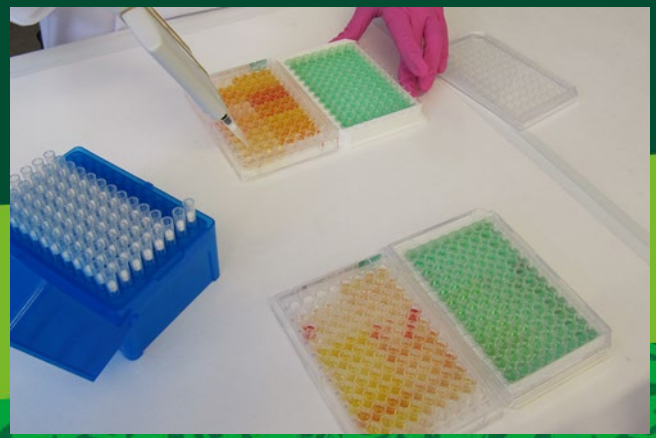
Interferon-gamma (IFNγ) blood test

What is it and why do we use it?



July 2021 (updated June 2026)

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IFNγ test

- A supplementary blood test for TB used in addition to the tuberculin skin test
- Approved for use in cattle under EU legislation and by the WOAH (World Organisation for Animal Health)
- It is the only blood test approved in the EU (and one of two blood tests approved in GB) to supplement the skin test for TB in cattle, water buffalo and farmed bison

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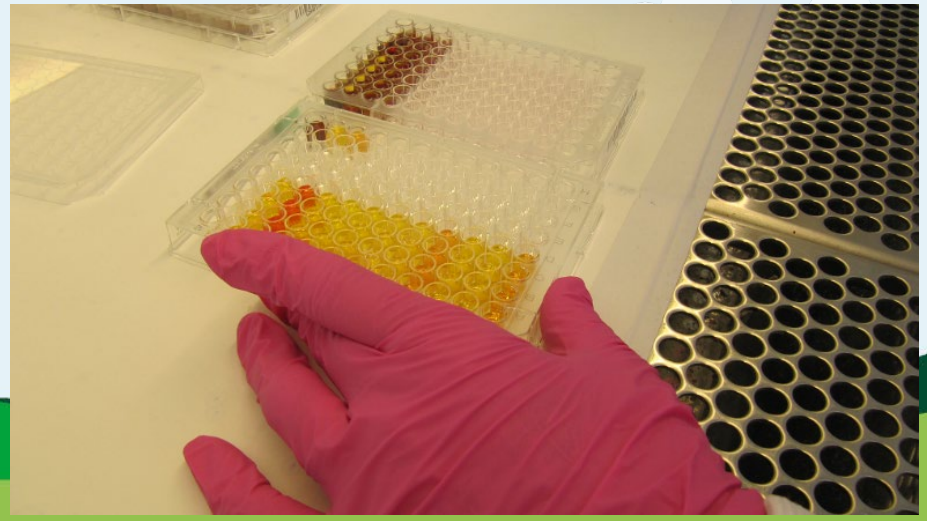
Why do diagnostic tests for bovine TB need to be approved?

- Diagnostic tests need to be fully **validated** as per WOAH guidelines, so that we can confidently interpret the results that they generate
- Validation involves multiple steps to assess the diagnostic performance characteristics of the test in the population of interest
- The test must also be shown to be standardised and repeatable, yielding consistent results when performed in different laboratories



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

World Organisation for Animal Health

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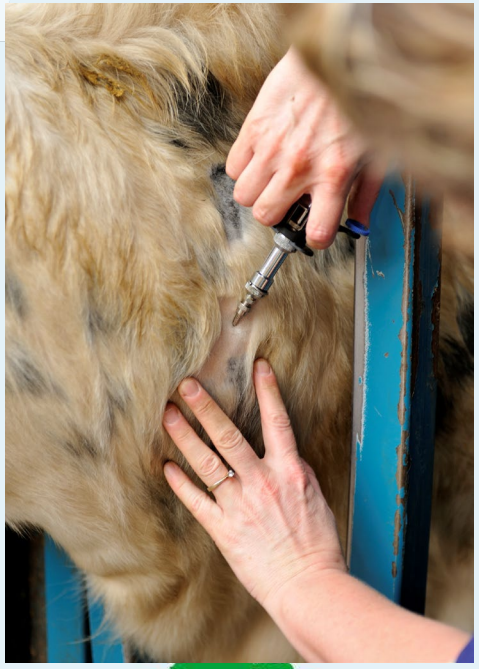
How does the IFNγ test work?

