DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS **CSG 15**

Research and Development

Final Project Report

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Project title	Cost-effectiveness of using the gamma interferon test in herds with multiple tuberculin reactors
DEFRA project code	**SE 3018**
Contractor organisation and location	Veterinary Laboratories Agency Weybridge
Total DEFRA project costs	£ 115,411
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Executive summary (maximum 2 sides A4)

INTRODUCTION AND OBJECTIVES

The intradermal comparative cervical tuberculin test (SICCT/skin test) is currently the only test used for the diagnosis of TB in cattle in Great Britain. In the late 1980s, a laboratory-based blood test, the gamma interferon (IFN) test, was developed in Australia for the diagnosis of bovine TB. As the IFN test is generally considered more sensitive but less specific than the skin test, it has been suggested that the IFN test could be used in infected herds to improve the detection of tuberculous cattle and so hasten the elimination of infection from herds. These advantages of the test would result in lower detection costs for government and cattle movement restriction costs for farmers. The main disadvantage of the IFN test is its lower specificity. In the UK where non-specificity is considered a problem, use of the IFN test could result in the unnecessary slaughter of more non-tuberculous animals. In this study, decision tree analysis was used to estimate the value of this trade-off in monetary terms. As well as concerns about cost, there are likely to be practical problems when a new test is used in the field. To investigate the feasibility of using the IFN test in herds in Great Britain, the test was used in combination with the skin test in five multiple tuberculin reactor herds. At the same time, the opportunity was taken to compare the performance of conventional PPD antigens used in the IFN test with synthetic antigens being developed at VLA (Weybridge). The specific objectives of the project were

1. To develop decision tree models to determine the cost-effectiveness of using the IFN test in herds with multiple tuberculin reactors in Great Britain compared to the current protocol of using the SICCT test alone.
2. To use the models and currently available IFN test data to determine whether use of the IFN test in multiple tuberculin reactor herds is likely to improve the speed and accuracy of detection at less cost to government and farmers than the current testing protocol.
3. To use the models to re-evaluate the cost-effectiveness of using the IFN test in multiple tuberculin reactor herds when IFN test data becomes available from field studies in Great Britain.
4. To evaluate the feasibility of using the IFN test in reactor herds as a basis for a larger field trial.

FINDINGS AND RECOMMENDATIONS

Objectives 1-3

- The study showed that use of the IFN test at around 10 days after the disclosure test reduced the number of tuberculous cattle in multiple tuberculin reactor herds more quickly than the current testing regime. Movement restriction orders were also lifted on fewer herds that still contained tuberculous cattle. However, the IFN test option cost between £1.0 million and £1.5 million more than the current testing regime over the period evaluated, that is, up to the fifth test after the disclosure test. Therefore, wholesale use of the IFN test in multiple tuberculin reactor herds should be approached with caution.
- One benefit from use of the IFN test that the economic analysis did not take into account was the savings associated with the likely reduction in the spread of TB within and between herds by removing tuberculous cattle from herds more quickly and releasing fewer tuberculous herds from movement restriction. However, to make use of the IFN test cost-effective, this benefit would have to exceed £1.0 million to £1.5 million. As this level of benefit is unlikely to be realised, priority should be given to conceiving alternative ways of using the IFN test in multiple tuberculin reactor herds and then evaluating their cost-effectiveness.
- To improve the decision-making value of the economic analysis, information on the magnitude of the losses caused by the spread of TB within and between herds should be sought from current projects modelling the spread of TB in cattle herds.
- As the cost of the IFN test comprised over 40% of the total cost of the IFN test option, attention should be focussed on improving the properties of the IFN test, especially its specificity, and on reducing its cost.
- The study showed that the number of cattle tested in a herd has a significant impact on herd outcomes when imperfect tests are used. As herd size increased, the probability that movement restriction orders would be lifted on herds decreased although the herds contained no tuberculous cattle. The problem is accentuated in multiple reactor herds as the proportion of large herds falling into this category is greater than in the general population. The models developed in this study could be used to explore the conditions necessary to improve the accuracy of herd outcomes in large herds.

Objective 4

- The trial demonstrated that it was feasible to perform the IFN test in farmers' herds in Great Britain.
- The IFN test detected animals infected with confirmed TB that were not detected by skin testing.
- The use of more specific antigens (ESAT6 protein, or ESAT6 and CFP10 peptides) could be useful. However, more detailed studies are required as this study was not designed to fully evaluate their usefulness.
- Co-ordination of tests between Animal Health Offices and VLA Laboratories is crucial and could be achieved with a network-based scheduling system.
- Blood sample consignments should be protected from cold environmental temperatures. Couriers should be supplied with detailed maps.
- The micro format test has been validated in Northern Ireland and elsewhere, and should be used as a routine at VLA since it offers a number of advantages:
    - Reduced volume of blood, thus reducing the time and stress for bleeding cattle.
    - Reduced quantity of antigens required.
    - Simplified transfer of plasma from the incubation plates to the ELISA plates.
- Omitting some antigens would reduce the cost of the test by saving on laboratory consumables and operations. Even so, about one in four samples should be monitored for lymphocyte viability with superantigen. The options are to reduce the test to an average of 4.5, 6.5 or 8.5 wells per sample, from the 12 in the trial. The biggest saving would be obtained with the peptide cocktail – 4.5 wells per sample, for which laboratory consumables would cost about £3.00 a sample.
- The maximum capacity of the Regional Laboratory performing the test (Luddington) has been currently estimated as just over 400 a week, or 20,000 a year. A very large demand for the test would call for rollout of the test to other Regional Laboratories, automated equipment, or outsourcing.
- More data are needed on the effectiveness of more specific antigens in the IFN test before this test can be included in the list of officially recognised tests for TB.
- It is likely that the IFN test detects cattle at earlier stages of infection than the skin test, and more studies (e.g. whole-herd slaughter studies) are required to examine this issue.

