A Review of the International Application of the Interferon-gamma Test

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Executive Summary

The interferon-gamma test is used in most developed countries which have significant levels of bovine tuberculosis.

The test can be used as both a serial and a parallel test. In summary where it is used serially it is used to increase the specificity (and therefore decrease the sensitivity) of the overall test, i.e. an animal is deemed positive if it tests positive to another test (typically an intradermal test) and to the interferon-gamma test. Where it is used as a parallel test, it is applied to increase sensitivity (and therefore will be associated with a decrease in specificity), i.e. if an animal tests positive to another test or the interferon-gamma test. A summary of the international use of the test can be found in table 1.

Serial testing is only used where there are concerns that the routine ante-mortem test lacks specificity e.g. where the Single Intradermal test is performed or in areas where there is a low likelihood of animals being truly infected. Parallel testing is used in converse situations i.e. where there is a higher likelihood of infection and where there is concern that infected animals might be missed by one test and where reduced specificity is deemed acceptable.

Nowhere is the test applied universally, i.e. used in a blanket national screening program.

The test readout is expressed as a net optical density (Net OD). This is a measure of the colour change which is related to the quantity of IFNg released by cells in the assay after taking away the background colour change of a negative control sample. Most countries use a net optical density cut-off of 0.1 compared to the cut-off currently in use in NI of 0.05. The only other known area to use a net cut off of 0.05 is the Republic of Ireland. The lower cut-off used in NI is likely to increase test sensitivity but decrease test specificity.

In most cases the reagents used for the test are the same as are used for intradermal testing i.e. the purified protein derivatives ‘a’ and ‘b.’ In some circumstances the specific antigens ESAT 6 and CFP 10 are used. These are used to increase test specificity but their use also reduces test sensitivity. Therefore they tend to be used in serial testing where there is a concern that there may be false positive reactions to other tests.

Specific antigens are used in some circumstances to clarify where there is suspicion that intradermal test reactions are spurious due to illegal test interference.

In areas where the distribution of infection is uneven the interferon-gamma test can be used as a parallel test in areas of high infection density and as a serial test in areas of low infection density. So, for example, in France in those départements with ongoing incidences of bovine TB the test is used as
a means of clarifying the status of single intradermal test inconclusive animals.

While the application of the interferon-gamma test varies between countries it is important to note that there are likely to be other critical differences between countries. Perhaps most notably there is a great variety of PPDs available for use internationally. The performance of these different reagents is likely to have significant effects on the performance of the test. Therefore comparing the test performance between countries which use different reagents could be misleading.

Numerous other factors such as background exposure to environmental mycobacteria, disease herd and animal prevalence or frequency of intradermal testing may have effects on the performance of the test in different regions. Therefore while broad conclusions can be drawn about how the test could be applied great care must be exercised in comparing details of test performance between different populations of animals.

