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The performance of the interferon gamma assay when used as a diagnostic or quality assurance test in *Mycobacterium bovis* infected herds

T.A. Clegg[a,*], M. Good[b], M. Doyle[c], A. Duignan[b], S.J. More[a], E. Gormley[c]

[a] Centre for Veterinary Epidemiology and Risk Analysis, UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[b] Department of Agriculture, Food & the Marine, Agriculture House, Kildare St., Dublin 2, Ireland
[c] Tuberculosis Diagnostics and Immunology Research Laboratory, UCD School of Veterinary Medicine, University College Dublin, UCD, Belfield, Dublin 4, Ireland

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ABSTRACT

There are two different contexts in the Irish bTB eradication programme in which the interferon-gamma assay (IFN-γ) is applied. Firstly, the IFN-γ assay is applied routinely to high risk cohorts in herds with four or more reactors to the SICTT. The IFN-γ test is then carried out on blood samples submitted to the laboratory within 8 h of collection (diagnostic testing). Secondly, the use of the IFN-γ assay has recently been extended to test SICTT reactors as part of a general quality assurance (QA) scheme to monitor the performance of the SICTT. Blood samples from reactors are tested one day after blood collection (QA testing). In this study, we analysed the relative performance of the SICTT and IFN-γ when used in parallel as an 8 h diagnostic test and as a 24 h QA test on SICTT reactors. A total of 17,725 IFN-γ tests were included in the analysis (11,658 diagnostic tests and 6067 QA tests). Of the samples submitted for diagnostic testing, the proportion positive to IFN-γ decreased with the severity of interpretation of the SICTT result. Of the standard reactors that were tested with IFN-γ in the QA programme, 92.2% were positive to the IFN-γ test. Among animals that were SICTT −ve/IFN-γ +ve, 18.9% were positive at *post-mortem* compared to 11.8% of those that were SICTT +ve (standard reactor)/IFN-γ −ve. These results highlight the risk associated with retaining SICTT −ve/IFN-γ +ve animals, and suggest that prompt removal of these animals is necessary to reduce the potential for future transmission.

1. Introduction

The tuberculin skin test in all of its forms is the most widely used field surveillance test for the detection of *Mycobacterium bovis* infection in live cattle. Where cross sensitization from environmental mycobacteria interferes with the specificity of the test, *M. avium*-derived tuberculin is included to perform the single intradermal comparative tuberculin test (SICTT). The SICTT has a median specificity approaching 100% based on studies of bovine tuberculosis (bTB)-free populations from several countries (de la Rua-Domenech et al., 2006; Gormley et al., 2006; Clegg et al., 2011). In Ireland, only 0.2% of animals in the national herd were reactors to the SICTT in 2015 (DAFM, 2016). The sensitivity of the SICTT in Ireland has an estimated median value of 80% (range 52%–100), based on many studies (Costello et al., 1997; de la Rua-Domenech et al., 2006; Gormley et al., 2006; Clegg et al., 2011). Results from a recent Bayesian latent-class analysis have suggested a lower test sensitivity for the SICTT (standard interpretation) in Ireland with 95% credibility interval: 64.5%, 73.0% (EFSA, 2012). In this analysis, the test specificity had 95% credibility interval: 99.3%, 99.96%. In order to improve test sensitivity, the interpretation criteria of the SICTT in bTB infected herds can be modified by applying a severe interpretation of the test (de la Rua-Domenech et al., 2006; Good et al., 2010). Nevertheless, a proportion of infected animals may still remain undetected.

In herds exposed to infection with *M. bovis*, the interferon-gamma assay (IFN-γ) can be used as an ancillary test in parallel with the SICTT to improve the sensitivity of diagnostic testing. The principle of the IFN-γ assay is to detect and quantify release of the IFN-γ cytokine when heparinised whole blood is incubated with bovine and avian tuberculin (PPD), normally within the first 8–24 h

* Corresponding author.
E-mail addresses: tracy.clegg@ucd.ie (T.A. Clegg), mgood2510@gmail.com (M. Good), mairead.b.doyle@ucd.ie (M. Doyle), Anthony.Duignan@agriculture.gov.ie (A. Duignan), simon.more@ucd.ie (S.J. More), egormley@ucd.ie (E. Gormley).