The IFNγ test, like the tuberculin skin test, measures the animal's **cell-mediated immune response** to the bovine TB bacterium (*Mycobacterium bovis* or *M. bovis*), rather than detect the bacterium itself



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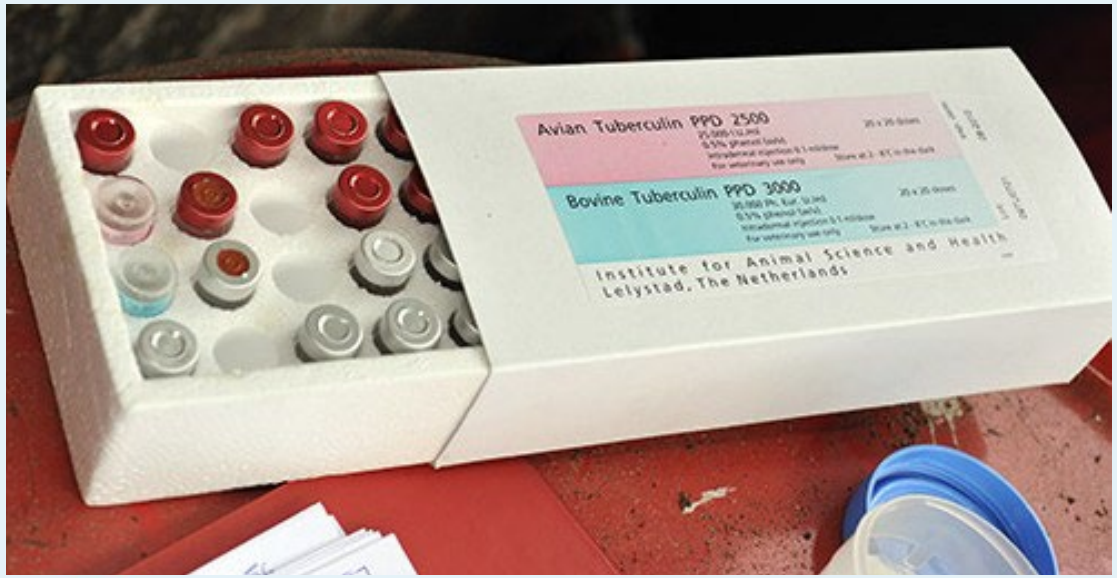
- The IFNγ test is a **comparative** test like the skin test
- It measures the animal's immune response to avian and bovine tuberculins (same as used in the skin test)
- Tuberculins are sterile, purified mixtures of proteins extracted from cultures of *M. bovis* and *M. avium and* bacteria grown in liquid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



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

Blood sample

White blood cell from an animal infected with TB

White blood cell releases the cytokine **IFNγ** (chemical messenger)

IFNγ

Levels of IFNγ measured

Stimulated by avian and bovine tuberculin

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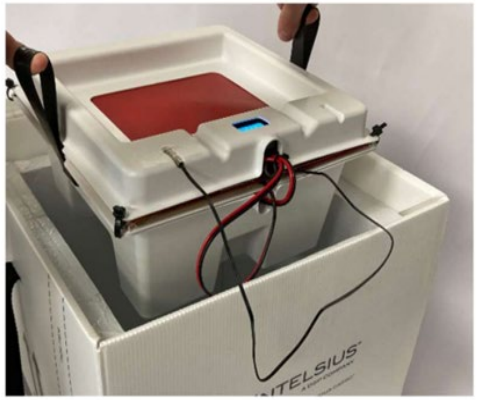
- Blood samples from *M. bovis*-infected animals sensitised to tuberculin will release greater amounts of IFNγ in response to bovine tuberculin compared to avian tuberculin
- Being a laboratory-based test, it is subject to **strict quality controls**, its readout is more objective, and its protocol is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



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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 wrongly classify uninfected animals as infected

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IFNγ test has 90% sensitivity

Or this is the probability that the 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)

This percentage is the national average sensitivity for the national cattle population in GB derived from a sample of selected infected and OTF herds, but the actual diagnostic performance of the IFN-γ test will vary from herd to herd.