There have been some reports of the possible private use of the IFNg test internationally. Clearly the private use of the IFNg test could be used by farmers to identify animals with a significant risk of infection and move or slaughter them before the competent authority could act. Current practice in N. Ireland is that testing for tuberculosis can only be carried out following authorisation by DARD. It is essential that this is maintained and that private IFNg testing continues to be prohibited and all testing only undertaken on behalf of the national or local competent authority and that all results are communicated through the competent authority.
<table>
<thead>
<tr>
<th>Glossary</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Antigen</td>
<td>A molecule that reacts with receptors on cells of the immune system eliciting immunological responses.</td>
</tr>
<tr>
<td>Bovigam</td>
<td>The commercial name of the interferon-gamma test (assay) which until recently was the only commercially available test.</td>
</tr>
<tr>
<td>CFP10</td>
<td>Cell filtrate protein. A <em>M. tuberculosis</em> complex specific protein with a size of 10 kilodaltons.</td>
</tr>
<tr>
<td>CFT</td>
<td>Caudal fold test. A version of the intradermal skin test where PPD is injected into the fold of skin at the base of the tail.</td>
</tr>
<tr>
<td>ESAT6</td>
<td>Early secreted protein. A <em>M. tuberculosis</em> complex specific protein with a size of 6 kilodaltons.</td>
</tr>
<tr>
<td>IFNg</td>
<td>Interferon gamma. A chemical messenger (cytokine) which plays a central role in controlling the cellular immune system.</td>
</tr>
<tr>
<td>Net OD</td>
<td>Net optical density. The difference in the quantity of IFNg released following blood stimulation with PPDa or PPDb</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density. The measure used as part of the IFNg test to quantify the amount of interferon-gamma released following blood stimulation</td>
</tr>
<tr>
<td>OTF (W/S)</td>
<td>Officially tuberculosis free (withdrawn/suspended). The status applied to a herd dependent upon presence/absence or suspicion of infection which determines whether animals can be moved between farms or not.</td>
</tr>
<tr>
<td>Parallel Testing</td>
<td>Where two or more tests are performed and only one of the tests must be positive in order to classify the result as positive.</td>
</tr>
<tr>
<td>PPD</td>
<td>Purified protein derivative. A complex mixture of components, including a range of antigens of the <em>M. bovis</em> (PPDb) or the <em>M. avium</em> (PPDa) organism used in intradermal tests and the IFNg assay.</td>
</tr>
<tr>
<td>SCITT</td>
<td>Single comparative intradermal tuberculin test</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>The proportion of infected animals that are test positive.</td>
</tr>
<tr>
<td>Serial testing</td>
<td>Where two or more tests are performed and both tests must be positive in order to classify the result as positive.</td>
</tr>
<tr>
<td>Specificity</td>
<td>The proportion of uninfected animals that are test negative.</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>SIT</td>
<td>Single Intradermal test</td>
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</table>
Introduction and Background

Terms of Reference

This report was written as part of the DARD funded Evidence and Innovation research project ‘An evaluation of interferon-gamma (IFN-g) testing for bovine TB in Northern Ireland.’ It fulfils one of the milestones of the project namely ‘a literature review on the application of the IFN-g test internationally.’

The Interferon-gamma Assay

The interferon-gamma test is a broad description of an assay designed to measure the dominant immunological response to tuberculosis in cattle, the cell mediated response. As such it broadly measures the same immune response as the intradermal tests. Briefly, blood samples are incubated with complex component mixtures of the causal agent of bovine TB (M. bovis) or avian TB (M. avium) or individual defined components of the M. tuberculosis complex of organisms (e.g. ESAT6/CFP10). In principle immune cells present in the blood that have encountered the organisms in the past then respond by releasing a cascade of chemical signals, most notably interferon-gamma. A positive result is seen where there is a preferential release of IFNg to constituents of M. bovis compared to M. avium or where there is a significant release of IFNg to the defined antigens (Wood and Jones 2001). Therefore the assay is a measure of the responsiveness of immune cells involved in the acquired immune response to the organism. The presence of reactive cells implies exposure (and by implication infection with) the organism.

Methods

Information was collected on the use of interferon-gamma testing in all the major developed countries where bovine tuberculosis infection remains an important endemic disease. These countries were identified through personal communication with senior scientific staff within AFBI. Countries that are now officially free of the disease but which had a history of infection were not included (for example Australia and Poland). Also those countries which have a history of occasional but rare outbreaks of bovine tuberculosis were not included as their programs tend to have relatively low levels of ante-mortem surveillance, relying more upon abattoir surveillance (for example Switzerland). Consequently the programs for control in these countries are very significantly different from that of N. Ireland and any application of the interferon-gamma test by them is not likely to be informative for the N. Ireland context. For the final list of countries, attempts were made to gain information on the use of the IFNg test. The methods used were an internet search of;

- The Web of Science database using ‘interferon’ and ‘country name’ and ‘bovine’ as the search term restricted to the most recent 10 years.
• Department of Agriculture or equivalent websites for each country using ‘TB’ or ‘tuberculosis’ as search terms.
• EFSA Website search using ‘interferon’ or ‘bovine and tuberculosis’ as search terms
• FVO website search for each country using ‘TB’ or ‘tuberculosis’ as search terms

For some countries, while there remain ongoing significant levels of bTB, there is a paucity of detail around how and in what circumstances the IFNg test is applied. This is likely to reflect a somewhat open ended approach to its use, the detail of its application very dependent upon local decision makers within the relevant competent authority.
**Countries Using the IFNg Assay**

**Great Britain**

In GB the IFN-g test has been used as part of the TB control program since 2002 (Vordermeier et al., 2006). It was first used as part of a field trial in infected herds in Wales and England and occasionally on an ad-hoc basis. A specificity trial was undertaken in England in 2005. Criteria for its structured application in the field were set in October 2006. The criteria for when it is used are set out below in table 1. Only animals over 6 months of age are tested. Due to the distances involved in transporting samples from farm to lab, samples are typically processed the day after collection and a positive result is defined as a net OD of greater than or equal to 0.1. Where an animal is found to be positive it is compulsorily slaughtered.

