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# Characteristics of *Mycobacterium bovis* infected herds tested with the interferon-gamma assay

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ABSTRACT

The IFN-γ (interferon gamma) assay is used in Ireland as an ancillary diagnostic test to the single intradermal comparative tuberculin test (SICTT) to maximise the detection of *Mycobacterium bovis* infected animals (bTB) in cattle herds. Understanding the relationships between herd and animal risk factors and IFN-γ test results is critical to enable the development and evaluation of policy measures on how best to use the test. In this study, we set out to characterise Irish herds with IFN-γ test positive animals in terms of herd size, number of SICTT reactors and number of IFN-γ positive tests, and to evaluate the IFN-γ test in terms of the test cut-off values. The results showed that larger herds with more SICTT reactors were likely to have more IFN-γ positives in the herd, and herds with an IFN-γ test positive animal that was also positive for bTB lesions at post-mortem had higher numbers of IFN-γ positive animals in the herd. Raising the cut-off values for the IFN-γ test only marginally decreased the combined sensitivity of the IFN-γ and the SICTT for diagnosis of bTB lesioned animals. The analysis has provided valuable information on the performance of the IFN-γ test as it is used under current bTB infection levels in Ireland.

## 1. Introduction

The IFN-γ diagnostic test was first developed in the late 1980s during the latter stages of the bovine tuberculosis (bTB) eradication programme in Australia (Rothel et al., 1990). Since then it has been used widely in many countries with ongoing bTB problems, including Ireland. The principle of the assay is to use ELISA methodology to detect and quantify release of the IFN-γ cytokine when heparinised whole blood is cultured with bovine and avian (PPD) tuberculin (Rothel et al., 1990; Wood et al., 1991). Results from experimental and natural infections of cattle with *Mycobacterium bovis* (the infectious cause of bTB) indicate that the assay can detect a cell-mediated immunological (CMI) response to infection as early as two weeks post-infection, and earlier than the single intradermal comparative tuberculin test (SICTT) (Buddle et al., 1995; Pollock et al., 2005; Waters et al., 2010).

The IFN-γ assay is most often used as an ancillary diagnostic test in parallel with the SICTT to detect the maximum number of infected animals in *M. bovis* exposed herds. This allows for the detection of additional infected animals that would otherwise be considered negative, if the SICTT alone had been used. Using this approach, the combined sensitivity of the IFN-γ test and the SICTT (applying severe interpretation) has been estimated in one study as 93% relative to lesion detection as the gold standard diagnostic (Gormley et al., 2006). As used in Ireland routinely, the IFN-γ diagnostic test has a sensitivity of between 63.1% and 88% (Gormley et al., 2006; Clegg et al., 2011) and a specificity of 90.77% (Gormley et al., 2013). A recent statistical meta-analysis of published results from a large number of studies in different countries has provided an estimate of 67% (95% credible interval [CrI] 49%, 82%) for sensitivity, and 98% (95% CrI 96%, 99%) for specificity of the test (Nunez-Garcia et al., 2018), although it should be noted that the data sets contributing to that study included some with different cut-off points, which could affect Se and Sp. Using data generated in Ireland, a Bayesian latent class analysis conducted on high and low TB prevalence herds provided a sensitivity estimate of 79%–86% (95% CrI) and specificity of 88%–91% (95% CrI) (EFSA, 2012). In Northern Ireland, the mean performance estimates were reported as 85.8–93.0% (sensitivity) and 75.6%– 96.2% (specificity) using the local cut-off values (Lahuerta-Marin et al., 2018).

The bTB situation in Ireland has improved in the past decade with reductions in both animal and herd incidence (Abernethy et al., 2013;

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More et al., 2018). The IFN-γ test is used in the Irish bTB eradication programme in a number of ways. As a diagnostic test, it is mostly applied to herds with evidence of within-herd spread and higher within-herd prevalence (generally four or more reactors to the SICTT) and is targeted at the cohort within the herd that has been exposed to a high risk of infection (Gormley et al., 2006). In this scenario and in order to maximise diagnostic sensitivity, the test is performed on samples submitted to the laboratory within 8 h of collection (Gormley et al., 2004). Secondly, the IFN-γ test is also carried out on blood samples submitted at 24 h post collection from SICTT reactors as part of the quality assurance scheme to monitor the performance of the SICTT. An analysis of 17,725 IFN-γ test results submitted for testing in 2015 showed that 18.9% of all SICTT − ve/IFN-γ +ve animals from bTB-infected herds were positive at post-mortem examination in contrast with 4.7% of SICTT -ve/ IFN-γ –ve animals (Clegg et al., 2017). It was concluded from this study that retaining these SICTT − ve/IFN-γ +ve animals in an infected herd might pose a high risk of recurrence or prolongation of restriction of the breakdown. In earlier studies, we showed that in infected herds, when compared to SICTT -ve/ IFN-γ -ve animals, SICTT -ve/ IFN-γ +ve animals were at significantly greater risk to become positive at a subsequent SICTT or at postmortem following initial IFN-γ disclosure: an odds ratio of 7.0–9.0 for a maximum follow-up period of 155 days post SICTT (Gormley et al., 2006).