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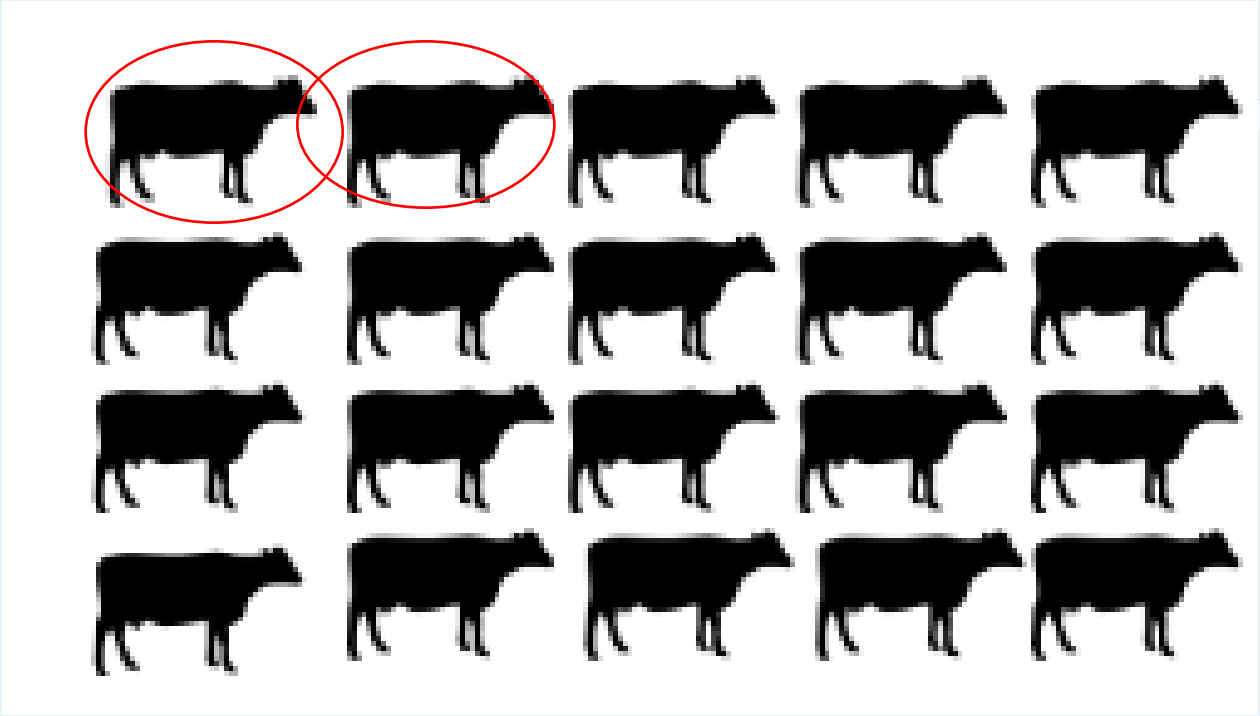
The IFNγ test is **90% sensitive** and will miss, on average, 10% of infected animals in a herd