The test is routinely used in parallel to the single comparative intradermal skin test in specified circumstances e.g. where there is a suspicion of spread of disease to a new area and on a discretionary basis where there are high disease incidences. On occasion it is also used as a serial test to improve test specificity where there are suspicions of non-specific skin test reactions or where there is suspicion of skin test interference.

In England, the test has been applied in every new OTFW breakdown herd in low TB risk areas since October 2006. These are defined as being areas where testing is undertaken every three or four years. The purpose of this approach is to minimise the likelihood of TB becoming established in new herds and areas.

It is also used in some OTFW herds with persistent and severe TB breakdowns in high TB risk areas where the application of the SCITT appears to have failed to remove residual infection. In these cases the test is applied as a parallel test, alongside the SCITT thereby improving the sensitivity of the TB testing regime. In this way it is believed that it assists in the identification of more infected animals more quickly, i.e. at an earlier stage of infection and for some infected animals detecting them before they could be by the SCITT.

Increasingly it is being used in two yearly testing areas as a means of buffering the spread of infection into these areas. These areas are by definition on the edge of regions where TB is endemic. The application of the IFNg test in these areas is seen as a means of reducing or slowing the further spread of disease into these areas (DEFRA 2011). In these cases the test is applied as a parallel test to herds with confirmed TB in order to maximise the likelihood of removing infected cattle (Anon 2011a).

Where TB has been found to be widespread in a herd, consideration is usually given to partial or total herd depopulation. In most circumstances to help inform this decision a parallel herd interferon gamma test is also undertaken to attempt to quantify the proportion of cattle that are infected.
The decision as to whether the herd is de-populated is taken at the local level by the competent authority.

The test is also used in two other circumstances using defined antigens. The first is in apparent chronic herd infections which fail to have TB confirmed either by bacteriology or by histopathology. In these cases a modified IFNg test can be applied using ESAT6 and CFP10 antigens to maximise the specificity of the test. Results from this are used by the competent authority to assess whether there is likely to truly be infection in the herd and if so to identify any animals apparently infected but not detected by the SCITT. The second circumstance is where there is suspicion that positive SCITT results are due to interference with the test.

**Republic of Ireland**

The interferon-gamma assay has been used as a statutory instrument since April 2005 (Anon 2005) which allows for the compulsory removal of animals testing positive.

Samples are processed within 8 hours of collection and are tested by two laboratories, TB Diagnostics & Immunology Research Centre, UCD and Sligo Veterinary Investigation centre. Decisions as to whether the test is to be performed on any herd is decided centrally by DAFM following dialogue with the referring DAFM veterinary inspection officer. In principle only those herds classified as high risk are considered for testing (approximately 30% of herd breakdowns). These are herds [or animal groups (E. Gormley pers. comm.)] with 2 or more skin test reactor animals after applying standard interpretation or where 2 or more animals are detected with lesions consistent with TB. This can include herds of origin where positive animals can be traced back to and epidemiological evidence exists to imply that the animals were infected on this farm.

The test is applied in parallel to the SICTT, typically on the first day of the follow-up skin test following infection disclosure (a minimum of 60 days after the first skin test). Sampling is also permitted 10 days following the initial skin test to allow for the more rapid removal of test positive animals. However this is less likely to be done due to the additional resources involved in testing and handling animals at an additional time point. An animal is classified as positive if the Net OD is greater than or equal to 0.05.