Scientific report (maximum 20 sides A4)

COST-EFFECTIVENESS AND OPERATIONAL FEASIBILITY OF USING THE GAMMA-INTERFERON TEST IN HERDS WITH MULTIPLE TUBERCULIN REACTOR ANIMALS

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INTRODUCTION

A compulsory national programme to control tuberculosis (TB) in cattle in Great Britain has operated since 1950. Cattle are tested using the single intradermal comparative cervical tuberculin (SICCT) test and animals that are positive to the test are declared reactors and slaughtered. Although the programme has reduced the incidence of TB in the cattle population to a low level, final eradication of *M. bovis* from cattle has proved elusive. One reason advanced for the lack of progress is the poor sensitivity of the SICCT test. A significant number of infected cattle will not be detected by a test of low sensitivity. These undetected tuberculous cattle serve as a reservoir of infection for other cattle and make the elimination of the organism from an infected herd more protracted. Therefore, use of a more sensitive test in infected herds should return considerable benefits.

The gamma-interferon (IFN) test is a laboratory-based blood test that was developed in Australia in the late 1980s for the diagnosis of TB in cattle [1] [2]. The test is generally considered more sensitive but less specific than the SICCT test [3] [4]. It may also detect infected cattle at an earlier stage of the disease than the SICCT test [5]. These positive attributes of the IFN test suggest that it could be used in infected herds to improve the detection of tuberculous cattle and so hasten the elimination of infection from herds. The faster clearing of infection from herds, and the reduced risk of infection spreading to other herds, would result in lower detection costs for government and cattle movement restriction costs for farmers. The main disadvantage of the IFN test compared with the skin test is its lower specificity. In the UK where non-specificity is considered a problem [6], replacing the SICCT test with the IFN test could result in more false positive cattle in the herds to which it was applied. Therefore, while using a more sensitive test in infected herds may hasten elimination of infection from a herd, it may also result in the unnecessary slaughter of more non-tuberculous animals. In this study, decision tree analysis [7] was used to estimate the value of this trade-off in monetary terms.

As well as concerns about cost, there are likely to be practical problems when a new test is used in the field. In the case of the IFN test, farmers must assemble cattle for blood sampling at about seven days notice and accept that more reactor animals than usual may be taken in the early stages of a TB incident. Blood samples collected for the IFN test must be kept in the correct environmental conditions and incubated at a laboratory within 30 hours of collection [8] [9]. To investigate the feasibility of using the IFN test in reactor herds, the test was used in combination with the SICCT test in five multiple tuberculin reactor herds. Interest centred on the logistical problems associated with use of the IFN test and its acceptabilty to farmers, the State Veterinary Service and laboratories. At the same time, the opportunity was taken to compare the performance of conventional PPD antigens used in the IFN test with synthetic antigens being developed at VLA (Weybridge).

The specific objectives of the project were

1. To develop decision tree models to determine the cost-effectiveness of using the IFN test in herds with multiple tuberculin reactors in Great Britain compared to the current protocol of using the SICCT test alone.
2. To use the models and currently available IFN test data to determine whether use of the IFN test in multiple tuberculin reactor herds is likely to improve the speed and accuracy of detection at less cost to government and farmers than the current testing protocol.
3. To use the models to re-evaluate the cost-effectiveness of using the IFN test in multiple tuberculin reactor herds when IFN test data becomes available from field studies in Great Britain.
4. To evaluate the feasibility of using the IFN test in reactor herds as a basis for a larger field trial.

Objectives 1 to 3 are covered in Part A and objective 4 is covered in Part B of this report. The IFN test is now used in over 20 countries [10] and DEFRA is under pressure to introduce it in the UK. This project was designed to provide information to help DEFRA formulate policy on the use of the IFN test within multiple tuberculin reactor herds.

PART A: COST-EFFECTIVENESS OF USING THE IFN TEST IN MULTIPLE REACTOR HERDS

Methodology

Overview

To determine whether it would be cost-effective to use the IFN test in herds with multiple tuberculin reactor animals, the cost of using the current testing regime (status quo option) in these herds was compared with the cost of using the IFN test (IFN test option) in the same population. A multiple tuberculin reactor (MTR) herd was defined as a herd that, at the disclosure test, had three or more reactors to the SICCT test, at least one of which had a visible lesion suggestive of TB found at slaughter. The disclosure test was any test at which a previously TB free herd was declared to have had a TB incident.

The population selected as the basis for the study was all herds in Great Britain that met the definition of a MTR herd at a disclosure test in 1997. The characteristics of these herds were extracted from the historic tuberculosis testing records on DEFRA's computerised database "Vetnet" up to March 2000. However, because the size of this population had more than doubled since 1997, the characteristics of the 1997 population were scaled up to the number of MTR herds in 2000 for the economic analysis.

The costs of the status quo option and the IFN test option were determined by constructing decision tree models for each option in MS Excel spreadsheets (Microsoft Corporation, Redmond, WA, USA) [7]. Each decision tree showed all the events and outcomes that were possible in a population of MTR herds between the disclosure test and the six-month check test. The cost of each event in the proportion of the population affected was calculated using 1998 prices. Events included imposition of movement restriction orders, herd and inconclusive reactor animal tests, slaughter of reactor animals and their subsequent post-mortem and laboratory examinations and DEFRA administration costs. To determine which testing regime was the most cost-effective, the total costs of each option were compared. The option with the lowest total cost was considered the most cost-effective.

Three outcomes were possible following the application of the SICCT test to a herd. If all the tested cattle were SICCT negative, the herd was classed as a non-reactor (NR) herd. If one or more cattle were inconclusive reactors and the remainder of the herd was SICCT test negative, the herd was classed as an inconclusive reactor only (IR) herd. If there were one or more reactors in the herd, it was classed as a reactor (R) herd. At the IFN test, herds were classed as IFN test negative (NGI) or IFN test positive (PGI) herds. NGI herds were those in which all the cattle gave a negative reaction to the IFN test. PGI herds had one or more cattle that gave a positive reaction to the IFN test.