Given the current widespread use of the IFN-γ diagnostic test in infected herds, it is important to evaluate the performance of the test at the cut-off point used. One of the objectives of this study therefore was to investigate the effects of varying the cut-off on test performance. In addition, the relatively high proportion of SICTT − ve/IFN-γ +ve animals that have no detectable lesions at slaughter has prompted suggestions that many of these animals may be uninfected, and therefore test false positive. The main purpose of this study was to investigate the profile of Irish herds undergoing IFN-γ testing as part of the national bTB eradication programme and to assess the performance of the test at animal level in terms of herd size and number of SICTT reactors.

## 2. Materials and methods

### 2.1. Study population

The SICTT and collection of blood samples on which the IFN-γ tests were carried out was conducted as part of the national bTB eradication programme, which is subject to the EU Trade Directive 64/432/EEC, which governs the nature and frequency of testing. The research described in this study relates to the analysis of results of these diagnostic samples recorded on databases. Blood testing for IFN-γ was approved by the UCD Animal Research Ethics Committee (AREC-E-16-34-Gormley).

### 2.2. Production and measurement of IFN-γ

Blood samples were tested using the IFN-γ assay on animals assigned to either diagnostic or quality assurance (QA) testing. For diagnostic testing, the assay was conducted on blood samples collected contemporaneous (just subsequent) to a SICTT test whilst the herd was under movement restriction because of bTB. The samples were submitted to the laboratory and stimulated with bovine and avian tuberculin within 8 h of blood collection (Gormley et al., 2004). The QA IFN-γ tests were conducted on blood samples collected subsequent to a SICTT at which the animals were classified as reactor (SICTT standard reactor or standard inconclusive reactor). The samples were posted to the laboratory and stimulated with tuberculin antigens the day after collection (24 h).

Aliquots of the heparinised blood (1.5 ml) were dispensed into individual wells of 24-well tissue culture plates (Cruinn, Ireland) containing either PPD-b (20 μg/ml final conc), PPD-a (10 μg/ml) (ThermoFisher Scientific, Lelystad, Netherlands) or phosphate buffered saline (PBS) as a non-stimulating control. The plates were incubated for 16 h at 37 °C with 5% $CO_2$ before harvesting of plasma supernatants by centrifugation. Prior to assay, samples were stored at +4 °C where appropriate. IFN-γ production was measured in duplicate samples by sandwich ELISA using a commercial diagnostic kit (Bovigam, ThermoFisher Scientific). Absorbance values at 450 nm were converted to OD units using the formula, $OD_{450} \times 1000$. A sample was considered positive when the $OD_{450}$ of the PPD-bovine stimulated sample exceeded 100 OD units (B > 100), was greater than the nil un-stimulated sample by 50 OD units (B–N > 50), and was greater than the PPD avian stimulated sample (B-A > 0).

### 2.3. Definition of a SICTT reactor

The SICTT was carried out by intradermal injection of cattle with 0.1 mL PPD-bovine and PPD-avian at sites 12 cm apart in the mid-neck region using a McLintock tuberculin syringe. Skin thicknesses were measured in mm at both sites before the intradermal injection and after 72 h in accordance with Council Directive 64/432/EEC (2015) and OIE (2009). Each animal was given a 'reactor status'. Based on the results of the SICTT, the animal was defined as a standard reactor if the bovine reaction was both positive and exceeded the avian reaction by > 4 mm; as a standard inconclusive reactor if the bovine reaction was either positive or inconclusive, > 1–4 mm above the avian reaction, and the criteria for a standard reactor were not met; as a severe inconclusive reactor if the bovine reaction was either positive or inconclusive, the avian reaction equalled the bovine reaction or exceeded it by = < 2 mm, and the criteria for a standard reactor or standard inconclusive were each not met; or as negative, in all other cases.

### 2.4. Characteristics of herds tested for IFN-γ

The unit of interest was the test with the study population including all diagnostic IFN-γ tests conducted during 2016 and 2017. A diagnostic IFN-γ test was considered eligible if conducted on an animal that had no positive SICTT result either at the SICTT test immediately preceding or, if tested again, at the first SICTT subsequent to the IFN-γ test, regardless of the time between the tests. In addition, any animals that were SICTT standard reactor at any time prior to the IFN-γ test were excluded. For each diagnostic IFN-γ test, the time between the test and the nearest SICTT, either before or after the IFN-γ test, was calculated. The total number of animals tested with, and positive to the IFN-γ test, and the proportion positive were analysed by herd size and the number of SICTT standard reactors during the full duration of the bTB herd restriction. The herd size was based on the maximum number of animals tested at a whole-herd SICTT test in either 2016 or 2017 or both, depending on when the IFN-γ tests were carried out (for tests taken in both years, the maximum herd size across both years was used) and falling during the period of herd restriction. Herds were categorised by whether any of the IFN-γ tested/positive animals were subsequently positive for bTB at post-mortem. The number of positive IFN-γ test animals within herds with and without a post-mortem positive animals were then compared using a Wilcoxon test.

### 2.5. Bovine-avian cut-points for classifying IFN-γ test positive animals

The unit of interest was the animal and the study population included all animals that tested positive to an IFN-γ test in 2016 or 2017, and were slaughtered by the end of 2017. The result of the last IFN-γ test prior to slaughter was used for those animals with more than one test. The proportion of animals with a positive post-mortem result is presented at varying levels of the B-A level of the IFN-γ test, both for diagnostic and QA IFN-γ tests.

