Monday 18th November 2024

TB Diagnostic Tests

Definitions:

- **Sensitivity (Sn)** = ability of a test to detect all infected animals.
  - **Ratio of the true negatives/test negatives.**
  - When Sn is low, false negatives are high and infections go unrecognised within a herd.
  - When Sn is high, false negatives are low and infected animals are recognised and appropriately removed from the herd.
- **Specificity (Sp)** = ability of a test to rule out the disease.
  - **Ratio of the true positives/test positives.**
  - When Sp is low, false positives are high and more cattle and sent to slaughter without the disease.
  - When Sp is high, false positives are low and the majority of cattle testing positive and sent to slaughter are truly infected with bTB.
- **Positive Predictive Value (PPV)** = probability of an animal testing positive that is truly infected:
  - True positive/True positive + False positive
  - Statutory skin test is estimated to be 92% at standard interpretation in the high-risk areas of England[1].
    - This means the vast majority of skin reactors removed in these areas represent true infection with *M.bovis* even if the animals show no visible lesions at slaughter.
    - Values in the edge areas and low-risk areas are 88% and 77%, respectively.
- **Negative Predictive Value (NPV)** = probability of an animal testing negative is truly free from infection:
  - True negative/True negative + False negative

Diagnostic Tests

Test		How it works	Stage of Infection	Use	Sensitivity	Specificity
Tuberculin Tests	**Single Intradermal Comparative Cervical Test[1] (SICCT)**	Measures the difference in inflammatory reactions 72 hours post injection of avium and bovine tuberculin in the side of the neck (cell-mediated immunity). If an animals immune system has been sensitised by *M.bovis* infection or exposure to	Identifies positive animals 3-6 weeks post infection[2].	Statutory herd-level test in the UK.	**Standard interpretation** (4mm response difference) **81%** 1 in 5 cattle which are positive for the disease test negative. Some suggest that in field sensitivity of the test is even lower i.e., **53-69.2%[3].** This lack of sensitivity has been implicated in	**99.98%** 1 in 5,000 cattle without the disease test positive. False positives can be attributed to environmental mycobacteria contaminations and interference from Johnes disease due to their similar

[1] https://tbhub.co.uk/tb-testing-cattle/skin-testing/tuberculin-skin-testing/#:~:text=The%20primary%20screening%20test%20for,animals%20and%20people%20for%20TB.
[2] https://www.nadis.org.uk/disease-a-z/cattle/bovine-tb/
[3] https://www.visavet.es/bovinetuberculosis/animal-tb/diagnosis.php

Test	How it works	Stage of Infection	Use	Sensitivity	Specificity
	other mycobacteria, then a delayed type IV hypersensitivity reaction will take place and a lump forms at the injection site. The use of avian tuberculin is to limit the false positive interpretation from high levels of environmental (avian) mycobacteria. Depending on the interpretation (standard <4mm or severe <2mm difference) used depends if the difference between avian and bovine lumps yields a positive reactor.			the UK's lack of progress in bTB eradication compared to other countries[4]. False negative can arise by missing early infections (pre-allergic phase), desensitisation (tests performed to close together), severe bTB infection (reduced immune response, anergy), incorrect storage/usage of tuberculin and/or immune system suppression (age, drugs, other disease)[5].	antigenic composition[3].
				Severe Interpretation (2mm response difference)	
				85%	**99.91%**
	Single Intradermal Cervical Test (SICT)[6]	Measures the cell-mediated delayed type IV hypersensitivity against bovine tuberculin injected in the mid-cervical region with **no comparison** to avium tuberculin.	Statutory test in Europe. Required for animals >42 days old in OTF herds for export to EU. Not validated in the UK.	Range between **52-100%.**	**99.5%** Slightly reduced sensitivity can be mitigated when combined with interferon gamma testing as recommended in the Godfray report.
	Caudal Fold Test (CFT)	Measures the cell-mediated delayed type IV hypersensitivity	Positive animals are either retested using INF gamma/SICCT or	**63.2-93%[8]**	**89.2-99%[9]**