Probability theory was used to predict herd outcomes and reactor numbers from applying the SICCT test and the IFN test to the population of MTR herds [11]. The predictions were based on knowledge of the sensitivity and specificity of the tests, the number of cattle tested and the true tuberculosis prevalence in the herds at the time of the test (Annex 1). The values used for the sensitivities and specificities of the SICCT and IFN tests are shown in Table 1. They were based on data from published trials for the SICCT test [6] [12] [13] and the IFN test [3] [4] [9] [14] [15]. Four herd-size categories were used in the analysis: 50, 150, 250 and 350 cattle.

TABLE 1: Sensitivity (Se) and specificity (Sp) of the SICCT test at standard and severe interpretations and of the IFN test interpreted according to the manufacturer's instructions

Test	Sensitivity		Test	Specificity	
	Standard	Severe		Standard	Severe
P(SICCT+/TB+)	0.593	0.714	P(SICCT-/TB-)	0.99	0.985
P(IR/TB+)	0.285	0.254	P(IR/TB-)	0.01	0.010
P(SICCT-/TB+)	0.122	0.032	P(SICCT+/TB-)	0.00	0.005
Se: (IR=SICCT+)	0.878	0.968	Sp: (IR=SICCT-)	1.00	0.995
Se: P(IFN+/TB+)	0.850	n/a	Sp: P(IFN-/TB-)	0.99	n/a
P(IFN-/TB+)	0.150	n/a	P(IFN+/TB-)	0.01	n/a

The prevalence of tuberculous cattle in a herd at the first short interval test (SIT1) was based on analysis of the 1997 population of MTR herds. It was assumed that all cattle with confirmed TB between the SIT1 and March 2000 were present in the herd immediately after the reactors at the disclosure test had been removed. In other words, there was no spread of infection within the herds after reactors at the disclosure test were removed and no new TB infections occurred within the herds after the initial TB incident. In the study, herds considered free from tuberculous animals after the reactors at the disclosure test were removed were termed TB negative herds. Herds that had further tuberculous cattle after the reactors at the disclosure test were removed were termed TB positive herds. The distribution of MTR herds by herd size and the number of tuberculous cattle remaining in the herd after the disclosure test are shown in Table 2. The study population of 305 herds comprised 95 TB negative herds and 210 TB positive herds with a total of 816 tuberculous cattle.

TABLE 2: Distribution of tuberculous cattle in 305 MTR herds by number of cattle tested at whole herd tests

No. of tuberculous cattle	50 cattle		150 cattle		250 cattle		350 cattle	
	No. of herds	% of herds	No. of herds	% of herds	No. of herds	% of herds	No. of herds	% of herds
0	31	43.7	41	36.3	12	21.0	11	17.2
1	12	16.9	18	15.9	11	19.3	11	17.2
2	8	11.3	11	9.7	7	12.3	8	12.5
3	6	8.4	7	6.2	5	8.8	5	7.8
4	6	8.4	7	6.2	5	8.8	5	7.8
5	4	5.6	7	6.2	5	8.8	5	7.8
6	4	5.6	7	6.2	4	7.0	5	7.8
7	0	0.0	4	3.5	2	3.5	5	7.8
8	0	0.0	4	3.5	2	3.5	3	4.7
9	0	0.0	4	3.5	2	3.5	3	4.7
10	0	0.0	3	3.5	2	3.5	3	4.7
Total no. of herds	71	100.0	113	100.0	57	100.0	64	100.0
Total no. cattle	2,000		10,800		11,250		18,550	
Total no. TB+ cattle	114		292		177		233	

The number of tuberculous cattle left in a herd following the removal of reactors at tests subsequent to the disclosure test was calculated according to the following rules. If the number of reactors at a test was greater than, or the same as, the number of tuberculous cattle in the herd, then the herd was considered free of tuberculous cattle when given the next test. If the number of reactors at a test was less than the number of tuberculous cattle in the herd, then the difference in the numbers of reactors and tuberculous cattle was the number of tuberculous cattle remaining in the herd at the next test. For example, if there were two tuberculous cattle in a herd at the first short interval test but only one reactor was detected, then the herd would have one tuberculous animal remaining in the herd at the second short interval test.

A summary of prices and other parameter values used in the models is shown in Table 3. The valuation of events and outcomes is described in more detail in Annex 2. Because of the uncertainty of most parameter values used in the analysis, sensitivity analysis was used to test the robustness of the economic evaluation of the use of the IFN test in the population of MTR herds.

<u>Description of the status quo option</u>

Under the current TB testing rules, a TB-free herd in which one or more reactor animals are detected is served with a TB2 notice, which restricts the movement of cattle on to and off the farm. The herd then undergoes a regular programme of testing until declared free from TB. All reactor animals are sent to slaughter and any visible lesions suggestive of TB that are detected at post-mortem examination are sent for histopathological and microbiological examination. Tissues from reactor animals with no visible lesions of TB are also sent to the laboratory to be cultured for *M. bovis*.

In the case of a confirmed TB incident, the SICCT test is applied to all cattle in the herd at intervals of 60 days until the herd has two consecutive short interval tests (SITs) free from reactor or inconclusive reactor (IR) animals. Following two clear consecutive SITs, a TB10 notice is served to lift the movement restriction order. The SICCT test is interpreted using severe interpretation at all SITs except at a SIT following a clear SIT, when standard interpretation is used.

If one or more IR animals only are detected at the second clear SIT, the IR(s) are re-tested with the SICCT test (standard interpretation) at 42 days. If all IRs in the herd are negative at the first inconclusive reactor retest (IRT1), the movement restriction order is lifted. If one or more IRs give an inconclusive reaction, the IR(s) are tested again at 42 days and any animals that are still inconclusive are declared reactors. The herd then resumes 60-day SITs. If all IRs are negative at the IRT2, the movement restriction order is lifted.