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post-collection (Rothel et al., 1990). Based on several studies from different countries, the estimated median specificity of the IFN-γ is 96.6% ranging between 85.0% and 99.6% (Monaghan et al., 1997; de la Rua-Domenech et al., 2006; Gormley et al., 2006; Schiller et al., 2010; Clegg et al., 2011; Gormley et al., 2013) which is lower than the SICTT and precludes it from use as an initial screening test for surveillance. In Ireland, the IFN-γ assay is performed within 8-h of sampling. Under these conditions, it has a sensitivity of between 63.1% and 88% (Gormley et al., 2006; Clegg et al., 2011), which is comparable to the observed sensitivity of the SICTT. In the Bayesian analysis, the 95% credibility intervals for Se and Sp of the IFN-γ were between 79.1% and 86.1% (Se) and 88.2% to 90.8% (Sp) (EFSA, 2012). In Ireland, when the SICTT (with severe interpretation) and IFN-γ test results are interpreted in parallel the combined tests have an estimated sensitivity of 93% (Gormley et al., 2006).

A national bovine tuberculosis eradication programme has been in place in Ireland since 1954. The programme consists of field and abattoir surveillance. Field surveillance involves the testing of all herds in Ireland at least annually using the SICTT. Movement restrictions are imposed on any herd when one or more animals test positive to the SICTT (reactor animals), these reactor animals are subsequently culled. Abattoir surveillance involves gross *post-mortem* examination, in accordance with Regulation (EC) No. 854/2004 (Council, 2015), to detect visible lesions, and is carried out at routine slaughter on all animals, including those that are removed as reactors.

There are two different contexts in the Irish bTB eradication programme in which the IFN-γ assay is applied. Firstly, the IFN-γ assay is applied routinely to herds with four or more reactors to the SICTT. A Veterinary Inspector (VI) employed by the Irish Department of Agriculture Food and the Marine (DAFM) evaluates the epidemiological evidence of bTB and identifies the high risk of infection cohort within the herd on which the IFN-γ assay is conducted for diagnostic purposes and termed diagnostic testing. The IFN-γ test is then carried out on blood samples submitted to the laboratory within 8 h of collection. Secondly, as part of a general quality assurance (QA) of SICTT testing, the IFN-γ assay is also conducted on blood samples from SICTT reactors to monitor the performance of the SICTT. As the correlation between IFN-γ and the SICTT test results is likely to be high in infected animals (Gormley et al., 2004), for convenience these tests are conducted on samples the day after blood collection (24 h tests). Subsequently, this is termed QA testing. The aim of this study was to describe the relative performance of the SICTT and IFN-γ when IFN-γ is used for diagnostic testing and for QA testing.

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, in compliance with the EU trade Directive 64/432/EEC, which governs the nature and frequency of testing. The results presented in this study are an analysis of national databases containing the test results on the national herd. The study population included all cattle tested using the IFN-γ assay in Ireland during 2015. The national databases used for this study were: the Animal Health Computer System (AHCS), with tuberculin testing data of animals (at test dates when inconclusive or reactor animals were disclosed) and gross *post-mortem* results for reactor animals slaughtered; the Animal Identification and Movement system (AIM), recording all cattle slaughtered in Ireland; a database of laboratory testing results from the national abattoir surveillance programme (histopathology and culture results from all non-reactor animals with a gross suspect-TB lesion(s) detected at slaughter); and a database of all IFN-γ tests held by the Tuberculosis and Immunology Research Laboratory at University College Dublin.

2.2. Definition of a SICTT reactor

The SICTT was carried out by intradermal injection of cattle with 0.1 mL PPD-bovine (30,000 I.U./ml) and PPD-avian (25,000 I.U./ml) tuberculin (Thermo-Fisher Scientific, Lelystadt, Netherlands) at sites 12 cm apart in the mid-neck region using McLintock tuberculin syringes. Skin thicknesses were measured in mm at both sites prior to the intradermal injection and at 72 ± 4 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. An 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.

