

## Testing to identify Bovine Tuberculosis in cattle

### Background

Bovine tuberculosis (TB) is a zoonotic notifiable disease in cattle caused by the bacteria *Mycobacterium bovis*.

Early efforts at detecting TB in cattle relied solely on physical examination of the animal, but despite the fact that there were many cattle with clinical TB, removal of such animals had no noticeable effect on the prevalence of the disease. Following the identification of a factor from broth culture filtrates, which halted the growth of tuberculosis bacilli, a factor known as tuberculin, Robert Koch, used tuberculin not to cure TB, but to diagnose TB in man. The technique was adapted for use in cattle when it was discovered that subcutaneous injections of 0.2-0.5ml tuberculin caused a temperature rise.

Tuberculin skin tests have now been used for the diagnosis of TB in cattle for more than 100 years.

Despite the high prevalence and incident of TB in cattle in the UK today, the majority of infected animals usually show no overt clinical signs of the disease and diagnosing the condition is reliant on ante-mortem active surveillance testing and the post-mortem examination of carcasses entering abattoirs.

### The Body's response to infection with *Mycobacteria*

The tests used to identify TB in the live animal are dependent on the body's response to the invading bacteria.

TB is considered to be primarily a respiratory pathogen gaining entry to the animal through inhalation, although transmission via other routes is also considered to occur.

Once the pathogen enters the lung, receptors on the surface of the cells recognise the foreign bacteria and trigger macrophages and other cells from the body's defence system, which engulf the bacteria, attempting to degrade and remove the foreign material, leading ultimately to the formation of a granuloma. At this point some of the defence cells will migrate to the lung associated lymph nodes where sites of infection are likely to form centres of immunological memory. An arms race ensues where the TB organism inhibits the degradation process and the immune system responds by producing chemical messengers to improve the defence process and recruit more cells into the fight. One such messenger is **interferon- $\gamma$**  (or interferon-gamma).

The formation of a granuloma is a protective immune-pathological response to the organism, as it is a means of curtailing spread of the organism to other tissues. It is these granulomas that are found in lymph nodes and tissues at post-mortem examination when a reactor is confirmed as having Visible Lesions (VL). These granulomas may vary in size from almost undetectable to very large. They may be small enough to not be identified at normal post-mortem examination. In an animal, where No Visible Lesion (NVL) is found, the absence of findings is clearly not a good indicator that the animal was not infected.

It is clear that in most cases of TB, the cellular immune response dominates and that there is ongoing stimulation of the immune system albeit at differing

magnitudes throughout the disease process, whether the animal is controlling infection, or not. A dominant antibody response may be an indicator of uncontrolled disease. Therefore any immune based test relies on its ability to detect some aspect of the acquired immune response.

## The Tests for TB

The **Single Intradermal Comparative Cervical Tuberculin Test (SICCT)**, an intradermal (skin) test, remains the standard test used for identifying TB in cattle in the EU. Both avian and bovine purified protein derivative tuberculins are injected into the skin in separate sites of the neck and reactions to the injections are measured and compared. The test relies on a delayed hypersensitivity reaction to injected tuberculin. Sensitised T cells recruit and orchestrate the infiltration of other cell types into the injection area thereby leading to a transient swelling, maximally measurable at 72 hours post injection. By utilising two different Mycobacterium species, the SICCT compares the reaction of the animal to each tuberculin. This increases the specificity of the test by differentiating between cattle infected with M.Bovis and those exposed to other mycobacterium and therefore sensitised to tuberculin. There is a standard operating procedure for the SICCT test which must be followed by vets to maximise the sensitivity and specificity.

The **Interferon-Gamma blood test** measures the cellular response to mycobacterial antigens and as such broadly measures the same immune response as the intradermal (skin) test. A positive response is seen where there is a preferential release of interferon gamma to constituents of M.Bovis compared to other mycobacteria. This test is approved for use as a complementary test to the SICCT, but not as a stand-alone test. IFN-Gamma has a number of advantages over the SICCT i.e. better sensitivity (as compared with standard interpretation), animals need only be handled once, the test can be repeated immediately & there is a less subjective interpretation. Limitations include lower specificity, particularly in young animals, the need for rapid laboratory processing, substantial laboratory expertise and significant test costs. A measurable interferon-gamma response can be detected as early as 3-5 weeks following infection, even when the level of exposure is very modest.

Antibody responses to the organism are generally regarded as muted in most animals. Antibody responses may be a marker for more advanced disease and therefore of animals more likely to be infectious. At any single point most infected animals will not be displaying a measurable specific antibody response. **Antibody testing** is mainly used currently to detect anergic animals.

### Performance of the tests in identifying disease

Two measurements used to indicate the performance of a test in identifying disease are:

**Sensitivity of a test** – this is the proportion of infected animals detected by the test.

**Specificity of a test** - this is the proportion of non-infected animals cleared by the test.

No test for TB, or combination of tests can provide 100% sensitivity and 100% specificity. In other words some truly infected animals may not be diagnosed and some truly non-infected animals may be identified as 'false-positives'.

In a recent paper (Goodchild et al, Veterinary Record - 2015), the specificity of the SICCT test was estimated at 99.98%. Sensitivity has been variously quoted as 55.1% - 90.9% (Costello et al, Neill



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et al. 1994). The sensitivity is currently considered to be approx. 80% in GB, but may be lower in certain scenarios.

In order to identify more “truly infected” animals, the sensitivity of the testing regime can be increased by using more severe interpretations of the SICCT test, or by using complementary Interferon-Gamma testing. This tends to be at the expense of reduced specificity. The Interferon-Gamma test is considered to have a higher sensitivity (88%) but a lower specificity (96.5%) than the SICCT (estimates can vary).

### **False negative reactions to the SICCT test**

Reasons for false negative reactions to the SICCT test may include:

- Recently infected cattle may fail to respond to tuberculin; reactivity is not usually apparent for 30-50 days (Francis, 1947).
- Cattle with advanced disease, so-called anergic animals.
- Use of low, or reduced potency tuberculin e.g. if tuberculin is stored incorrectly.
- Injection of insufficient tuberculin e.g. malfunctioning syringes.
- Desensitisation i.e. a reduction in the ability of an infected animal to react for a period following injection e.g. a test performed before a 60 day period has elapsed since the preceding test.
- Immunosuppression in the early post-partum period e.g. within 4-6 weeks of calving.
- The presence of other underlying disease, or severe nutritional stress compromising an animal's immune response.
- Infection of cattle with *Mycobacterium avium*, subspecies paratuberculosis which causes Johne's disease, and also vaccination against this can cause cross reactivity with the SICCT and Interferon-Gamma tests, reducing test sensitivity.
- Observer variations in reading the test results - recent analysis of historical testing data has indicated that animals, in long term herd breakdowns, that miss becoming an inconclusive reactor by 1mm may be significantly more likely to become a reactor at future testing than a clear tested animal (unpublished).

### **Conclusion**

The presence of TB in a bovine animal may not be straightforward to diagnose. Welsh Government and APHA use a combination of tests and test interpretations to vary specificity and sensitivity of the testing regime, depending on the disease situation in the region and present on the farm, in order to ensure the best possible options are used to identify disease and remove infected cattle from the farm.

### **Other factors**

To find out more about bTB in Wales look out for the Farming Connect events on Animal Health with the Office of the Chief Veterinary Officer. For more information and practical advice on TB in general visit:

[www.tbhub.co.uk](http://www.tbhub.co.uk) ,

or <http://gov.wales/topics/environmentcountryside/ahw/disease/bovinetuberculosis>





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