**New Zealand**

The program for the control of bovine TB in New Zealand is set out in the National Operation Plan of the Animal Health Board parts A and B. (Anon 2011a & b). The interferon-gamma test is available to be used as both a serial and a parallel test. As a parallel test it uses the standard format of the Bovigam test with PPDa and PPDb. In addition to the standard format, additional antigens ESAT 6 and CFP 10, can be used to improve test specificity and applied as a serial test to the skin test.
As a serial test it is only used in animals positive to the caudal fold test in order to confirm positivity. In these cases it is used 13 to 33 days following the skin test and blood samples are processed within 30 hours of collection having been stored between 10 and 26°C. A positive reaction is defined as being when the PPDb-PPDa net optical density is greater than or equal to 0.1. All animals testing positive are declared reactors and slaughtered. It should be noted that not all caudal skin test positive animals necessarily will be tested using the interferon-gamma test, a local decision is made based upon the history of the animal and herd, i.e. **where there is good reason for the presence of bTB the animal is likely to be removed without any serial testing taking place.** The comparative skin test is available to be used as a primary or a serial test but only following approval of the National Disease Control Manager (NDCM).

The use of the specific antigens ESAT 6 and CFP 10 in the interferon-gamma test are only permitted to be used as a serial test and only after permission of the NDCM. It is only used in animals with a positive CFT test in an area that is deemed not to have a TB infected possum population, i.e. to maximise the specificity of ante mortem diagnosis. Where this is done a combined ESAT 6/CFP 10 cocktail is used as the stimulating antigen and a positive test is defined as one having a net optical density (ESAT6/CFP10 antigen minus negative control) greater than 0.04. All test positive animals are deemed reactors and culled.

The standard Bovigam test can be applied as a parallel test where animals test negative to the CFT. Typically it is applied at the discretion of the competent authority as a pre-movement test from 'High Risk' herds with an ongoing history of infection. In this way it is used to maximise detection. An animal is defined as high risk where there is a net OD of greater than or equal to 0.07 and medium risk where the net OD is greater than or equal to 0.04 and less than 0.07. An animal is only permitted to move if it has a negative test result. In addition a parallel bovigam test can be used as part of the second herd caudal fold test used to declare a herd free for TB. This is at the discretion of the competent authority.

**Spain**

In Spain the test is applied to animals over 6 months of age. The test is used as a parallel test using a Net OD cut-off of 0.1. It is usually applied in regions with a perceived high incidence of TB infection (herd prevalence of greater than 1%) although it can be applied in herds in other regions at the discretion of the competent authority (Anon 2008). Samples are processed within 8 hours of collection and all positive animals are compulsorily slaughtered (Anon 2012). However the application of the test is not entirely uniform and is related to the practical difficulty of getting blood samples from the farm to the lab within 8 hours.

**France**

The use of the interferon-gamma test is authorised for use in nine départements (Anon 2010a). Samples must be processed within 6-8 hours of
collection and a net OD of greater than 0.1 is used to classify positive reactions. Bloods are taken no later than at the time of the skin test reading. It is applied as a serial test following either a single or a comparative intradermal test (Anon 2011b). Whether a single or a comparative test is used is determined by the competent authority based upon whether there is a requirement to maximise sensitivity (i.e. where the SIT is used) or maximise specificity (where a comparative skin test is used). The IFNg test is used to clarify the status of all SIT inconclusive animals and any positive SIT test results where there is a query over the validity of the skin test result after considering the epidemiological context of the result. If the animal tests positive the herd’s official TB status is classified as withdrawn. If the animal tests negative the herd’s status is suspended and all animals within the herd are required to undertake one comparative skin test. Where the comparative skin test is applied, animals with inconclusive reactions are subjected to a follow-up interferon-gamma test. The result of this IFNg test is used to classify the trade status of the farm, i.e. where a SCITT inconclusive animal tests negative to the IFNg test the herd is classified as OTFS.