### 2.6. Combined diagnostic sensitivity of the SICTT and IFN-γ test

The unit of interest was the animal and the study population

included all animals that were tested using both the SICTT and the IFN-γ test during 2016 or 2017, that were slaughtered prior to the end of 2017, and were bTB positive at post-mortem. All IFN-γ tests were considered, i.e. both QA and diagnostic tests. A combined sensitivity for both tests was calculated at varying B-A cut-off points (0, 50, 80 and 100) of the IFN-γ test, using the post-mortem status as a gold standard.

## 3. Results

### 3.1. Characteristics of herds with IFN-γ test positive animals

During the 2016–2017 study period, there were a total of 68,993 diagnostic IFN-γ tests conducted on cattle in Ireland. The median time between the IFN-γ test and the nearest SICTT was 18 days (ranging from 0 to 96 days). In total, 7874 (11.4%) of these IFN-γ tests were positive. These tests were conducted on 64,405 animals, of which 7852 (12.2%) were positive at one or more tests, and 22 animals were positive on 2 IFN-γ tests. The maximum number of times an animal was tested with IFN-γ (of those negative to SICTT) was 4 (1 animal, which was negative on all 4 tests).

There were 1104 bTB restrictions within 1083 herds with one or more IFN-γ tests conducted on SICTT negative animals (Table 1). The start year of these restrictions varied from 2009 until 2017, with the majority (94%) starting in either 2016 or 2017. The median number of tests per bTB restriction was 39 with a median of 4 IFN-γ test positives per restriction. Both the number of IFN-γ tests and the number of IFN-γ positives increased with the number of SICTT reactors during the restriction (Table 1). The highest number of tests (a median of 64.5) were carried out in herds with > 13 SICTT reactors. Similarly the proportion of IFN-γ tests that were positive also increased with the number of reactors; the highest median of 8 test positives per restriction was recorded when there were > 13 SICTT reactors, resulting in a median 12.14% positive IFN-γ tests per herd.

The number of IFN-γ positives per herd in the study period is presented in Fig. 1, highlighting a skewed distribution towards lower numbers of IFN-γ positives per herd. The number of IFN-γ positives tended to increase with the number of SICTT standard reactors per herd (Fig. 2), with a correlation (Spearman's) coefficient of 0.32, indicative of a moderate correlation. Similarly the number of IFN-γ positives tended to increase as the herd size increased (Fig. 3), with a Spearman's correlation coefficient of 0.46.

There were 83 bTB restricted herds that had not slaughtered any of the animals that had been tested with an eligible diagnostic IFN-γ test by the end of 2017, however, only 5 of these had an IFN-γ positive animal (ranging from 1 to 5 IFN-γ positive animals). Of the remaining 1002 herds (with 1021 separate bTB restrictions), 532 restrictions had no PM positive animals among those IFN-γ tested and a median number of 2 IFN-γ positives per restriction (Table 2). There were 489 restrictions with one or more animals tested with IFN-γ that were PM positive, with a median of 7 IFN-γ positives per herd. Of these, 454 restrictions had one or more IFN-γ test positive animals that were also PM positive, with a median of 7 IFN-γ positive animals per herd. The median number of IFN-γ positives per restriction was significantly higher (Wilcoxon test $p < 0.001$) when comparing the 454 restrictions with at least one IFN-γ positive/PM positive animal to the 532 restrictions with no IFN-γ tested/PM positives (Table 2). In other words, those herds with an IFN-γ test positive animal that was also PM positive in the herd also had a significantly higher number of IFN-γ positives in the herd.

### 3.2. Bovine-avian cut-points for determining IFN-γ test positives

The post-mortem result for all IFN-γ positive animals tested in either 2016 or 2017 and slaughtered by end of 2017, by the bovine-avian (B-A) difference at the IFN-γ test and reason for testing (diagnostic, quality assurance) is presented in Table 3. As expected, and for the same range of B-A readings, the proportion of animals positive at post-mortem was much higher during quality assurance testing conducted on SICTT standard reactors. When B-A > 200, 49.5% of SICTT positive animals were lesion positive at post-mortem compared with a cumulative 28.4% when B-A ranged from 0 to 200 (that is, the total number of animals with a B-A difference up to and including 200). For animals with a positive IFN-γ test during diagnostic testing (SICTT negatives), the proportion positive at post-mortem was largest when B-A > 200 (29.8% lesion positive). For those animals with a cumulative B-A of 0–200, 5231 animals were positive to the IFN-γ test, however, only 387 (7.4%) were lesion positive at post-mortem.

### 3.3. Combined diagnostic sensitivity of the SICTT and IFN-γ test

A total of 8357 of the animals tested by both IFN-γ and the SICTT in 2016–2017 were positive at post-mortem (Table 4). Of these, 1283 animals (15.4%) were negative to the SICTT, and 84.6% were positive to the SICTT (either standard reactor, standard inconclusive or severe inconclusive).The proportion positive to the IFN-γ was 91.0% when the B-A cut-off was > 0 and decreased to 78.8% when the cut-off was increased to ≥100. The combined sensitivity for the two tests (IFN-γ and the SICTT) relative to post-mortem was 98.6% at a cut-off for IFN-γ of 0 and decreased to 96.4% when the IFN-γ cut-off increased to ≥100. The standard reactors included in this analysis were the IFN-γ QA tests and would have been tested 24 h after the sample was taken. Therefore the sensitivity may be slightly higher for these animals if the test had been carried out at 8 h after taking the sample. A further 116 (1.4%) animals positive at post-mortem were negative to both tests at the test prior to slaughter.