20 truly infected animals – 2 missed by the IFNγ test

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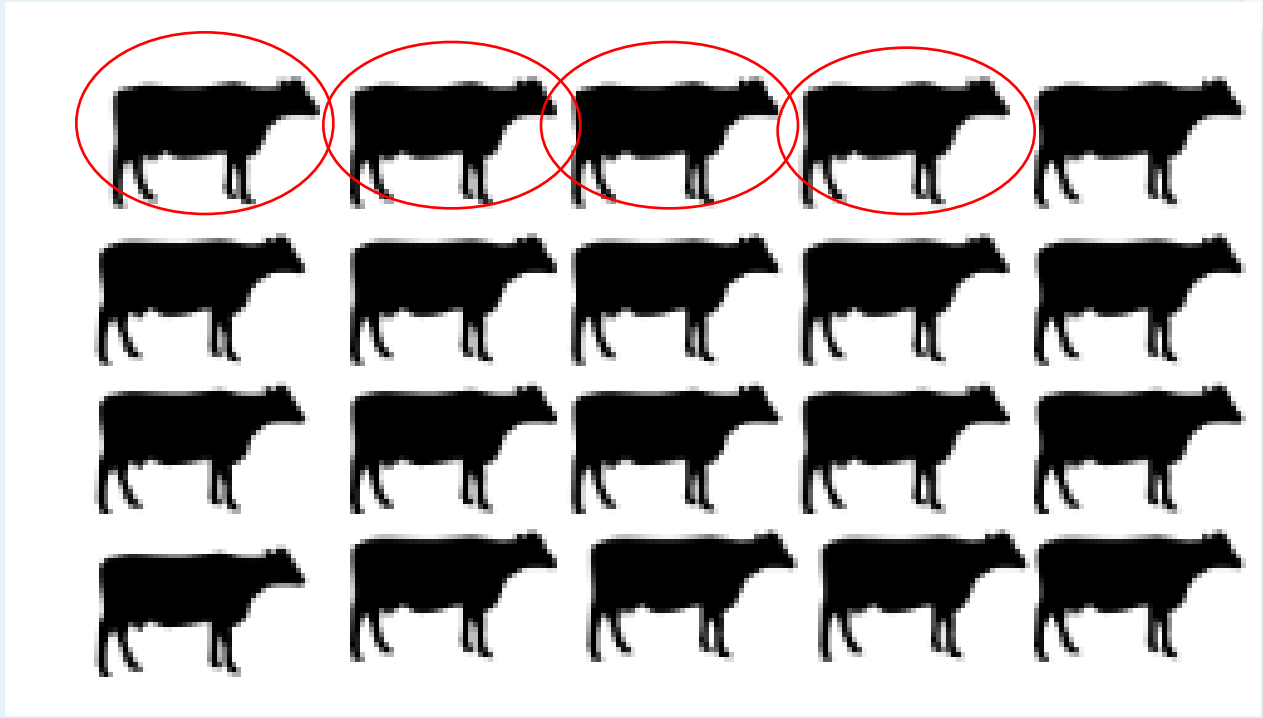
How does this compare with the skin test?

The IFNγ 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

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IFNγ test has 96.5% (minimum) specificity

Or this is the probability that the 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)

Again, this percentage is the national average specificity for the national cattle population in GB derived from a sample of selected infected and OTF herds, but the actual diagnostic performance of the IFN-γ test will vary from herd to herd.

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How does it compare to the skin test?

- With the IFNγ 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 IFNγ 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 result per 5,000 disease-free animals tested (false positive rate of 0.02%)

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So, why use IFNγ if there is a higher probability of false positives?

- Due to a higher probability of false positive results, the IFNγ 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 PCR(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

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So, why use IFNγ if there is a higher probability of false positives?

- The overall proportion of IFNγ test-positive results in England in 2024 was 4.61%*
- The IFNγ test is used in a targeted, proportionate way in TB breakdown herds to maximise the sensitivity of testing
- The IFNγ 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 January 2024
https://www.data.gov.uk/dataset/7182ee44-b080-4573-8bb5-fb1158348a2a/interferon-gamma-testing-for-bovine-tuberculosis-monthly-reports-from-january-2024

What are the benefits?

The skin and IFNγ tests are used together to **maximise the sensitivity** of testing and increase the probability of finding all infected animals in the affected herd

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What are the benefits? (2)

- The IFNγ test can identify cattle at an **earlier stage of infection,** as early as 3-4 weeks post exposure, while the skin test generally detects infection a bit later, from 6 weeks post exposure
- Studies in GB, NI & RoI consistently show that IFNγ-positive (but skin test-negative) cattle have a higher risk of becoming skin test reactors and develop visible lesions if not removed, compared with IFNγ and skin test-negative animals in the same herds

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What are the benefits? (3)

- Using the IFNγ test at the beginning of a new TB breakdown can shorten its duration and the time under movement restrictions by accelerating the detection of infected animals
- It can also reduce the likelihood of residual infection in herds that regain OTF status after a breakdown
- In 2023, nearly 60% 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 2023 - Epidemiological analysis of the 2023 data and historical trends https://www.gov.uk/government/publications/bovine-tb-epidemiology-and-surveillance-in-great-britain-2023

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‘De-coupling’ of skin and IFNγ tests

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

- Increases the chances of finding infected animals. The IFNγ test is more sensitive than the skin test and identifies a different population of infected animals
- IFNγ 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 IFNγ tests (2)

- To achieve this, the default position is to 'de-couple' the IFNγ 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 IFNγ test is to be undertaken very close to the next skin test

What about ‘no visible lesion’ (NVL) IFNγ and/or skin-test positive animals?