**Italy**

The distribution of bovine tuberculosis in Italy is highly regionalised with controls devolved to different regions. Therefore there is no single approach to TB control across the country with differing levels of IFNg testing applied (Anon 2010b). In Lombardia and Valle D’Aosta the test is applied to herds where there is confirmed presence of bTB (i.e. culture positive). In these cases it is applied as a parallel test and any test positive animals are slaughtered. In the latter area there have been reports of farmers applying private IFNg tests and slaughtering or selling positive test animals before an official test can be undertaken. In Sicily there is plans to apply the IFNg test in a similar way to the other regions although at the time of writing it is uncertain whether this has been implemented yet. The test is carried out using the Bovigam recommended Net OD cut off of 0.1 and samples must be processed within 8 hours of collection (Anon 2009).
<table>
<thead>
<tr>
<th>Country</th>
<th>Serial/Parallel</th>
<th>Reason for Test use</th>
<th>Standard Test Cut-off</th>
<th>Time to sample processing</th>
<th>Action taken with test positive animals</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Ireland</td>
<td>P</td>
<td>Improve bTB detection and eradication in herds with large or unresolved disease outbreaks.</td>
<td>Net OD ≥0.05</td>
<td>≤ 8 hours</td>
<td>Voluntary slaughter of test positives.</td>
<td>ESAT6 antigen used in addition to PPDa and PPDb to help inform DARD of animal disease status.</td>
</tr>
<tr>
<td>GB</td>
<td>P</td>
<td>Prevent spread of infection to low incidence areas in herds with confirmed infection</td>
<td>Net OD ≥0.1</td>
<td>Next day</td>
<td>Test positives slaughtered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Resolution of SCITT inconclusive animals</td>
<td></td>
<td></td>
<td></td>
<td>Use of specific antigens</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Ongoing large unresolved infection outbreaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Herds with ongoing SCITT positive animals without other evidence of <em>M. bovis</em> infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Republic of Ireland</td>
<td>P</td>
<td>Application in 'High Risk' herds/groups i.e. herds with 2 or more SCITT reactors using standard interpretation or herds with 2 or more LRS animals</td>
<td>Net OD ≥0.05</td>
<td>≤ 8 hours</td>
<td>Test positives slaughtered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>To help resolve animals with intradermal PPDb rises where there is doubt about the correct application of the test (e.g. failure to inject PPDa) or where test interference is suspected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>S</td>
<td>To confirm SIT testing positive animals where there is lack of epidemiological support for actual infection</td>
<td>Net OD ≥0.1</td>
<td>≤ 30 hours</td>
<td>Test positives slaughtered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>As a pre-movement test for cattle from 'High Risk' areas</td>
<td>Net OD ≥0.07 high risk</td>
<td></td>
<td>Only used as a means of classifying infection risk.</td>
<td>Only low risk animals permitted to move</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Net OD ≥0.04 &lt;0.7 medium risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>In SIT positive cattle in areas where the possum population is thought to be TB free i.e. where there is doubt over the validity of the SIT result</td>
<td>Net OD ≥0.04 than negative background</td>
<td></td>
<td>Test positives slaughtered</td>
<td>ESAT6/CFP10 antigen used at the discretion of the local competent authority</td>
</tr>
<tr>
<td>Spain</td>
<td>P</td>
<td>In regions with high TB herd prevalence (&gt;1%)</td>
<td>Net OD ≥0.1</td>
<td>≤ 8 hours</td>
<td>Test positives slaughtered</td>
<td>In many cases the distance from farm to laboratory sets a practical limit to the use of this test</td>
</tr>
<tr>
<td>France</td>
<td>S</td>
<td>To clarify the status of SCITT or SIT inconclusive animals</td>
<td>Net OD ≥0.1</td>
<td>≤ 8 hours</td>
<td>Test positives slaughtered</td>
<td>Reports of private testing leading to inappropriate movement of high risk animals</td>
</tr>
<tr>
<td>Italy</td>
<td>P</td>
<td>To maximize detection in herds with confirmed infection</td>
<td>Net OD ≥0.1</td>
<td>≤ 8 hours</td>
<td>Test positives slaughtered</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Summary of the Application of the Interferon-gamma test internationally.
Conclusions

The interferon gamma test is used in virtually every developed country as an ancillary test to various forms of the intradermal skin test. Nowhere is it currently used as a widespread screening tool due to its recognised sub-optimal specificity. However it is used in many circumstances to enhance the overall detection of infection. It can be used as either a serial test to confirm infection or as a parallel test to increase overall infection detection sensitivity. How it is applied is very strongly influenced by the known or suspected prevalence of infection in the animal populations it is being applied to. Typically it is used as a serial test in circumstances where there is doubt over the specificity of the test routinely being used. For example where the SIT is applied in areas where disease has a low incidence it is used to more accurately categorise skin test positive animals as infected or not. As a parallel test it is typically applied in areas or herds with a high disease prevalence in order to maximise the likelihood of disease detection. Invariably this leads to false positive animals although this will to some extent be mitigated by the moderately high positive cut-off used by most countries (net optical density of 0.1). Most countries use the recommended net OD cut-off of 0.1 (unlike N.I. where a cut-off of 0.05 is used). The higher cut-off used internationally will have the effect of increasing test specificity but reducing test sensitivity.