<u>Description of the IFN test option</u>

Under the IFN test option, blood samples are taken for the IFN test from all cattle in MTR herds at around 10 days after the disclosure test is read. The IFN test is performed and interpreted according to the manufacturer’s instructions. Cattle that give a positive reaction to the IFN test are slaughtered and any visible lesions suggestive of TB that are detected at post-mortem examination are sent for histopathological and microbiological examination. Tissues from reactor animals with no visible lesions of TB are also sent to the laboratory to be cultured for *M. bovis*. Regardless of the result of the first IFN test, all herds continue to follow the status quo testing regime with two consecutive, clear SITs required to lift the movement restriction order.

TABLE 3: Summary of 1998 prices and other parameter values used in the economic analysis

Item	Price
Cost to farmer of herd movement restriction order	£7.79 per herd per day
Bovine and avian tuberculins for SICCT test	£0.13 per test
LVI fee for SICCT testing of 50 cattle in a herd	£143.16 per herd
LVI fee for SICCT testing of 150 cattle in a herd	£303.34 per herd
LVI fee for SICCT testing of 250 cattle in a herd	£463.33 per herd
LVI fee for SICCT testing of 350 cattle in a herd	£623.33 per herd
LVI fee for SICCT testing of 5 or fewer inconclusive reactor cattle in a herd	£35.49 per herd
IFN test - materials and labour (laboratory)	£15.00 per test
LVI fee for blood sampling 50 cattle for IFN test	£122.97 per herd
LVI fee for blood sampling 150 cattle for IFN test	£354.17 per herd
LVI fee for blood sampling 250 cattle for IFN test	£584.73 per herd
LVI fee for blood sampling 350 cattle for IFN test	£814.73 per herd
LVI travel cost for farm visit	£0.45 per mile
Average miles travelled by LVI to and from farm for testing or sampling	25 miles per herd visit
Hours of farm labour associated with SICCT testing or blood sampling	Not calculated
Cost of farm labour	Not priced
Reactor depreciation	£41.00 per reactor animal
Post-mortem inspection of reactor cattle	£45.00 per reactor animal
Laboratory confirmation of tuberculosis	£122.00 per reactor animal
DEFRA administrative cost for herd restriction	£5.88 per herd restriction
DEFRA administrative cost for SICCT testing	£11.38 per herd
DEFRA administrative cost for IFN testing	£11.98 per herd
DEFRA administrative cost for slaughter of reactor animals	£12.60 per reactor animal
Number of days between disclosure test and first short interval test	60 days
Number of days between short interval tests	60 days
Number of days between disclosure test and IFN test	10 days
Number of days between IFN test and first short interval test	50 days
No. of days between a short interval test and an inconclusive reactor re-test	42 days

Results

Effect of applying the IFN test to the study population of 305 MTR herds

In the 210 herds that still contained tuberculous cattle when given the IFN test (IFNT1), application of the IFN test resulted in 165 (79%) herds becoming TB negative by the SIT1. The average within-herd prevalence of tuberculous cattle in the 210 TB positive herds was reduced from 1.9% at the IFNT1 to 0.2% at the SIT1 (Table 4). The probability of at least one animal in a herd giving a positive reaction to the IFN test increased with herd size. Therefore, larger TB positive herds had a greater likelihood of becoming free from tuberculous animals by the SIT1 than smaller herds. In the 95 TB negative herds, use of the IFN test resulted in 147 animals being slaughtered unnecessarily because they gave a positive reaction to the IFN test (Table 4).

TABLE 4: Results of applying the IFN test to 210 TB positive and 95 TB negative, MTR herds at 10 days after the disclosure test. There were a total of 816 tuberculous cattle in the 210 TB positive herds at the IFNT1.

Herd size	Total no. of herds	No. of PGI[1] herds	No. of PGI herds that were TB+ at SIT1	No. of PGI herds that were TB- at SIT1	No. of NGI[2] herds	Total no. of herds TB+ at SIT1	Total no. of herds TB- at SIT1	Per cent of TB+ cattle at IFNT1	No. of IFN test positive cattle	Per cent of TB+ cattle at SIT1
TB positive herds										
50	40	36	11	25	4	15	25	5.70	116	1.20
150	72	70	16	54	2	18	54	2.70	353	0.32
250	45	44	6	38	1	7	38	1.57	261	0.11
350	53	53	5	48	0	5	48	1.26	381	0.05
Totals	210	203	38	165	7	45	165	1.92	1111	0.19
TB negative herds										
50	31	12	0	12	19	0	31	0.00	16	0.00
150	41	32	0	32	9	0	41	0.00	62	0.00
250	12	11	0	11	1	0	12	0.00	30	0.00
350	11	11	0	11	0	0	11	0.00	39	0.00
Totals	95	66	0	66	29	0	95	0.00	147	0.00

[1]A PGI herd had at least one animal with a positive reaction to the IFN test. [2]An NGI herd was one in which all animals had a negative IFN test.

Effect of changes in IFN test sensitivity and specificity on herd outcomes at the IFN test

The model of the IFN test option was run using IFN test sensitivities of 75%, 85% and 95% and specificities of 94%, 96.5% and 99%. The values represented the sensitivity and specificity ranges of the IFN test reported in the literature. The effects of IFN test sensitivity and specificity on outcomes at the IFNT1 in TB positive and TB negative herds are shown in Tables 5 and 6 respectively. The shaded row shows the outcomes at the chosen "baseline" IFN test values (Table 1).

TABLE 5: Effect of IFN test sensitivity (Se) and specificity (Sp) on the number of herds becoming TB negative by the SIT1 and on the prevalence (%) of tuberculous cattle at the SIT1 in 210 TB positive MTR herds.