[4] de la Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Clifton-Hadley RS. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Res Vet Sci. 2006 Oct;81(2):190-210. doi: 10.1016/j.rvsc.2005.11.005. Epub 2006 Mar 2. PMID: 16513150.
[5] https://www.visavet.es/bovinetuberculosis/animal-tb/diagnosis.php
[6] https://www.visavet.es/bovinetuberculosis/animal-tb/diagnosis.php
[8] O'Brien, A., Clarke, J., Hayton, A. *et al.* Diagnostic accuracy of the Enferplex Bovine Tuberculosis antibody test in cattle sera. *Sci Rep* **13**, 1875 (2023). https://doi.org/10.1038/s41598-023-28410-9
[9] O'Brien, A., Clarke, J., Hayton, A. *et al.* Diagnostic accuracy of the Enferplex Bovine Tuberculosis antibody test in cattle sera. *Sci Rep* **13**, 1875 (2023). https://doi.org/10.1038/s41598-023-28410-9

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	against bovine tuberculin injected in the flap at the base of the tail[7]. This method is deemed to be faster and safter than the SICT.		sent to slaughter. (USA/Canada/NZ/ Australia) Not approved in EU.		
Interferon (INF) Gamma[10]	Blood test against bovine and avian tuberculin purified protein derivatives to assess the immune response by measuring the release of interferon gamma (increased release in response to bovine tuberculin if the animal is infected with *M.bovis*) – cell-mediated immunity.,	1-4 weeks post infection. Not suitable for animals <6 months old.	EU/UK approved to **supplement** the SICCT test (improves sensitivity) in breakdown herds. (identifies early-stage disease and animals which don't respond to the SICCT). Permitted in herds with persistent breakdown >18 months, new breakdowns with lesions at PMI or culture positive or in edge areas/low risk areas, recurrent breakdown within 18 months of securing OTF status[10]. Private testing with prior approval from the APHA.	**90%** 1 in 10 which are positive for the disease test negative.	**96.6%** 1 in 30 cattle without the disease test positive.
IDEXX ELISA[11]	Detects the presence of serum antibodies against two *M.bovis* antigens – serological test.	Possibly later stage of disease as requires the development of antibodies.	Role in ruling out false negatives (don't react to SICCT/interferon gamma) – quicker and cheaper than other tests.	**64.6%** This can be increased by prior injection of tuberculin 10-30 days before testing to increase antibody	**98%**

[7] https://www.visavet.es/bovinetuberculosis/animal-tb/diagnosis.php
[10] https://tbhub.co.uk/wp-content/uploads/2020/01/Factsheet_gamma_test_TB_hub.pdf
[11] https://tbhub.co.uk/tb-testing-cattle/blood-testing/the-idexx-antibody-test/

Test	How it works	Stage of Infection	Use	Sensitivity	Specificity
			WOAH approved as a supplementary test in 2012. Not recognised by the EU. Third-line test in chronic bTB breakdown herds where there is suspected cattle-cattle transmission. Needs special permission from the APHA for use in cattle in England. In Wales, it is considered a relevant test under bTB legislation and therefore doesn't need prior approval for usage.	production in infected animals.	
Enferplex bTB Antibody Test	Enzyme linked immunoassay of serum or milk to detect antibodies to 11 *M.bovis* antigens.		**WOAH approved** as a supplementary test in 2019. Not recognised by the EU/UK. Can only be used privately with prior permission from the APHA.	**83.6-97.2%**[12] Depending on the extent of disease in the animal. Sensitivity is improved by prior tuberculin boosting.	**99.7%**[13]
Actiphage[14]	Incubating blood/milk samples with a bacteriophage that infects **live and viable** *M.bovis* cells and causes replication and release of the phage to infect	Any stage of infection.	Used for rapidly detecting low quantity of *M.bovis*. Can only be used privately with prior permission from the APHA.	**95%***	**99%***
				*Data taken from Swift et al 2019. However the study had: • Low population number • Tested on SICCT positive cattle – may have had a higher bacterial burden for detection by later tests.	

