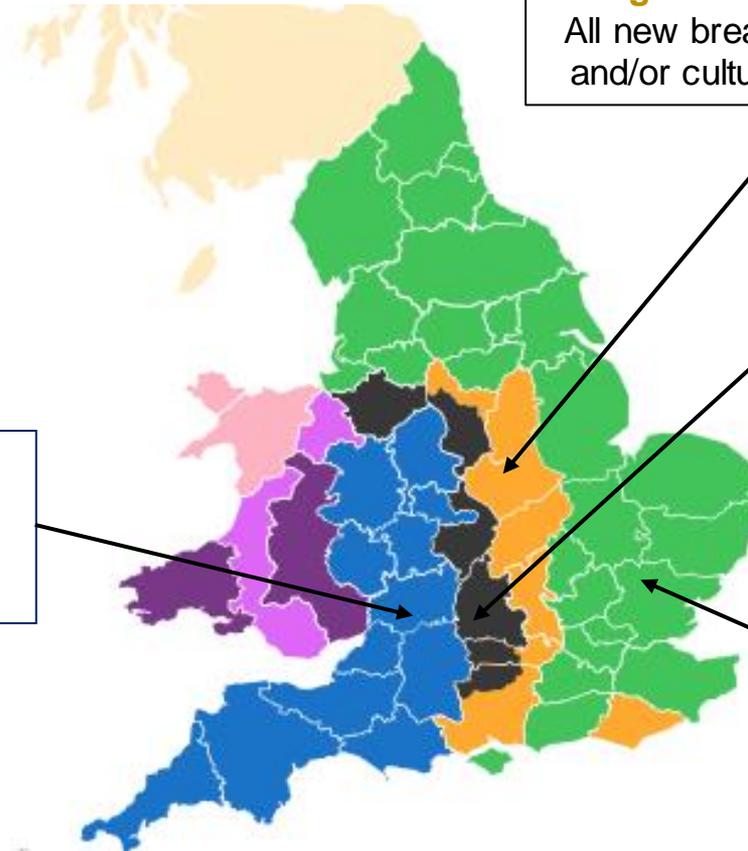
Persistent TB breakdowns with lesion and/or culture positive animals in all risk areas

Edge Area (annual testing)
All new breakdowns with lesion and/or culture positive animals

Edge Area (six-monthly testing)
Recurrent TB breakdowns with lesion and/or culture positive animals

High Risk Area
Recurrent TB breakdowns with lesion and/or culture positive animals

Low Risk Area
All new breakdowns with lesion and/or culture positive animals



Edge Area

Annual surveillance testing parts

All new breakdowns with lesion and/or culture positive animals require a mandatory herd IFN γ test

Six-monthly surveillance testing parts

From 12 July 2021, all new breakdowns with lesion and/or culture positive animals are assessed against the following criterion for recurrence. If a herd meets the criterion then it will require a mandatory IFN γ test:

The breakdown occurred within 18 months of the herd regaining officially TB free (OTF) status following a previous breakdown with lesion and/or culture positive animals.

High Risk Area (HRA)

From 12 July 2021, all new breakdowns with lesion and/or culture positive animals are assessed against the following criterion for recurrence. If a herd meets the criterion then it will require a mandatory IFN γ test:

The breakdown occurred within 18 months of the herd regaining officially TB free (OTF) status following a previous breakdown with lesion and/or culture positive animals.