2.3. IFN-γ assay groups

Blood samples were tested using the IFN-γ assay on animals assigned to either of the following categories: (1) Diagnostic testing (in parallel): The IFN-γ test was conducted on blood samples collected contemporaneous (either just prior or subsequent) to a SICTT on non-reactor animals whilst the herd was restricted due to bTB. The samples were submitted to the laboratory and stimulated with bovine and avian tuberculin, using the same tuberculin PPDs as for the SICTT, within 8-h of blood collection. (2) QA testing: The IFN-γ test was conducted on blood samples collected subsequent to a SICTT at which the animals were classified as reactor (standard reactor or standard inconclusive reactor). The samples were posted to the laboratory and stimulated with antigens the day after collection (24 h). Occasionally samples for QA testing were taken at the same time as samples for diagnostic testing and the latter were antigen-stimulated at 24 h. Where this was evident, these samples were assigned to the test category for which they were submitted.

2.4. Production and measurement of IFN-γ

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) (Thermo-Fisher Scientific, Lelystadt, 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, Thermo-Fisher Scientific, Schlieren, Switzerland). 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, was greater than the nil un-stimulated sample by 50 OD units and was greater than the PPD avian stimulated sample. The formula to define the test result for each animal is optimized for sensitivity with respect to lesion detection and positive *M. bovis* culture, and is currently adopted as the official interpretation in the Irish bovine TB eradication programme (More and Good, 2006).

2.5. Data analysis

Each IFN-γ result was paired with an associated SICTT result as follows: the nearest SICTT result within 14 days (either before or after the IFN-γ test) was used unless the animal had previously been classified as a reactor at any test prior to the IFN-γ, in which case that reactor result was used. Correlations between IFN-γ and SICTT were measured by reactor category (standard reactor, standard inconclusive reactor) and tester type including Private Veterinary Practitioner (PVP) and whole time government paid temporary Veterinary Inspector (WTVI)/Veterinary Inspector (VI) using Spearman's measure of rank correlation. Correlations were also measured for the subset of reactor animals with a SICTT within 14 days of the IFN-γ test. The difference between bovine and avian readings for IFN-γ positive animals and standard reactor animals were plotted to explore any correlation between the readings.

2.6. Post-mortem data

When reactor bovines are slaughtered, Regulation 854/2004 (2015) requires that incision of specified head and thoracic lymph nodes and particular attention is paid to the palpation of tongues, lungs and visual inspection of the mesenteric lymph nodes. When suspect bTB lesions are found, specific additional carcass lymph glands are opened and inspected depending on the location of the suspect lesion. Slaughter data was used up to 31st December 2015. For animals tested more than once with IFN-γ, the most recent IFN-γ and corresponding SICTT result prior to slaughter was used. All animals slaughtered as non-reactors were routinely inspected for the presence of visible granulomatous lesions indicative of bTB. These lesions were then sent to the laboratory for follow-up confirmatory testing (histopathology and/or culture) (Frankena et al., 2007). Any slaughtered animal with no *post-mortem* result recorded was assumed to have no visible lesions at *post-mortem*.

3. Results

A total of 17,725 IFN-γ tests were included in the analysis (11,658 diagnostic tests conducted at 8 h and 6067 QA tests conducted at 24 h), taken from 1453 herds (287 herds had diagnostic tests and 1348 herds had QA tests), with a median of 3 samples per herd ranging from 1 to 403 samples per herd (Diagnostic testing herds: median = 16, range 1–324; QA testing herds: median = 3, range 1–81). Of the standard reactors that were tested with IFN-γ in the QA programme, 92.2% were IFN-γ positive (Table 1). A further 469 standard reactors were submitted as diagnostic tests, with significantly fewer (86.8%) of these being positive to the IFN-γ test (chi-square test: p-value < 0.001). Among 1187 standard inconclusive reactors submitted for QA testing, 72.2% were positive for IFN-γ. Of the samples submitted for diagnostic testing, the proportion positive to IFN-γ decreased with the severity of the SICTT reaction with 64.6% of standard inconclusive reactors positive to IFN-γ and 48.9% of severe inconclusive reactors positive to IFN-γ, and 13.3% of SICTT negatives (Table 1).