IFN test		Herd size								All TB positive herds (n=210)		
		50 (n=40)		150 (n=72)		250 (n=45)		350 (n=530				
Se (%)	Sp (%)	No. (%) of TB- herds	% of TB+ cattle	No. (%) of TB- herds	% of TB+ cattle	No. (%) of TB- herds	% of TB+ cattle	No. (%) of TB- herds	% of TB+ cattle	No. (%) of TB- herds	% of TB+ cattle	No. of IFN test positives
75	99.0	22 (55)	1.47	49 (68)	0.42	36 (80)	0.15	46 (87)	0.07	153 (73)	0.25	1030
85	99.0	25 (63)	1.20	54 (75)	0.32	38 (84)	0.11	48 (91)	0.05	165 (79)	0.19	1111
95	99.0	27 (68)	0.96	57 (79)	0.24	40 (89)	0.08	49 (93)	0.04	173 (82)	0.14	1194
75	96.5	31 (78)	0.70	68 (94)	0.06	45 (100)	0.00	53 (100)	0.00	197 (94)	0.05	2075
85	96.5	33 (83)	0.55	69 (96)	0.04	45 (100)	0.00	53 (100)	0.00	200 (95)	0.04	2156
95	96.5	34 (85)	0.42	70 (97)	0.03	45 (100)	0.00	53 (100)	0.00	202 (96)	0.03	2237
75	94.0	36 (90)	0.32	71 (99)	0.02	45 (100)	0.00	53 (100)	0.00	205 (98)	0.02	3120
85	94.0	37 (93)	0.24	72 (100)	0.00	45 (100)	0.00	53 (100)	0.00	207 (99)	0.01	3201
95	94.0	38 (95)	0.18	72 (100)	0.00	45 (100)	0.00	53 (100)	0.00	208 (99)	0.01	3283

Increasing the sensitivity and decreasing the specificity of the IFN test accelerated the clearance of tuberculous cattle from the 210 TB positive herds (Table 5). If the sensitivity of the IFN test was increased from 75% to 95% and the specificity of the IFN test was decreased from 99% to 94%, the per cent of herds that were free from tuberculous cattle at the SIT1 increased from 73% to 99%. Use of the IFN test also reduced the average within-herd prevalence of tuberculous animals from 1.92% at the IFNT1 to between 0.01% and 0.25% at the SIT1. The downside of this change in IFN test sensitivity and specificity was the increased number of non-tuberculous cattle in TB positive herds that were slaughtered unnecessarily because they gave a positive reaction to the IFN test.

TABLE 6: Effect of IFN test specificity (Sp) on the number of PGI herds and IFN test positive cattle at the IFNT1 in 95 TB negative MTR herds.

IFN test	Herd size								All TB negative herds (n=95)	
	50 (n=31)		150 (n=41)		250 (n=12)		350 (n=11)			
Sp (%)	No. (%) of PGI herds	No. of IFN test positives	No. (%) of PGI herds	No. of IFN test positives	No. (%) of PGI herds	No. of IFN test positives	No. (%) of PGI herds	No. of IFN test positives	No. (%) of PGI herds	No. of IFN test positives
99.0	12 (39)	16	32 (78)	62	11 (92)	30	11 (100)	39	66 (69)	147
96.5	26 (84)	54	41 (100)	215	12 (100)	105	11 (100)	135	90 (95)	509
94.0	30 (97)	93	41 (100)	369	12 (100)	180	11 (100)	231	94 (99)	873

In the 95 TB negative herds, the number of cattle that gave a positive reaction to the IFN test increased with decreasing IFN test specificity (Table 6). At an IFN test specificity of 99%, 147 cattle gave a false positive reaction to the IFN test and 69% of herds had at least one IFN test-positive animal. At an IFN test specificity of 94%, 873 cattle gave a false positive reaction to the IFN test and 99% of herds were PGI. (IFN test sensitivity did not affect the number of PGI herds and IFN test-positive cattle in TB negative herds.)

<u>Herd outcomes at the second short interval test (SIT2)</u>

Under the official TB testing rules, all herds with a confirmed TB incident require two consecutive short interval tests with no reactor animals before the movement restriction order is lifted from the herd. Therefore, the SIT2 is the first test at which movement restriction orders may be lifted. To farmers, it is important that movement restriction orders are lifted quickly. Every day that they are unable to sell and buy in cattle may cost them money. Therefore, the test and testing regime used on MTR herds that are TB negative after the disclosure test should keep to a minimum the number of herds that are restricted past the required 120 days. However, it is equally important that a movement restriction order is not lifted on a herd that still contains tuberculous cattle. A herd that is incorrectly identified as being non-tuberculous at a test may cost farmers and government money from the spread of TB within the missed herd and from the missed herd to other TB-free herds.

Number and per cent of MTR herds that had movement restriction orders lifted at SIT2

Under the status quo option, 21 (7%) of the 305 MTR herds had the movement restriction order lifted immediately after the SIT2 (Table 7). The 21 herds comprised 16 herds each with 50 cattle and five herds each with 150 cattle. None of the 121 herds with either 250 or 350 cattle had a clear SIT1 and SIT2 even though 23 of these herds were TB negative.

The number of herds on which the movement restriction order was lifted at the SIT2 under the IFN test option varied between 37 (12%) and 45 (15%) herds across the range of IFN test sensitivities and specificities that was investigated (Table 7). Generally, the movement restriction order was lifted on more herds when the IFN test sensitivity was increased and the specificity was decreased. The number of cattle tested in a herd had a bigger impact on the number of herds on which the movement restriction order was lifted than the properties of the IFN test. As the number of cattle tested in a herd increased, the probability that the movement restriction order would be lifted at the SIT2 decreased until no herds with 350 cattle had a clear SIT1 and SIT2.

Over all values of IFN test sensitivity and specificity, around twice as many MTR herds had the movement restriction order lifted at the SIT2 under the IFN test option than under the status quo option (Table 7).