If a skin test reactor or IFNγ test-positive animal does not have visible lesions of TB at slaughter it does not mean that it was not infected

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What about ‘no visible lesion’ (NVL) IFNγ and/or skin-test positive animals (2)?

- 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 test false positives i.e. the animal was not infected but tested positive

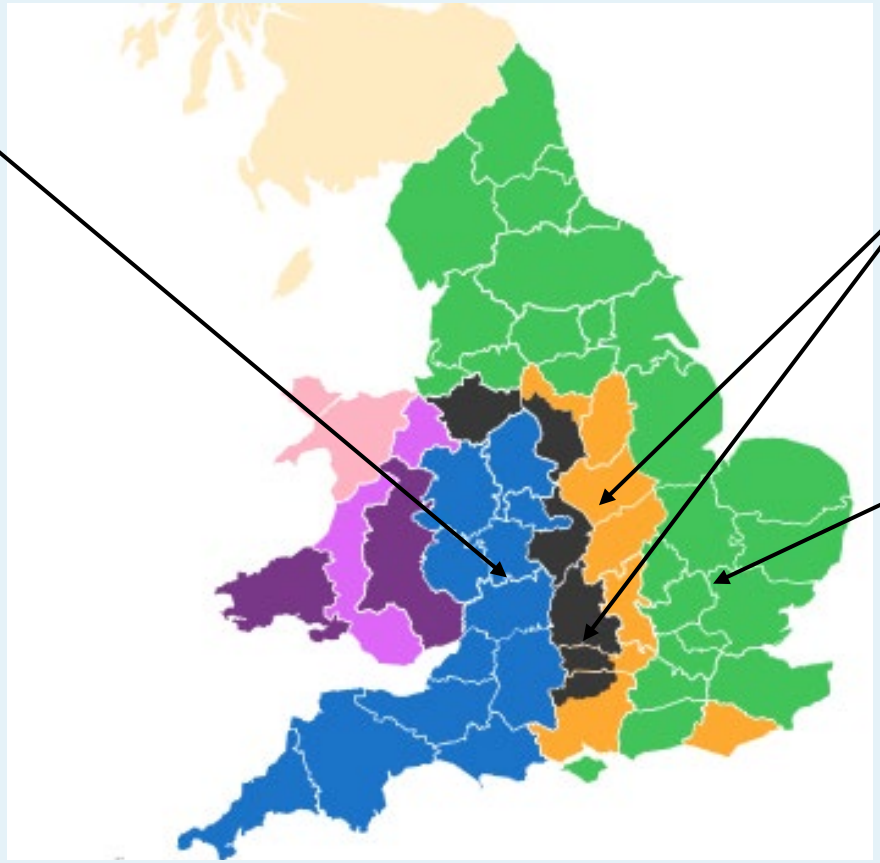
What about further IFNγ positive results after a skin test?

- Residual infection, or the reintroduction of infection in the herd is often a reason for further positives
- The skin & IFNγ tests identify slightly different populations of infected individuals
- 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. Among those that have reacted to the skin or IFNγ test, there may still be others that have only just been infected and not yet detectable by either test

When do we use the IFNγ test?

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

Since **1 September 2025,** APHA offers Government funded IFNγ testing on voluntary basis to keepers of herds in England with lesion and/or PCR (or culture) positive animals that do not qualify for compulsory testing



Edge Area
All new breakdowns with lesion and/or PCR (or culture) positive animals (**from 1st July 2026**)
* *Prior to this date the recurrence criteria applies to the **six-monthly** testing parts (as per High Risk Area)*

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

Chronic and persistent TB breakdowns with lesion and/or PCR (or culture) positive animals in all risk areas

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Edge Area

Annual surveillance testing parts

All new breakdowns with lesion and/or PCR (or culture) positive animals require a mandatory herd IFNγ test

Six-monthly surveillance testing parts

From 01 July 2026 (and before Jul 2021), all new breakdowns with lesion and/or PCR (or culture) positive animals require a mandatory herd IFNγ test.