In most countries there is a requirement for samples to be processed within 8 hours of collection in order to minimise the proportion of cells unresponsive to stimulation when being processed by the lab. However there are two notable exceptions to this. In both GB and New Zealand samples must be processed by the end of the next day. This is a consequence of the practical limitations of the time delay between sampling and arrival of samples to the lab in order for them to be processed.

While it is generally agreed that the delay in sample processing has an effect on the characteristics of the test, there remains some controversy concerning the impact of next day testing on the test's overall specificity and sensitivity. The original recommendation for the assay was that samples should be processed within 8 hours of sampling (Rothel et al., 1992). This was supported by one study which found that approximately 50% of animals that were SCITT positive and IFN-g positive using 8 hour old blood samples remained IFN-g positive using blood samples 24 hours old (Gormley et al., 2004). However the same study found no reduction in sensitivity in detecting cattle with confirmed infection. This could suggest that next day testing reduced the sensitivity of the test to detect early-stage infection (i.e. cattle with limited pathology) and/or that it increases test specificity (i.e. any cattle with false positive reactions at eight hours fail to remain positive where the blood is stored for longer). In work carried out at the VLA (now AHVLA) no effect of time was seen in the sensitivity of the test to detect experimentally infected cattle but there was a modest decrease in sensitivity to detect field reactors, although with this category of animal there was an increase in test specificity (Vordermeier et al., 2006). When the antigens ESAT-6 and CFP-10 were used in assays comparing 8 hours and 24 hours
blood storage the longer storage period resulted in a reduction in the predictive value of the test (Whelan et al., 2004).

It is now well recognised that there is considerable variation in the performance of tuberculins available internationally and this is likely to have a significant effect on the relative performance of the assays as they are used across the world. While there is very little available information on the particular tuberculin sources used by each country it is clear that a variety is available and used. One study found that the use of different PPDs led to significant differences in test outcome using both SICCT and the IFN-g test when applied to both experimentally and naturally infected cattle (Schiller et al., 2010). This study found a 17 fold difference in the potency of the PPDs between the best and worst performing ones. Another study found that most internationally available tuberculins, if used at a dose rate of 1mg/ml, would have failed to meet the minimum dose of 2000 international units prescribed in legislation (Bakker et. al., 2009). The international standard in vivo potency testing method of PPDs in guinea pigs is known to give variable results and so a wide potency range is acceptable for skin test reagents (Council Dir 64/432). However it is unclear if this method is the most appropriate way of assessing PPD potency for either intradermal or IFN-g tests (Schiller et. al., 2010).

Current practice in N. Ireland is to use dialysed Weybridge tuberculin as the stimulating antigen for the interferon-gamma test. This is in contrast to the use of Lelystad tuberculins for the SCITT. The effect of using a different source of tuberculin for the IFN-g test from that used in the field is uncertain. It is possible that this variation has a negligible overall effect or it could be that it leads to a greater overall detection rate given that there could be variation between infected cattle in which tuberculin source they preferentially respond to, i.e. using two sources of tuberculin for the SCITT and gamma interferon tests identifies infected animals that respond to either source. However this remains conjecture and would be worthy of further investigation.

Tuberculins used in the N. Ireland program are dialysed to remove the preservative phenol before use in the interferon-test assay. Phenol is cytotoxic and its removal is believed necessary to avoid affecting the viability and reactivity of cells during the incubation stage of the test. However it is likely that this dialysis process also removes potential antigens. Whether this is likely to have any effect on the test’s characteristics is currently unknown.

While all countries use the PPDs as the primary antigen many also on occasion use the M. tuberculosis complex specific antigens ESAT6 or CFP10. However there is currently no consensus on how they are used or in what circumstances for example which cut-offs to use to classify animals positive, or whether to use them individually or in combination. Generally they are used to attempt to maximise the specificity of the test. However their use is also associated with an overall reduction in test sensitivity.
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