**Table 1**
Number of eligible diagnostic IFN-γ tests[a] conducted per herd in 2016 and 2017, and the number and percentage of IFN-γ positives, by number of SICTT standard reactors throughout the bTB herd restriction.

| Number of SICCT standard reactors[b] | No. of restrictions[c] | Number of IFN- γ tests | | | Number of IFN-γ positives | | | Percentage of positive IFN- γ tests | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Median | Min. | Max. | Median | Min. | Max. | Median | Min. | Max. |
| 0-2 | 126 | 21 | 1 | 401 | 1 | 0 | 23 | 5.43 | 0.00 | 100.00 |
| 3 | 134 | 32.5 | 1 | 289 | 2 | 0 | 45 | 6.54 | 0.00 | 100.00 |
| 4-5 | 256 | 29.5 | 1 | 740 | 3 | 0 | 65 | 9.31 | 0.00 | 100.00 |
| 6-7 | 165 | 42 | 2 | 354 | 4 | 0 | 45 | 10.00 | 0.00 | 100.00 |
| 8-13 | 209 | 34 | 1 | 484 | 4 | 0 | 43 | 10.08 | 0.00 | 100.00 |
| > 13 | 214 | 64.5 | 1 | 692 | 8 | 0 | 162 | 12.14 | 0.00 | 66.67 |
| All herds | 1104 | 39 | 1 | 740 | 4 | 0 | 162 | 9.64 | 0.00 | 100.00 |

[a] An IFN-γ test was considered eligible if conducted on an animal that had no positive SICTT either at the SICTT test immediately preceding or, if tested again, at the first SICTT subsequent to the IFN-γ test, regardless of the time between the tests, were included. In addition, any animals that were standard reactor at any time prior to the IFN-γ test were excluded.
[b] With the exception of the first 2 groups, the remainder are based on the quintiles of the distribution of the number of standard reactors.
[c] 1083 herds had 1104 restrictions (21 herds had 2 restrictions).

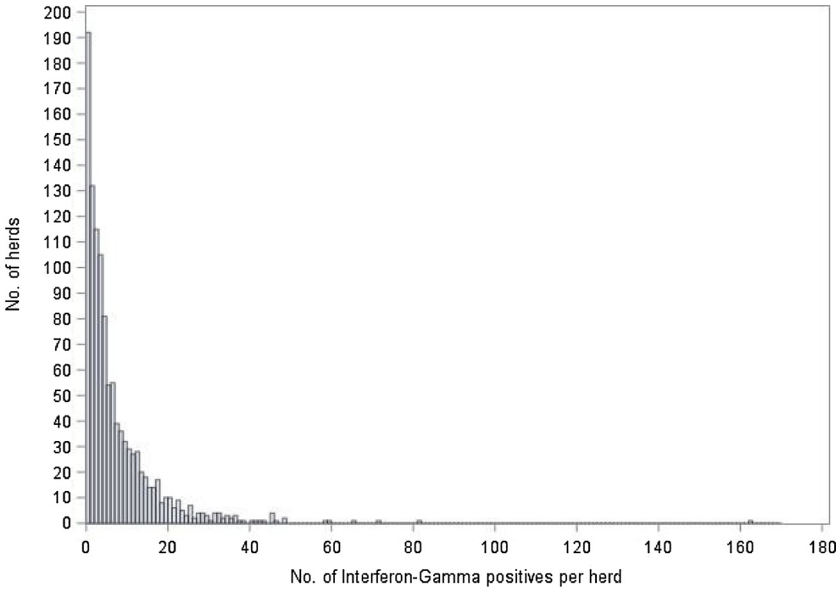


**Fig. 1.** Number of eligible diagnostic IFN-γ tests that were positive per herd in 2016 and 2017. An IFN-γ test was considered eligible if conducted on an animal that had no positive SICTT either at the SICTT test immediately preceding or, if tested again, at the first SICTT subsequent to the IFN-γ test, regardless of the time between the tests, were included. In addition, any animals that were standard reactor at any time prior to the IFN-γ test were excluded.

## 4. Discussion

There is evidence of a continuing significant decreasing trend in herd recurrence of bTB in Ireland from 1998 until 2015 (More et al., 2018; Houtsma et al., 2018). However, despite these improvements, the issue of undisclosed or residual infection remains a problem, with 30.2% of herds derestricted in 2012 being re-restricted over the following three years (Houtsma et al., 2018). There is now increased