More information about the IFN γ testing policy in England can be found on the TB hub

<https://tbhub.co.uk/tb-policy/england/refinements-to-the-interferon-gamma-testing-policy-in-the-high-risk-and-edge-area-of-england/>



Persistent TB breakdowns

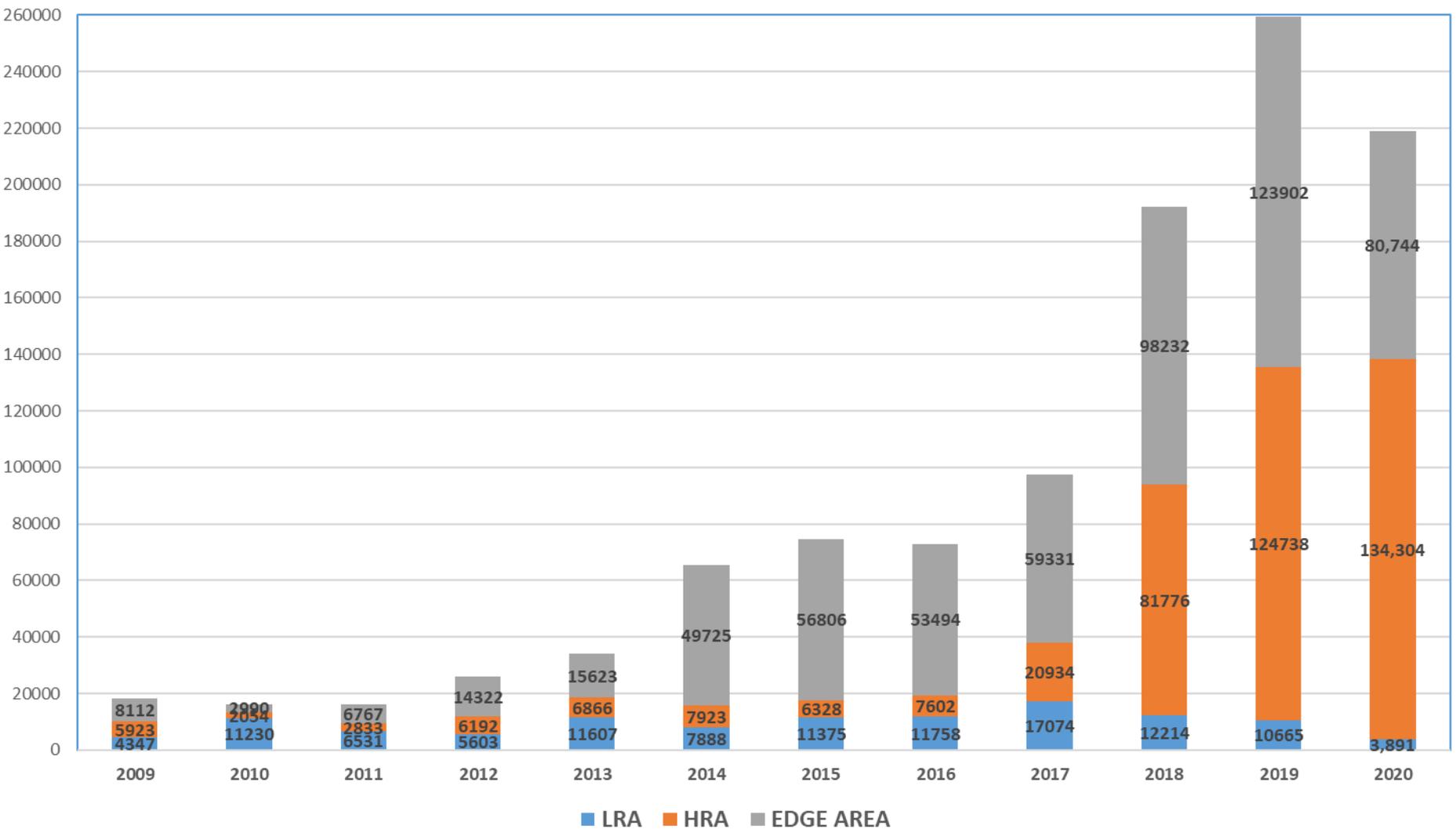
- Persistent TB breakdown herds are those that have been under movement restrictions for at least 18 months
- These herds are assessed at 17 months under restrictions for a mandatory herd IFN γ test
- Owners of persistent breakdown herds receive a letter at 12 and 18 months under restrictions advising of the potential need for a mandatory IFN γ test
- There are also other interventions that keepers are expected to implement alongside supplementary blood testing, such as enhancing on-farm biosecurity.
- Further advice and support can be sought from APHA, private vets and the TB Advisory Service

Discretionary use by APHA

- Discretionary IFN γ testing is applied in certain situations where the TB breakdown is not automatically eligible for mandatory testing, e.g. in breakdowns with high numbers of reactor animals
- Also used by APHA in other ad hoc scenarios e.g. informing decisions about partial or whole herd slaughter for TB control reasons.