Essentially, application of the IFN test 10 days after the disclosure test enabled the removal of most of the remaining tuberculous cattle from the TB positive herds. Therefore, many TB positive herds were TB negative by the time of the SIT1 and so were more likely to meet the test criteria for the lifting of the movement restriction order. Under both the status quo and IFN test options, the models showed that few large herds would have the movement restriction order lifted at the SIT2 even though they were free from tuberculous cattle at the SIT1 and SIT2.

TABLE 7: Effect of IFN test sensitivity (Se) and specificity (Sp) on the number of MTR herds that had the movement restriction order lifted at the SIT2 under the IFN test option. The number of MTR herds that had the movement restriction order lifted at the SIT2 under the status quo (SQ) option is shown at bottom of table.

IFN test option		Herd size								All herds in 2000 population	
IFN test		50		150		250		350			
Se	Sp	No. herds (n=71)	% total herds[1]	No. herds (n=113)	% total herds[1]	No. herds (n=57)	% total herds[1]	No. herds (n=64)	% total herds[1]	No. herds (n=305)	% total herds[1]
75	99.0	26	36.6	10	8.8	1	1.8	0	0.0	37	12.1
85	99.0	27	38.0	10	8.8	1	1.8	0	0.0	38	12.5
95	99.0	28	39.4	11	9.7	1	1.8	0	0.0	40	13.1
75	96.5	30	42.2	12	10.6	1	1.8	0	0.0	43	14.1
85	96.5	31	43.7	12	10.6	1	1.8	0	0.0	44	14.4
95	96.5	31	43.7	12	10.6	1	1.8	0	0.0	44	14.4
75	94.0	32	45.1	12	10.6	1	1.8	0	0.0	45	14.8
85	94.0	32	45.1	12	10.6	1	1.8	0	0.0	45	14.8
95	94.0	32	45.1	12	10.6	1	1.8	0	0.0	45	14.8
SQ option		16	22.5	5	4.4	0	0.0	0	0.0	21	6.9

[1]Number of herds with movement restriction order lifted at SIT2 as a per cent of all MTR herds (n) in class.

Error rates in herd outcomes at the SIT2

The ideal outcome at the SIT2 is that movement restriction orders should be lifted on all herds that contained no tuberculous cattle at the SIT1 and SIT2 but on no herds that still contained tuberculous cattle. However, because the SICCT test is not 100% sensitive and specific, errors will occur.

Under the status quo option, movement restriction orders were lifted at the SIT2 on two herds that still contained tuberculous cattle (Table 8). Therefore, an incorrect decision was made in nearly one in 10 herds that had the TB10 lifted at the SIT2. The error rate decreased with increasing herd size (Table 9).

TABLE 8: Herd outcomes at the SIT2 in 305 MTR herds under the status quo and IFN test options

Movement restriction (TB10) status at SIT2	Eligibility of herd to have TB10 lifted at SIT2: Not eligible (TB+ herds)	Eligible (TB- herds)	Total no. of herds	% correct decision	% wrong decision
Status quo option					
TB10 lifted at SIT2	2	19	21	90.5	9.5
TB10 not lifted at SIT2	208	76	284	73.2	26.8
Total no. of herds	210	95	305		
% correct	99.0	20.0			
% false negative/positive	1.0	80.0			
IFN test option					
TB10 lifted at SIT2	1	37	38	97.4	2.6
TB10 not lifted at SIT2	44	223	267	16.5	83.5
Total no. of herds	45	260	305		
% correct	97.8	14.2			
% false negative/positive	2.2	85.8			

Under the IFN test option at baseline sensitivity and specificity values, one herd out of the 38 herds that had the movement restriction order lifted at the SIT2 still contained tuberculous cattle. Therefore, under the IFN test option, an incorrect decision was made in 2.6% of herds compared with 9.5% of herds under the status quo option (Table 8). The error rate increased with decreasing sensitivity and increasing specificity of the IFN test (Table 9). However, at all sensitivity and specificity values tested, the probability that a herd on which the movement restriction order was lifted at the SIT2 was TB positive was low at under 3%. When the population of MTR herds was stratified by herd size, the error rate in herds with 150 or more cattle was zero (Table 9).

TABLE 9: Effect of IFN test sensitivity (Se) and specificity (Sp) on the error rate in herds that had the movement restriction order lifted at the SIT2 under the IFN test option. The error rate in herds that had the movement restriction order lifted at the SIT2 under the status quo option is shown at the bottom of the table.

IFN test option		Herd size								All herds in 2000 population	
IFN test		50		150		250		350			
Se	Sp	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]
75	99.0	1/26	3.8	0/10	0.0	0/1	0.0	0/0	n/a	1/37	2.7
85	99.0	1/27	3.7	0/10	0.0	0/1	0.0	0/0	n/a	1/38	2.6
95	99.0	1/28	3.6	0/11	0.0	0/1	0.0	0/0	n/a	1/40	2.5
75	96.5	1/30	3.3	0/12	0.0	0/1	0.0	0/0	n/a	1/43	2.3
85	96.5	1/31	3.2	0/12	0.0	0/1	0.0	0/0	n/a	1/44	2.3
95	96.5	0/31	0.0	0/12	0.0	0/1	0.0	0/0	n/a	0/44	0.0
75	94.0	0/32	0.0	0/12	0.0	0/1	0.0	0/0	n/a	0/45	0.0
85	94.0	0/32	0.0	0/12	0.0	0/1	0.0	0/0	n/a	0/45	0.0
95	94.0	0/32	0.0	0/12	0.0	0/1	0.0	0/0	n/a	0/45	0.0
SQ option		1/16	6.3	1/5	20.0	0/0	n/a	0/0	n/a	2/21	9.5

[1]Number of herds not eligible to have movement restriction order lifted at SIT2 (i.e., TB positive)/Number of herds that had movement restriction order lifted at SIT2.
[2]Probability that a herd is TB positive when the movement restriction order is lifted at the SIT2.