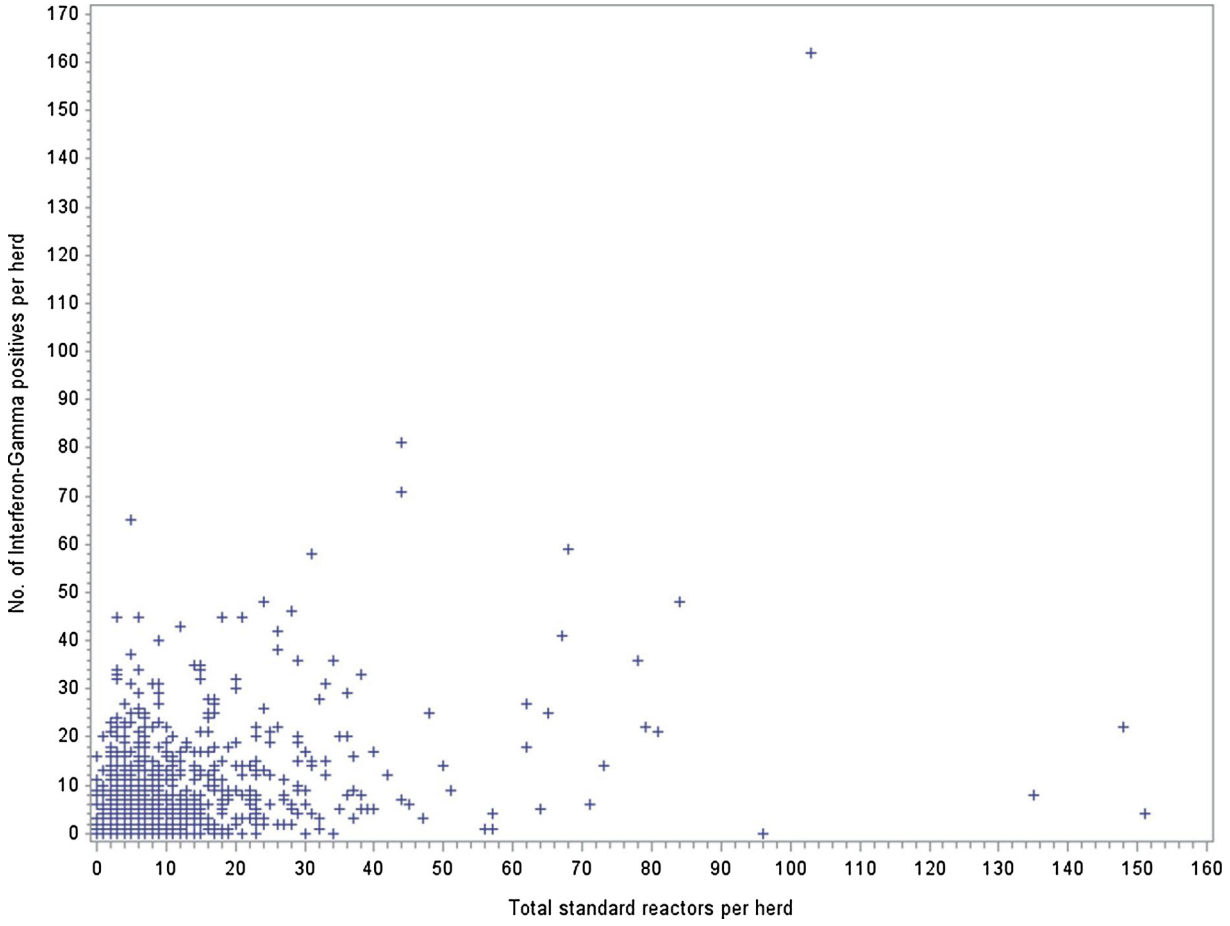


**Fig. 2.** Association between the total number of SICTT standard reactors during a bTB herd restriction and the number of eligible diagnostic IFN-γ tests that were positive per herd in 2016 and 2017. An IFN-γ test was considered eligible if conducted on an animal that had no positive SICTT either at the SICTT test immediately preceding or, if tested again, at the first SICTT subsequent to the IFN-γ test, regardless of the time between the tests, were included. In addition, any animals that were standard reactor at any time prior to the IFN-γ test were excluded.