The correlation between IFN-γ readings (the difference between $OD_{450} \times 1000$ Bovine and Avian readings) and the SICTT reading (B-A skin thickness difference) for all QA tests are given in Table 2, a plot of IFN-γ readings by SICTT results are given in Fig. 1. All correlations were significant, the correlation with IFN-γ readings was higher in standard SICTT reactors (0.36, Spearman's rank correlation (Spr)) compared with standard inconclusive reactors (Spr = 0.17) with the highest correlation (Spr = 0.46) achieved when both reactor types were combined (Table 2a). There was very little difference in correlation between the tester types (Table 2b). When the correlation analysis was restricted to standard reactors with an IFN-γ test within 14 days of the SICTT there was no change to the correlation estimates. The plot of B-A readings for animals positive to both tests reflects the moderate correlation between the two tests (Fig. 1) and shows the wide variation in readings for the two tests, ranging from 1 to 3800 OD units for IFN-γ and 5 mm to 90 mm for the difference in skin thickness in the SICTT.

In animals with a positive IFN-γ result (either diagnostic or QA) and negative SICTT, 94.4% were slaughtered by the end of 2015 compared to 98.0% of standard reactors that were negative at the IFN-γ test (Table 3).

Of the animals slaughtered, 42.0% of those that were positive to the IFN-γ were positive at *post-mortem* compared to only 7.4% of those that were negative to the IFN-γ (Table 4). For animals that were classified as standard reactors and positive to the IFN-γ, 52.3% were positive at *post-mortem* compared to only 11.8% of standard reactors that were negative to the IFN-γ. For animals that were negative to the SICTT, the odds of being positive at *post-mortem* were nearly five times higher for IFN-γ positive animals compared to IFN-γ negative animals.

Of those animals that were positive to the IFN-γ and negative to the SICTT, 18.9% were positive at *post-mortem* compared to 11.8% of those that were standard reactor to SICTT and negative to IFN-γ falling to 9.2% of those that were standard inconclusive to SICTT and negative to IFN-γ.

4. Discussion

In this study, we have compared the relative performance of the IFN-γ assay when used in parallel with the SICTT as a diagnostic test and when used with the SICTT as a quality assurance measure for monitoring the integrity of the skin test. The proportions of animals positive to the IFN-γ test decreased with the severity of the SICTT reaction irrespective of timing (8 h or 24 h) of the first stage of the assay (antigen-stimulation) post-collection of blood samples (Table 1). Among the standard SICTT reactors tested for QA using IFN-γ at 24 h post-collection of blood, 92.2% were positive to both tests. In the development stage of the IFN-γ assay it was concluded that IFN-γ samples should be processed on the same day as collection, as a delay of 24 h in processing the blood resulted in a 30% decrease in the ELISA OD values (Rothel et al., 1992). In a study conducted on Irish cattle (Gormley et al., 2004) there was also a significant drop in the ELISA OD values when the blood processing of diagnostic samples was delayed for 24 h, with this effect greatest among animals negative to the SICTT. These authors suggested that the likelihood of a blood sample remaining IFN-γ positive for 24 h post-collection is dependent on the stage of disease in the infected animal and on the magnitude of the IFN-γ responses. The results of the current study, where 92.2% of standard SICTT reactors were positive to the IFN-γ test when the IFN-γ assay was carried out 24-h after collection, is consistent with this proposition. We expected an increased sensitivity of the IFN-γ test at 8 h compared to 24 h testing. However, as highlighted in Table 1, significantly fewer standard SICTT reactors were positive to the IFN-γ at 8 h diagnostic testing (86.8%) compared with standard SICTT reactors submitted for 24 h QA tests (92.2%). We note though that the correlation between the IFN-γ and SICTT in this group of animals was significantly lower (Fisher z-transformation: p = 0.014) for the diagnostic test (Spr = 0.25; 95% CI: 0.17–0.34) compared to those submitted for a QA test (Spr = 0.36; 95% CI: 0.34–0.39), pointing to an uncertainty in the performance or interpretation of the SICTT result in the reactor animals submitted for diagnostic testing. We also cannot rule out the possibility of an anamnestic IFN-γ response in the QA animals following tuberculin injection; blood samples are collected from QA reactor animals typically within 7–10 days of tuberculin injection, whereas samples for diagnostic testing may be collected

Table 1
SICTT and IFN-γ result for animals tested with both tests in Ireland in 2015, by test type.