In terms of the proportion of TB positive herds that had movement restriction orders lifted at the SIT2, the status quo option performed better than the IFN test option (Table 8). However, when the weight of infection was considered, the IFN test option performed better. Movement restriction orders were removed on one or two fewer tuberculous herds under the IFN test option, which reduced the likelihood of spread of TB within and between herds. However, of perhaps more importance was the early reduction in the number of TB infected herds under the IFN test option. Use of the IFN test reduced the number of infected herds at the SIT1 from 210 herds under the status quo option to between two and 57 herds under the IFN test option, depending on the sensitivity and specificity of the IFN test.

Under the status quo option, movement restriction orders were not lifted on 284 MTR herds at the SIT2 (Table 8). However, 76 of these herds were eligible to have the movement restriction order lifted at the SIT2, giving an error rate of 26.8% in the herds that remained under restriction past the SIT2 (Table 10).

Under the IFN test option, movement restriction orders were not lifted on 267 MTR herds at the SIT2 (Table 8). However, 223 of these herds were eligible to have the movement restriction order lifted at the SIT2, giving an error rate of 83.5% in the herds that remained under restriction past the SIT2 (Table 10). The error rate in herds that did not have the movement restriction order lifted at the SIT2 increased with increasing sensitivity and decreasing specificity of the IFN test (Table 10). However, at all sensitivity and specificity values tested, the probability that a herd on which the movement restriction order was not lifted at the SIT2 was TB negative was high at 80% or more. This error rate was around three times higher than under the status quo option

In terms of the proportion of TB negative herds that had movement restriction orders lifted at the SIT2, there was no significant difference between the status quo option and the IFN test option (Table 8). (The difference seen in Table 8 was due to rounding up fractions of herds in the models to whole numbers.) This was because, regardless of the option applied, all herds had to pass two SITs 60 days apart for the TB10 to be lifted. Therefore, the probability that the TB10 would be lifted on eligible (TB negative) herds was the same under both options. However, because there were more eligible herds under the IFN test option than under the status quo option (260 herds versus 95 herds), the probability that an eligible herd would remain restricted at the SIT2 was higher under the IFN test option (83.5%) than under the status quo option (26.8%).

TABLE 10: Effect of IFN test sensitivity (Se) and specificity (Sp) on the error rate in herds that did not have the movement restriction order lifted at the SIT2 under the IFN test option. The error rate in herds that did not have the TB10 lifted at the SIT2 under the status quo option is shown at the bottom of the table.

IFN test option		Herd size								All herds in 2000 population	
IFN test		50		150		250		350			
Se	Sp	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]	No. herds[1]	Error rate (%)[2]
75	99.0	28/45	62.2	81/103	78.6	47/56	83.9	57/64	89.1	213/268	79.5
85	99.0	30/44	68.2	85/103	82.5	49/56	87.5	59/64	92.2	223/267	83.5
95	99.0	31/43	72.1	88/102	86.3	51/56	91.1	60/64	93.8	230/265	86.8
75	96.5	33/41	80.5	98/101	97.0	55/56	98.2	64/64	100.0	250/262	95.4
85	96.5	34/40	85.0	99/101	98.0	55/56	98.2	64/64	100.0	252/261	96.6
95	96.5	34/40	85.0	99/101	98.0	56/56	100.0	64/64	100.0	253/261	96.9
75	94.0	36/39	92.3	101/101	100.0	56/56	100.0	64/64	100.0	256/260	98.5
85	94.0	36/39	92.3	101/101	100.0	56/56	100.0	64/64	100.0	257/260	98.8
95	94.0	36/39	92.3	101/101	100.0	56/56	100.0	64/64	100.0	257/260	98.8
SQ option		16/55	29.1	37/108	34.3	12/57	21.1	11/64	17.2	76/284	26.8

[1]Number of herds eligible to have the movement restriction order lifted at the SIT2 (i.e., herds TB negative at SIT1 and SIT2)/Number of herds that did not have the movement restriction order lifted at the SIT2.
[2]Probability that a herd is TB negative at the SIT1 and SIT2 given that the movement restriction order is not lifted at the SIT2.

Cumulative herd outcomes up to the fifth test after the disclosure test or IFN test

In the models, herd outcomes were calculated at all tests up to the fifth test after the disclosure test or IFN test. Outcomes at the fifth test and beyond were not calculated due to the increasing complexity of the models.

Under the status quo option, 137 (45%) of the 305 MTR herds had the movement restriction order lifted by the fourth test post-disclosure compared with 187 (61%) herds under the IFN test option (Table 11). The probability that a movement restriction order would be lifted on a herd by the fourth test post-disclosure decreased with increasing herd size. Generally, movement restriction orders were lifted on more herds when the IFN test sensitivity was increased and the specificity was decreased. However, the number of herds on which the movement restriction order was lifted by the fourth test after the IFN test only varied between 184 herds and 198 herds across the range of IFN test values investigated. Over all values of IFN test sensitivity and specificity, more herds had the movement restriction order lifted by the fourth test under the IFN test option than under the status quo option.

TABLE 11: Cumulative number and per cent of herds that had movement restriction orders lifted at each test post-disclosure and the error rate (% TB positive) under the status quo and IFN test options

Test number	**Status quo option**				**IFN test option**			
	No. of herds with TB10 lifted	% of all herds	No. TB+ herds	% error rate	No. of herds with TB10 lifted	% of all herds	No. TB+ herds	% error rate
2nd test	21	6.9	2	9.5	38	12.5	1	2.6
3rd test	70	23.0	3	4.3	125	41.0	1	0.8
4th test	137	44.9	6	4.4	187	61.3	2	1.1

Under the status quo option, movement restriction orders were lifted by the fourth test post-disclosure on six herds that still contained tuberculous cattle compared with two herds under the IFN test option (Table 11). In contrast to earlier tests, 140 eligible herds had not had movement restriction orders lifted by the fourth test under the status quo option compared with 114 herds under the IFN test option (Table 12).