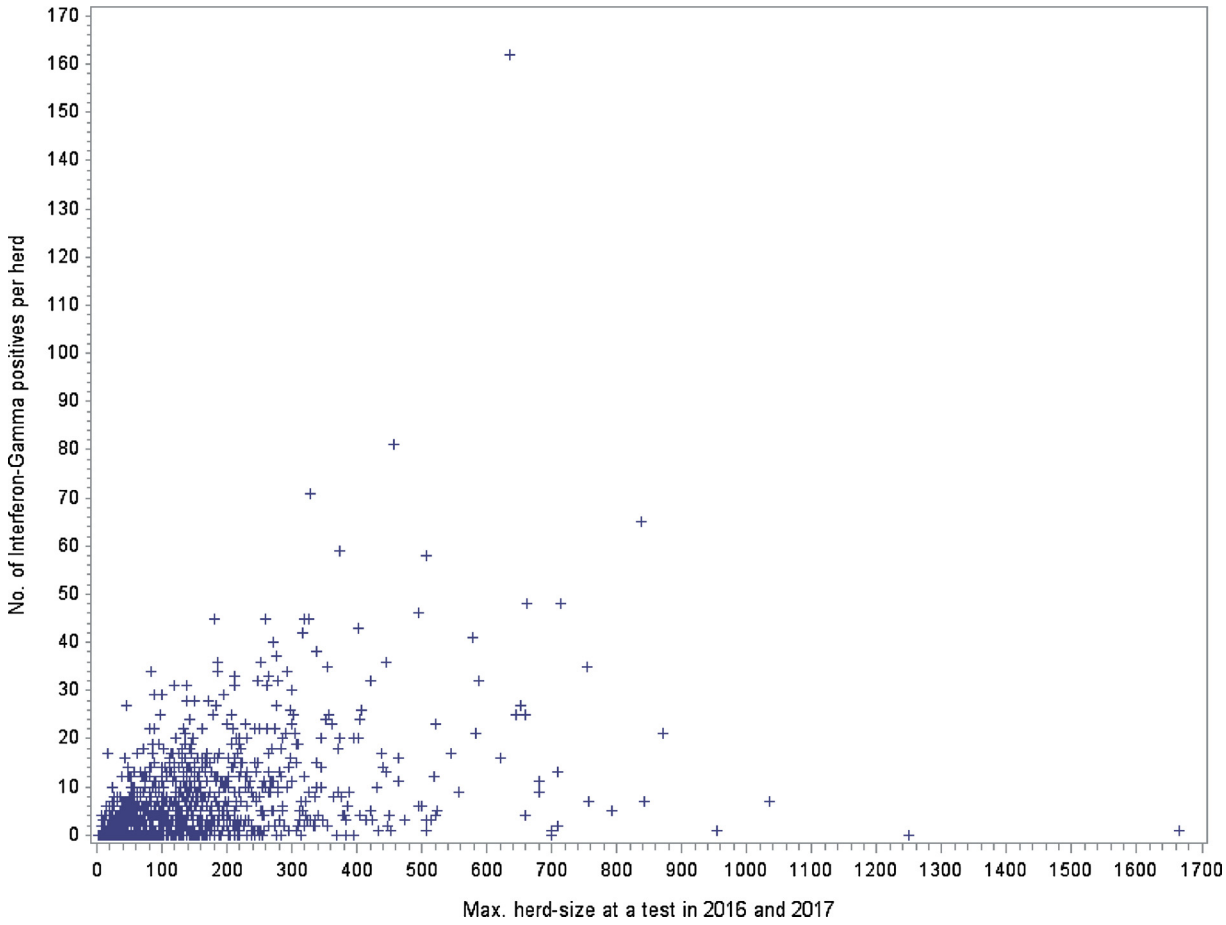


**Fig. 3.** Association between maximum herd size and the number of eligible diagnostic IFN-γ tests that were positive per herd in 2016 and 2017. An IFN-γ test was considered eligible if conducted on an animal that had no positive SICTT either at the SICTT test immediately preceding or, if tested again, at the first SICTT subsequent to the IFN-γ test, regardless of the time between the tests, were included. In addition, any animals that were standard reactor at any time prior to the IFN-γ test were excluded.

**Table 2**
Number of bTB restrictions where 1 or more of the eligible diagnostic IFN-γ tests[a] in 2016 and 2017 were positive, by post-mortem result.

| At least one animal with an eligible IFN-γ test was also positive at post-mortem? | At least one animal with an eligible IFN-γ test was both IFN-γ positive and positive at post-mortem? | No. of bTB restrictions | No. of eligible IFN-γ tests per herd that were positive | | | No. of eligible positive IFN- γ tests per herd where the animal was positive at post-mortem | | |
|---|---|---|---|---|---|---|---|---|
| | | | Min. | Median | Max. | Min. | Median | Max. |
| No | No | 532 | 0 | 2 | 27 | 0 | 0 | 0 |
| Yes | No | 489 | 0 | 7 | 162 | 1 | 2 | 41 |
| Yes | Yes | 454 | 1 | 7 | 162 | 1 | 2 | 41 |

[a] An IFN-γ test was considered eligible if conducted on an animal that had no positive SICTT either at the SICTT test immediately preceding or, if tested again, at the first SICTT subsequent to the IFN-γ test, regardless of the time between the tests, were included. In addition, any animals that were standard reactor at any time prior to the IFN-γ test were excluded.

knowledge about infection sources involved in bTB persistence in infected herds. Previous work on Irish data attributed 15% of bTB episodes (periods of herd restriction following bTB identification in a herd) to residual infection (White et al., 2013). In Britain, it is estimated that up to 20% of breakdowns have at least one infected animal present at the time of derestriction (Conlan et al., 2012). With its higher diagnostic sensitivity relative to the SICTT, the IFN-γ test is applied strategically in infected herds to identify additional infected animals that are false negative to the SICTT. Given the improvements in the Irish bTB situation, it is important to understand the performance of the IFN-γ test against the background of the decreased animal and herd incidence. This study set out to evaluate the characteristics of herds where the test was applied and to evaluate the impact of different cut-off points on test performance. The results of the analysis have provided new insights into the performance of the test and how it helps to underpin the diagnostic programme for eradication of tuberculosis from the Irish national herd.

Herd size is recognized as a significant risk factor for recurrence of bTB (Griffin et al., 1996; Brooks-Pollock and Keeling, 2009; Broughan et al., 2016). Among the key findings, we found that the number of animals testing IFN-γ positive correlated moderately with the size of the herd and the severity of the breakdown (as measured by number of SICTT standard reactors and detection of lesions at slaughter); that is, larger herds with more severe breakdowns were likely to have more IFN-γ positives in the herd, with a median of 4 IFN-γ positive animals per restriction. Herds with an IFN-γ test positive animal that was also PM positive in the herd also recorded higher numbers of IFN-γ positives in the herd. These results conceivably reflect an increase in testing

**Table 3**
The post-mortem result for all IFN-γ positive animals tested in either 2016 or 2017 and slaughtered by end of 2017, by the bovine-avian difference in the IFN-γ test and reason for testing; Diagnostic, Quality Assurance (QA).