SICTT result	Test type[a]	IFN-γ result			95% Confidence interval	
		Total	No. Positive	% positive	Lower	Upper
Standard Reactor	Diagnostic	469	407	86.8	83.7	89.8
	QA	4880	4499	92.2	91.4	92.9
	Total	5349	4906	91.7	91.0	92.5
Standard Inconclusive	Diagnostic	237	153	64.6	58.5	70.6
	QA	1187	857	72.2	69.7	74.7
	Total	1424	1010	70.9	68.6	73.3
Severe Inconclusive	Diagnostic	393	192	48.9	43.9	53.8
Negative	Diagnostic	10559	1404	13.3	12.6	13.9

[a] QA = Quality Assurance.

Table 2
Correlation between the IFN-γ and SICTT Bovine-Avian readings for all Quality Assurance tests, by reactor type and, for standard reactors only, by tester type

a) by reactor type

	Spearman's rank correlation (no. of animals)	p-value	Spearman's rank correlation (no. of animals) based on tests <= 14 days apart	p-value
All tests	0.46 (6067)	<0.001	0.46 (5774)	<0.001
By Reactor type				
Standard. Reactor	0.36 (4880)	<0.001	0.36 (4587)	<0.001
Standard Inconclusive	0.17 (1187)	<0.001	0.17 (1187)	<0.001

b) by tester type for standard reactors only

Tester	Spearman's rank correlation (no. of animals)	p-value	Spearman's rank correlation (no. of animals) based on tests <= 14 days apart	p-value
PVP	0.36 (4276)	<0.001	0.36 (4040)	<0.001
WTVI/VI	0.35 (604)	<0.001	0.35 (547)	<0.001