[12] O'Brien, A., Clarke, J., Hayton, A. *et al.* Diagnostic accuracy of the Enferplex Bovine Tuberculosis antibody test in cattle sera. *Sci Rep* **13**, 1875 (2023). https://doi.org/10.1038/s41598-023-28410-9
[13] O'Brien, A., Clarke, J., Hayton, A. *et al.* Diagnostic accuracy of the Enferplex Bovine Tuberculosis antibody test in cattle sera. *Sci Rep* **13**, 1875 (2023). https://doi.org/10.1038/s41598-023-28410-9
[14] https://tbhub.co.uk/wp-content/uploads/2020/03/Phage_factsheet_13.02.2020_TB_hub.pdf

Test	How it works	Stage of Infection	Use	Sensitivity	Specificity
	more bacteria. PCR then analyses the DNA released from the bacteria to confirm presence of *M bovis.*			• Unable to extrapolate for the viability of detecting early stage infections. <u>Non-validated</u> – no definitive values of sensitivity or specificity.	
PCR[15]	Detect *M.bovis* DNA collected from tissue samples at post mortem inspection. Can reveal the presence of living/recently living bacteria from very small amounts of material.		Validated by the APHA. Used in tissue samples from bTB positive animals (SICCT/interferon), direct contact cattle, compulsory/private slaughter of inconclusive reactors and lesions typical of bTB found at routine PMI. Also used in non-bovid reactors, direct contacts, clinical suspect at AMI or PMI of TB and in domestic pets. Test time take approximately 3 weeks therefore can reduce the time a herd is under movement restrictions compared to traditional culture techniques or introduce additional controls to herds faster.	**Low** - the number of circulating M.bovis within the blood is low and therefore often results are no valid.	**Very high** – detects DNA sequences from *Mycobacterium tuberculosis* complex (*M. microti*, *M., caprae* and *M. tuberculosis*) and a separate sequence specific to *M. bovis*. Positive results must be positive to both target sequences. If any other result is obtained (i.e., positive for one sequence and not the other), the test is invalid and samples are subjected to traditional culture techniques.
Culture[16]	Growth of *M.bovis* on agar medium.		Tissue samples where a bovine/non-bovine animal has		

[15] https://tbhub.co.uk/tb-testing-cattle/pcr-test-for-detection-of-m-bovis-in-post-mortem-tissue-samples/
[16] https://tbhub.co.uk/tb-testing-cattle/pcr-test-for-detection-of-m-bovis-in-post-mortem-tissue-samples/

Test	How it works	Stage of Infection	Use	Sensitivity	Specificity
	A very challenging and slow process, which can take up to 22 weeks.		visible lesions at PMI for which the PCR test is negative. If growth is achieved (i.e., positive for M. bovis) the sample will go through **WGS** analysis.		
Whole Genome Sequencing (WGS)[17]	Molecular technique to characterise the entire DNA content of an organism. Enables characterisation of recurrent bTB breakdowns due to residual infection in the herd or via new introductions (brought in cattle, wildlife).		Routinely carried out on **culture positive** cattle at PMI to separate bacteria into clades and track the spread of disease across the UK[18]. Also used for surveillance in wildlife and non-bovine species to assess the transmission relationship between wildlife and cattle and visa versa.		
Post-mortem Inspection (PMI)	Identification of pathological lesions in bTB positive and normal slaughter animals. Animals are classified as having visible lesions (VL) or non-visible lesions (NVL).	NVL are often attributed to an early stage of infection (bTB lesions are too small), the slow progression of infection or visible lesions which are present but they are missed on PMI. VLs are becoming increasingly rare.	Samples from suspect lesions can be taken for PCR and culture for definitive diagnosis.	Unclear specificity, most cases identified at early stage – 40% of skin test reactors show visible lesions at slaughter, and/or isolation of *M. bovis*. Gold standard for confirming infection in a herd.	