| IFN-γ B-A difference | Post-mortem result | | | Cumulative post-mortem results | | | |
|---|---|---|---|---|---|---|---|
| | Total Post-mortems | Number Positive | % positive at post-mortem | IFN-γ B-A difference | Total post-mortems | Number positive | % positive at post-mortem |
| **Diagnostic** | | | | | | | |
| 0-20 | 90 | 7 | 7.8 | ≤20 | 90 | 7 | 7.8 |
| 21-40 | 752 | 42 | 5.6 | ≤40 | 842 | 49 | 5.8 |
| 41-60 | 1,123 | 66 | 5.9 | ≤60 | 1,965 | 115 | 5.9 |
| 61-80 | 831 | 48 | 5.8 | ≤80 | 2,796 | 163 | 5.8 |
| 81-100 | 662 | 63 | 9.5 | ≤100 | 3,458 | 226 | 6.5 |
| 101-120 | 508 | 43 | 8.5 | ≤120 | 3,966 | 269 | 6.8 |
| 121-140 | 397 | 38 | 9.6 | ≤140 | 4,363 | 307 | 7.0 |
| 141-160 | 348 | 31 | 8.9 | ≤160 | 4,711 | 338 | 7.2 |
| 161-180 | 278 | 26 | 9.4 | ≤180 | 4,989 | 364 | 7.3 |
| 181-200 | 242 | 23 | 9.5 | ≤200 | 5231 | 387 | 7.4 |
| > 200 | 3,892 | 1,160 | 29.8 | All | 9,123 | 1,547 | 17.0 |
| Total | 9,123 | 1,547 | 17.0 | | | | |
| **QA** | | | | | | | |
| 0-20 | 1055 | 415 | 39.3 | ≤20 | 1,055 | 415 | 39.3 |
| 21-40 | 555 | 149 | 26.8 | ≤40 | 1,610 | 564 | 35.0 |
| 41-60 | 410 | 85 | 20.7 | ≤60 | 2,020 | 649 | 32.1 |
| 61-80 | 350 | 72 | 20.6 | ≤80 | 2,370 | 721 | 30.4 |
| 81-100 | 340 | 83 | 24.4 | ≤100 | 2,710 | 804 | 29.7 |
| 101-120 | 323 | 69 | 21.4 | ≤120 | 3,033 | 873 | 28.8 |
| 121-140 | 257 | 58 | 22.6 | ≤140 | 3,290 | 931 | 28.3 |
| 141-160 | 209 | 66 | 31.6 | ≤160 | 3,499 | 997 | 28.5 |
| 161-180 | 254 | 71 | 28.0 | ≤180 | 3,753 | 1,068 | 28.5 |
| 181-200 | 227 | 63 | 27.8 | ≤200 | 3,980 | 1,131 | 28.4 |
| > 200 | 9,954 | 4,923 | 49.5 | All | 13,934 | 6,054 | 43.4 |
| Total | 13,934 | 6,054 | 43.4 | | | | |

frequency and the likelihood of finding an IFN-γ positive animal as the size of the herd and severity of the restriction increases. The low median number (n = 4) of IFN-γ test positives, based on testing an average of 39 'targeted high-risk' animals per herd and an average test positive rate of 10% within the infected herds may help to address perceptions among some herdkeepers and veterinarians that the IFN-γ test regularly identifies unacceptably large numbers of animals whose disease status is uncertain (e.g., false positives).

It has been shown in a number of countries that IFN-γ +ve animals in infected herds are at greater risk of becoming positive at SICTT or postmortem in subsequent years (Coad et al., 2008; Lahuerta-Marin et al., 2015; Sinclair et al., 2016; Clegg et al., 2017). Elsewhere we have shown that more severe breakdowns are at higher risk of recurrence of herd restriction when compared with smaller breakdowns (Gallagher et al., 2013; Byrne et al., 2014; Houtsma et al., 2018) suggesting that the failure to detect infected animals (false-negatives) is at least partly responsible for recurrence of restricted herds. This is consistent with our previous finding where the odds of SICTT -ve/ IFN-γ +ve animals being positive at post-mortem within the same calendar year, was 5 times higher compared to SICTT -ve/ IFN-γ -ve animals (Clegg et al., 2017). Other than in New Zealand, there is limited information on the herd level impact of IFN-γ testing (Sinclair et al., 2016). Further work is needed to characterise the contribution of the use of IFN-γ testing to reducing future herd risk of bTB recurrence in Ireland. The impact of

**Table 4**
SICTT and IFN-γ test results for animals positive at post-mortem that were tested in 2016/2017 and slaughtered prior to the end of 2017. For both the SICTT and IFN-γ test, the test result was at the last test prior to slaughter.

| SICTT result | No. (%) of animals | IFN-γ cut-point | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 50 | | 80 | | 100 | |
| | | No. positive | % positive | No. positive | % Positive | No. positive | % positive | No. Positive | % Positive |
| Negative[a] | 1283 (15.4) | 1167 | 91.0 | 1093 | 85.2 | 1032 | 80.4 | 982 | 76.5 |
| Severe inconclusive | 279 (3.3) | 232 | 83.2 | 217 | 77.8 | 198 | 71.0 | 191 | 68.5 |
| Standard Inconclusive | 800 (9.6) | 653 | 81.6 | 584 | 73.00 | 558 | 69.8 | 539 | 67.4 |
| Standard reactor[b,c] | 5995 (71.7) | 5549 | 92.6 | 5026 | 83.8 | 4,934 | 82.3 | 4874 | 81.3 |
| Total | 8357 | 7601 | 91.0 | 6920 | 82.8 | 6,722 | 80.4 | 6586 | 78.8 |
| Relative sensitivity[d] | | | | | | | | | |
| Combined (SICTT and IFN-γ) | | **98.6** | | **97.7** | | **97.0** | | **96.4** | |
| IFN-γ | | 91.0 | | 82.8 | | 80.4 | | 78.8 | |
| SICTT | | 84.7 | | 84.7 | | 84.7 | | 84.7 | |

[a] The total of SICTT negatives / PM positives is less than the equivalent number arising from diagnostic tests shown in Table 3 (1547). This is due to some SICTT positives being submitted as diagnostic tests or the positive SICTT was carried out after the IFN-γ diagnostic test.
[b] The SICTT standard reactors that were tested with the IFN-γ test were the QA tests and were carried out at 24-hours after the sample was taken.
[c] The total standard reactors is less than the QA total in Table 3 (6054) due to some inconclusives/negative SICTT animals being submitted as QA tests.
[d] Relative to the post-mortem result.