Number of IFNG tests completed in England by bTB Risk Area (Jan 2009 - Dec 2020)



De-coupling of skin and IFN γ tests

In England, IFN γ testing in TB breakdown herds is undertaken as soon as practicably possible for disease control reasons:

- Increases the chances of finding infected animals. The IFN γ test is more sensitive than the skin test and identifies a different population of infected animals
- IFN γ test can identify cattle at an earlier stage of infection than the skin test – infected animals are removed earlier (less time for spread of infection to other cattle and wildlife)
- Potentially reduces the duration of the breakdown and the chances of residual infection remaining when movement restrictions are lifted

De-coupling of skin and IFN γ tests (2)

- To achieve this, the default position is to 'de-couple' the IFN γ test from the skin test and complete it as a stand-alone test as soon as practicably possible, giving the farmer a reasonable period of notice
- By de-coupling, we are essentially removing infected animals that were missed by the previous skin test earlier, thus maximising the probability that the herd goes clear at its next skin test
- On some occasions it may not be practical to decouple e.g. if the IFN γ test is to be undertaken very close to the next skin test

- Animals under six months of age are excluded (their developing immune system interferes with the test)
- TB breakdown herds eligible for IFN γ testing will initially undergo one round of blood testing
- The IFN γ test is repeated up to three times as long as standard interpretation skin test reactors and/or animals with visible lesions (including slaughterhouse cases) continue to be found after application of the blood test
i.e. if there is still evidence of **residual infection** in the herd which was not picked up by previous skin and IFN γ testing



Private IFN γ testing (England only)

- Private vets can submit samples for IFN γ test at keeper's cost with prior approval from APHA
- Limited to specific scenarios outside of the government-funded testing programme where owners seek additional assurances as to the TB-free status of animals over and above statutory testing
 - e.g. to supplement pre/post movement testing, screen animals joining high value herds, test animals following a negative routine or tracing skin test

More information at:

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

Private IFN γ testing (2)

Cattle not eligible for private IFN γ testing:

- Test reactors & other cattle awaiting slaughter for TB control purposes
- Cattle from TB breakdown herds undergoing government-funded IFN γ testing
- Cattle from herds under restrictions for overdue skin testing

If a **positive result** is received, the animal is (with few exceptions) compulsorily slaughtered with compensation paid, the herd will be placed under movement restrictions and normal breakdown procedures followed.

Private IFNy test cost (as of July 2020)

Cost per test (£)	Single test	5+ tests	10+ tests
High specificity test	24.00	19.50	18.40
High sensitivity test	17.50	14.10	13.00

Take-home messages

- IFN γ test is **more sensitive** than the skin test. It can identify TB infected cattle that are earlier in the course of infection and those missed by the skin test
- It is **less specific** than the skin test and so it is used in TB breakdown herds with lesion and/or culture positive animals. It cannot be used for surveillance testing or as a replacement for the skin test
- **IFN γ test positive animals do not necessarily show visible lesions at post mortem.** This is because they are often in the early stages of infection when TB lesions are too small to be seen by the naked eye and sometimes lesions are missed at the slaughterhouse

Further information

More information on bovine TB, including further background on the IFN γ test is available on the TB hub.



<http://www.tbhub.co.uk/>

The TB Advisory Service provides free bespoke advice via advisory visits and over the phone in England.

<http://www.tbas.org.uk/>