Difficulties in Current Surveillance

[17] https://tbhub.co.uk/wp-content/uploads/2021/04/TB-WGS-factsheet.pdf
[18] https://tbhub.co.uk/tb-testing-cattle/pcr-test-for-detection-of-m-bovis-in-post-mortem-tissue-samples/

- For tuberculin tests, the tuberculin mixture is a cocktail of proteins derived from *M.bovis*. These proteins are difficult to standardise and therefore the inflammatory reaction elicited varies and potentially alters test interpretation[19].
    - Specifically, Johnes disease complicates tuberculin test interpretation as there is cross-reactivity between the proteins within the tuberculin mixture and the Mycobacterium which causes Johnes.
- Stage of infection affects test sensitivity:
    - Tuberculin based tests require development of host immune response to disease; if the animal is infected early, there will be no immune response and therefore a positive animal will not be detected.
    - However, recent studies suggest that antibody responses can appear as early as 3-8 weeks post infection and therefore challenges the assumption that antibody-based tests will only detect animals in later stage of disease[12].
- Human error:
    - Improper storage, too little injected, wrong injection technique, reading the test too early/late, errors in recording skin or identifying reactors.
- Sensitivity and specificity can be influenced by the stage and severity of the disease and exposure to environmental mycobacteria.
- The most common test detected the body's immune response to bTB and not the bacterium itself – there is concern that infected animals can become anergic and therefore won't respond to bTB tests despite being highly infected with bTB[20].

Test Validation

- Currently in the UK only two tests are validated:
    - SICCT is the statutory test for all routine testing.
    - Interferon Gamma is used as a supplementary test to the SICCT in certain areas of the country or breakdown herds (see section above).
- The IDEXX ELISA test is approved as a third-line test (only after the use of interferon gamma if required by Defra) in persistent breakdown herds but requires prior permission from the APHA for its use.
    - In this case, prior to the test the farmer must agree to surrender any positive animal for slaughter, with compensation paid by the APHA.
- Non-validated tests:
    - Include the **Actiphage** test and **Enferplex.**
    - These are classified as non-validated as their performance characteristics, for example their sensitivity, specificity, PPV and NPV are not fully understood.
    - Requires prior approval from the APHA for their use.
        - It is a breach of the Animal Health Act 1981 if these tests are used without prior APHA approval.
    - Animals which test positive do not require compulsory slaughter and can be restricted to that holding for life. If the farmer does decide the cull the animal, Defra is no liable to pay any compensation. Furthermore, testing from non-validated tests does not contribute to the lifting of movement restrictions and OTF status.

Godfray Report Recommendations for Diagnostics:

- Changing to EU SICT in high-risk areas and edge areas to enable early detection → would increase 'infections' and herd breakdowns in the short-term but this is epidemiological and not a failure of policy.

[19] https://assets.publishing.service.gov.uk/media/5beed433e5274a2af111f622/tb-review-final-report-corrected.pdf
[20] https://tbhub.co.uk/tb-testing-cattle/skin-testing/tuberculin-skin-testing/#:~:text=The%20primary%20screening%20test%20for,animals%20and%20people%20for%20TB.

- After a herd has broken down → use gamma interferon to retest reactor animals → reduces false positives as it is a more sensitive test.
- Identifying all the positive individuals within a herd once break down by using the skin test, interferon gamma test and IDEXX ELIZA in combination. This will come at increased costs to the industry but will reduce false negatives.
- Tuberculin control:
    - Skin tests and interferon test relies on difficult to standardise proteins from Mycobacterium → aim to replace tuberculin with defined antigens → would also provide a DIVA function to tests to enable viable cattle bTB vaccination.