Between July 2021 and July 2026, all new breakdowns with lesion and/or PCR (or culture) positive animals are assessed for recurrence as defined below:

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.

If a herd meets this criterion, then it will require a mandatory IFNγ test

High Risk Area (HRA)

Since July 2021, all new breakdowns with lesion and/or PCR (or culture) positive animals are assessed for recurrence as defined below:

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.

If a herd meets this criterion, then it will require a mandatory IFNγ test

More information about the IFNγ 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/

Chronic TB breakdowns

- Chronic TB breakdown herds are those that have lasted for less than 18 months but have had
  - one or more VL and/or PCR (or culture) positive reactors and/or unresolved IRs at a third (or later) short interval test (SIT), and/or
  - a PCR (or culture) positive TB slaughterhouse or clinical case identified after the second (or later) SIT
- Chronic OTFW breakdown herds meeting the above criteria must undergo IFN-y testing.

Persistent TB breakdowns

- Persistent TB breakdown herds are those that have lasted for at least 18 months
- These herds are assessed at 17 months under restrictions for a mandatory herd IFNγ test
- Owners of persistent breakdown herds receive a letter at 12 and 18 months under restrictions advising of the potential need for a mandatory IFNγ 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 IFNγ 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.
- APHA usually only apply IFNγ testing in OTFW TB breakdowns (those with lesion and/or PCR (or culture) positive animals). Exceptionally, IFNy testing is also applied in OTFS breakdowns herds (not disclosing lesion and/or PCR (or culture) positive animals).

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Other principles

- Animals under six months of age are excluded (their developing immune system interferes with the test)
- TB breakdown herds eligible for IFNγ testing will initially undergo one round of blood testing
- The IFNγ 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 IFNγ testing

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Voluntary IFNγ testing

- Since **1 September 2025**, APHA offers Government funded IFNγ testing on voluntary basis to keepers of herds in England with lesion and/or PCR (or culture) positive animals that do not qualify for compulsory testing.

Basic principles:

- The herd must be in the HRA (or in the six-monthly testing parts of the Edge area of England if the breakdown became OTFW before 1st July 2026)
- The herd must have had at least one skin test reactor with visible lesions of TB and/or animals with PCR (or culture) positive results disclosed at some point during the breakdown.
- Only animals aged six months or older are eligible.
- The IFNγ test will be applied to the whole herd, any exemptions from this must be approved by an APHA vet. Exclusion of newly purchased or fattening animals very close to slaughter still apply.

Voluntary IFNγ testing (2)

Herds are not eligible for voluntary IFNγ testing, if they are:

- pending just one clear short interval test (SIT) and they have not had any reactors at their last skin test
- only pending an inconclusive reactor (IR) retest, or
- the issue of TB10 lifting restrictions notice is only awaiting the submission of a BT05 cleansing and disinfection declaration

Any animals with a **positive IFNγ test result** are removed, and the usual valuation and compensation procedures apply.

Private IFNγ testing (England only)

- Private vets can submit samples for IFNγ testing 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:

https://www.gov.uk/guidance/private-interferon-gamma-ifn-y-testing-for-bovine-tuberculosis-tb-in-england

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Private IFNγ testing (2)

Cattle not eligible for private IFNγ testing:

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

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

Private IFNγ test cost (as of July 2025*)

Cost per test (£)	1 to 4 blood samples	5 or more blood samples
High specificity format (TC0751)	£30.00	£24.50
High sensitivity format (TC0651)	£23.60	£19.20
There is also a box return fee (TC0251) of £24.20 per box.		
*Please refer to latest rates at https://www.gov.uk/guidance/private-interferon-gamma-ifn-y-testing-for-bovine-tuberculosis-tb-in-england		

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Take-home messages

- The IFNγ 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 PCR (or culture) positive animals. It cannot be used for surveillance testing or as a replacement for the skin test
- **IFNγ 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.
- The IFNγ test is usually deployed **after at least one skin herd test**, which means that many infected animals with advanced TB pathology (visible lesions) will have already been removed from the affected herd.

Further information

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

TBhub
The home of UK TB information

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/

TBAS
TB ADVISORY SERVICE

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