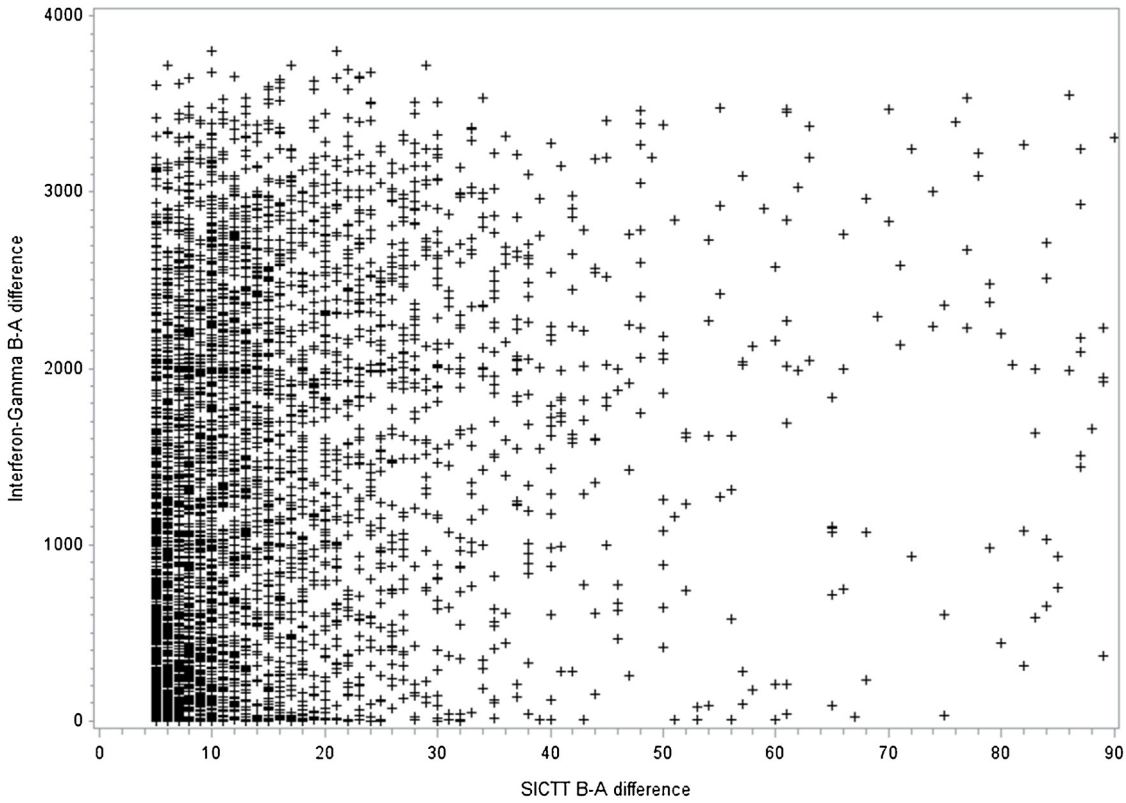

Fig. 1. Interferon Gamma readings (bovine – avian) for positive animals against SICTT readings (bovine – avian) for standard reactors, for animals submitted as QA samples (n = 3220).

Table 3
Proportion of study animals slaughtered by 31 December 2015, by IFN-γ and SICTT result.

IFN-γ	SICTT	Alive	Dead	Total	% dead	95% confidence interval	
						Lower	Upper
Neg	All	7480	1941	9421	20.6	19.8	21.4
	Standard Reactor	9	434	443	98.0	96.7	99.3
	Standard Inconclusive	54	358	412	86.9	83.6	90.2
	Severe Inconclusive	117	67	184	36.4	29.5	43.4
	Negative	7300	1082	8382	12.9	12.2	13.6
Pos	All	191	7280	7471	97.4	97.1	97.8
	Standard Reactor	82	4824	4906	98.3	98.0	98.7
	Standard Inconclusive	26	984	1010	97.4	96.4	98.4
	Severe Inconclusive	6	184	190	96.8	94.4	99.3
	Negative	77	1288	1365	94.4	93.1	95.6

Table 4
Post-mortem results for study animals slaughtered before the end of 2015, by IFN-γ and corresponding SICTT result prior to slaughter.

IFN-γ	SICTT	*Post-mortem* result			95% confidence interval	
		Total	No. Positive	% Positive	Lower	Upper
Neg	All	1941	144	7.4	6.3	8.6
	Standard Reactor	434	51	11.8	8.7	14.8
	Standard Inconclusive	358	33	9.2	6.2	12.2
	Severe Inconclusive	67	9	13.4	5.3	21.6
	Negative	1082	51	4.7	3.5	6.0
Pos	All	7280	3059	42.0	40.9	43.2
	Standard Reactor	4824	2524	52.3	50.9	53.7
	Standard Inconclusive	984	264	26.8	24.1	29.6
	Severe Inconclusive	184	28	15.2	10.0	20.4
	Negative	1288	243	18.9	16.7	21.0

14-days prior to, on the day of tuberculin injection or even weeks after injection.

We also compared the IFN-γ readings (B-A OD_{450} values) and the SICTT reading (B-A skin test difference) for all of the 24 h QA tests (Table 2). Despite the high level of agreement between the test result outcomes, we found only moderate correlation between the B-A values of each of the tests. There are two components that are likely to impact on the correlation. The first is the difficulty in achieving consistent readings in the SICTT arising from inter-operator variability, though in this study there was very little difference in the correlation whether the SICTT was conducted by a PVP or WTVI/VI (Table 2b). The second is the underlying biological difference where one test is measuring a more general inflammatory reaction in the skin and the other a specific quantity of a single cytokine (IFN-γ) in a small volume of peripheral blood, which could influence the relative performance of the tests.