IFN-γ testing is likely to be confined to a reduction in the risk associated with undisclosed infected animals that test false negative to the SICTT test, which may subsequently manifest as residually infected animals. The use of IFN-γ testing would be unlikely to reduce the aspects of future breakdown risk associated with wildlife, local spread or introduction of infection.

Among countries that carry out IFN-γ testing on infected herds, a variety of cut-off points are employed to determine the infected status of the animal and the test interpretation is adapted to the local environment and disease levels (EFSA, 2012). Changing the differential between the PPD-bovine and PPD-avium OD values can be used to modify the specificity and sensitivity of the test, with increasing differences correlated with higher specificity and lower sensitivity. Setting the cut-off point for high sensitivity will increase the likelihood of detecting infected animals in high prevalence herds, but where infection levels are low, high specificity may be more important to reduce the number of false positive test results. The presence of other non-tuberculous mycobacteria in the environment may reduce specificity because of cross-reactivity with antigens used for blood stimulation in the IFN-γ assay (Jenkins et al., 2018). The IFN-γ test readouts may also be influenced by the individual immunological profile of the host animal, and also immuno-suppression caused by anti-inflammatory cytokines that are produced by stimulation of blood with tuberculin (Sheridan et al., 2017).

In this study we investigated the impact of altering the B-A differential on the detection of animals with lesions at post-mortem. The disclosure of lesioned animals was highest for animals that were positive to both the SICTT and the IFN-γ test: with a B-A differential > 200, 49.5% of animals had a bTB lesion at post-mortem examination and 28.4% when B-A < 200. However, among the SICCT –ve and IFN-γ +ve cohort of animals only 7.4% of animals were cumulatively positive at post-mortem when B-A < 200, whereas 29.8% of animals were positive at post-mortem when B-A was > 200. The difference in lesion rate between the SICTT reactor and non-reactor animals plausibly represents a more advanced stage of disease that is more likely to be detected at post-mortem. Reliance on lesion detection as a gold standard will inevitably lead to overestimation of diagnostic test performance as it does not take into account undisclosed or sub-clinical infection. The proportion of PM positive animals that were negative to both tests (1.4%) is much lower than that indicated in the previous study where 4.7% of animals that were negative to both the IFN-γ and SICTT were positive at post-mortem (Clegg et al., 2017). This is partly because the SICTT employed in the earlier study was conducted as the test closest in time to the IFN-γ test. However, with retrospective analysis, some of these animals went on to have a positive SICTT result after the IFN-γ test. When these animals were taken into account and excluded, the proportion of those animals negative to both tests and positive at post-mortem falls to 2.3%, closer to the rate observed in the current study.

Given that higher B-A differential values appeared to preferentially identify animals with a high risk of having a lesion, and a potentially increased impact of future risk at herd level, the analysis presented an opportunity to investigate the influence of changing B-A values on the performance characteristics of the test. As expected, and when using lesion detection as the gold standard diagnosis, the relative sensitivity of the IFN-γ test decreased from 91.0% when the B-A = 0 cut-off point was applied, to 78.8% when B-A > 100. However, when the combined sensitivity (IFN-γ and SICTT) was determined, there was only a small reduction in overall sensitivity from 98.6% (B-A = 0) to 97.0% (B-A > 80), and 96.40% (B-A > 100). Any increase in the cut-off point that reduces sensitivity will also result in an increase in the combined specificity levels. However, since data used in this study comes from cattle in infected herds, it was not possible to estimate the combined specificity. Based on similar data that included bTB free herds from Ireland, and analysed by the European Food Safety Authority (EFSA), the combined specificity for SICTT (standard interpretation) and IFN-γ test (B-A = 0) ranged from 87.58% to 90.96% when using the estimated credible interval range of reported specificities and covariances of the two tests (EFSA, 2012). In other words, altering the B-A cut-off between 0–100 appears to maintain sensitivity of detection at almost the same level while increasing the specificity above 91%. Any changes to the criteria for interpretation of the test will need to be monitored in the appropriate cohorts of animals in order to generate confidence that the test is optimized and provides a beneficial impact to reducing future risks to derestricted herds.

## 5. Conclusions

This analysis has provided us with an update of the performance of the IFN-γ test as it is used under current infection levels in Ireland. It is consistent with the knowledge that large herds are at higher risk of experiencing more severe bTB breakdowns and that the IFN-γ test can be a very useful tool for identifying infected animals that are missed (false-negative) by the skin test. As progress towards disease eradication advances there will an ongoing requirement to monitor all aspects of IFN-γ diagnostic testing to ensure that it remains fit for purpose.

## Competing interests

The authors declare that they have no competing interests

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