The proportion of SICTT +ve (standard reactor)/IFN-γ −ve animals that were positive at *post-mortem* (11.8%) was much lower when compared with SICTT +ve (standard reactor)/IFN-γ +ve (52.3%) (Table 4). This reflects the more advanced stage of disease in animals positive to both tests. We note that the failure to detect visible lesions at *post-mortem* does not necessarily indicate absence of infection. A review of many studies has found that 50% to 80% of reactor animals had no visible lesions (de la Rua-Domenech et al., 2006). In a recent study carried out in Northern Ireland, only 43% of reactors had visible lesions detected at slaughter (O'Hagan et al., 2015). There are multiple reasons as to why reactor animals do not present with visible lesions, these include early infection, poor examination technique, lesion presence in a site not subject to examination or not examined in sufficient detail, latent *M. bovis* infection, or infection with other mycobacteria (Corner, 1994; de la Rua-Domenech et al., 2006). In a study by Mcllroy et al. (1986) where lungs from reactor cattle were sliced into 0.5 cm thick sections, in 63% of tuberculous lungs only a single lesion was present in the caudal lobe and 70% of lesions were less than 1 cm in diameter. In addition, *ante mortem* tests do not identify all infected animals (Clegg et al., 2016). In the current study, of the 1082 animals that were negative to both the SICTT and IFN-γ, 51 (4.7%) were positive at *post-mortem* at varying times post-test. Risk factors identified for animals with a lesion at slaughter (with no *ante mortem* response) include previous bTB exposure history, previous inconclusive reactor result to the SICTT, number of herd movements and herd-type/size (Clegg et al., 2016).

A key finding from this study was that among animals that were SICTT −ve/IFN-γ +ve, 18.9% were positive at *post-mortem* compared to 11.8% of those that were SICTT +ve (standard reactor)/IFN-γ −ve (Table 4). In addition, of 7280 IFN-γ +ve animals, 1045 (14%) were negative to the SICTT and at *post-mortem* (Table 4). It is probable that some of these latter animals were infected given the earlier response detectable by IFN-γ post infection (compared with SICTT) (Neill et al., 1994; de la Rua-Domenech et al., 2006) and the lower sensitivity of *post-mortem* inspection at routine slaughter (Corner et al., 1990; Asseged et al., 2004; Teklul et al., 2004; Biffa et al., 2010; Bekele and Belay, 2011). In infected herds, a positive IFN-γ test can identify additional high-risk animals that if left in the herd might either prolong the restriction or result in a future breakdown (Lahuerta-Marin et al., 2015; Sinclair et al., 2016). There can be a reluctance to remove animals that are SICTT −ve/IFN-γ +ve, even in infected cohort groups, whereas, possibly due to greater familiarity with the SICTT, there is less reluctance to remove SICTT + ve/IFN-γ −ve animals. In the current study, significantly (chi-square test: p = 0.002) fewer SICTT −ve/IFN-γ +ve animals (94.4%) were slaughtered by the end of the study compared to SICTT +ve (standard reactor)/IFN-γ −ve animals (98.0%) (Table 3). With a longer follow-up period of observation before slaughter, it is likely that many of the SICTT −ve/IFN-γ +ve would have progressed to either develop a positive response to SICTT or to present lesions detectable at slaughter. Studies in Ireland (Gormley et al., 2006; Gormley et al., 2013) have shown that SICTT −ve/IFN-γ +ve animals have up to 10 fold greater risk of being positive to a SICTT or at *post-mortem* for

up to 32 months after initial IFN-γ +ve disclosure compared with SICTT −ve/IFN-γ −ve animals. In Northern Ireland, SICTT −ve/IFN-γ +ve animals that remained alive in the initial herd of disclosure were found to be twice as likely to become SICTT reactors compared with SICTT −ve/IFN-γ −ve animals from the same herd over a 5 year follow-up period, which increased to 3.7 times the risk when the follow-up period was reduced to 18 months (Lahuerta-Marin et al., 2015). In the current study, the odds of being positive at *post-mortem*, within the same calendar year, for SICTT −ve/IFN-γ +ve was 5 times higher compared to SICTT −ve/IFN-γ −ve animals. Given the high level of risk associated with retaining SICTT −ve/IFN-γ +ve animals in an infected herd, prompt removal of these animals appears to be the most judicious course of action to reduce the potential for within-herd transmission and the future risk of recurrence, of prolonging a restriction or of causing a restriction in another herd following movement.

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