Under the status quo option, three herds that remained under movement restriction after the fourth test post-disclosure still contained tuberculous cattle following removal of reactor animals at the fourth test. No herds that remained under movement restriction after the fourth test under the IFN test option contained tuberculous cattle.

TABLE 12: Cumulative number and per cent of herds that did not have movement restriction orders lifted at each test post-disclosure and the error rate (% eligible at each test) under the status quo and IFN test options

Test number	**Status quo option**				**IFN test option**			
	No. of herds still restricted	% of all herds	No. eligible herds	% error rate	No. of herds still restricted	% of all herds	No. eligible herds	% error rate
2nd test	284	93.1	76	26.8	267	87.5	223	83.5
3rd test	235	77.0	149	63.4	180	59.0	166	92.2
4th test	168	55.1	140	83.3	118	38.7	114	96.6

Total costs

Costs were computed up to and including the cost of carrying out the fifth test after the disclosure test (status quo option) or IFN test (IFN test option). Details of the items of expenditure and their prices are given in Table 3. The total cost of each option included the cost of:

(a) all tests between the disclosure test and the test at which the TB10 was lifted,
(b) restricting herds between the disclosure test and the test at which the TB10 was lifted,
(c) all animals taken as reactor animals (reactor depreciation), and
(d) post-mortem and laboratory examinations of reactor animals.

The total cost of the IFN test option was £2,339,989 compared with a total cost of £1,371,133 for the status quo option to give a net benefit from the status quo option of £968,856 (Table 13). Under the IFN test option, the average cost per TB positive herd was £8,456 compared with £5,939 per TB negative herd (Table 14). Under the status quo option, the average cost per TB positive herd was £5,208 compared with £2,920 per TB negative herd (Table 14).

TABLE 13: Total costs (£) of status quo and IFN test options by herd size and for all MTR herds

Herd size	IFN test option			Status quo option			Net benefit from IFN test option		
	TB+ herds	TB- herds	All herds	TB+ herds	TB- herds	All herds	TB+ herds	TB- herds	All herds
50	147,796	84,213	232,009	133,238	53,011	186,249	-14,558	-31,202	-45,760
150	497,254	239,607	736,861	341,596	118,317	459,913	-155,658	-121,290	-276,948
250	443,430	107,664	551,094	257,435	48,750	306,185	-185,995	-58,914	-244,909
350	687,288	132,737	820,025	361,510	57,276	418,786	-325,778	-75,461	-401,239
All herds	1,775,768	564,221	**2,339,989**	1,093,779	277,354	**1,371,133**	-681,989	-286,867	**-968,856**

TABLE 14: Total costs (£) per herd of status quo and IFN test options by herd size and for all MTR herds

Herd size	IFN test option			Status quo option			Net benefit/herd from IFN test option		
	TB+ herds	TB- herds	All herds	TB+ herds	TB- herds	All herds	TB+ herds	TB- herds	All herds
50	3,695	2,717	3,268	3,331	1,710	2,623	-364	-1,006	-645
150	6,906	5,844	6,521	4,744	2,886	4,070	-2,162	-2,958	-2,451
250	9,854	8,972	9,668	5,721	4,063	5,372	-4,133	-4,910	-4,297
350	12,968	12,067	12,813	6,821	5,207	6,544	-6,147	-6,860	-6,269
All herds	8,456	5,939	**7,672**	5,208	2,920	**4,496**	-3,248	-3,020	**-3,177**

The total cost of the IFN test option increased with increasing sensitivity and decreasing specificity of the IFN test (Table 15). Over the range of IFN test sensitivity and specificity values investigated, the total cost of the IFN option varied between £2,336,075 and £2,930,518. For any given specificity of the IFN test, a change in the sensitivity of the IFN test had only a small impact on the overall cost of the IFN test option. An increase in the test sensitivity from 75% to 95% increased the total cost by £10,378 at a specificity of 99% and by £33,594 at a specificity of 94%. However, when the specificity of the test was decreased for a given sensitivity, the impact on the total cost was much greater. For example, a decrease in test specificity from 99% to 96.5% increased total costs by £270,406 at a sensitivity of 85%. A decrease in test specificity from 99% to 94% increased total costs by £573,587 at a sensitivity of 85%.

TABLE 15: Effect of IFN test sensitivity (Se) and specificity (Sp) on the total cost (£) and net benefit of the IFN test option by herd size category and for all herds in the year 2000 population (n = number of herds). The total cost (£) of the status quo (SQ) option is shown at the bottom of the table for comparison.

IFN test option		Herd size				All herds in population (n=305)	Net benefit from IFN test option	All TB-herds (n=95)
IFN test		50 (n=71)	150 (n=113)	250 (n=57)	350 (n=64)			
Se	Sp	Total cost	Total cost	Total cost	Total cost	Total cost		Total cost
75	99.0	233,122	736,757	549,480	816,716	2,336,075	-964,942	564,221
85	99.0	232,009	736,861	551,094	820,025	2,339,989	-968,856	564,221
95	99.0	231,354	738,079	553,209	823,811	2,346,453	-975,320	564,221
75	96.5	240,986	804,319	618,196	932,614	2,596,115	-1,224,982	644,464
85	96.5	241,336	809,332	621,990	937,737	2,610,395	-1,239,262	644,464
95	96.5	242,068	814,778	625,826	942,867	2,625,539	-1,254,406	644,464
75	94.0	254,322	892,297	695,526	1,054,824	2,896,969	-1,525,836	724,707
85	94.0	255,653	898,534	699,429	1,059,960	2,913,576	-1,542,443	724,707
95	94.0	257,243	904,850	703,332	1,065,093	2,930,518	-1,559,385	724,707
SQ option		186,249	459,913	306,185	418,786	1,371,133	n/a	277,354

The total cost of the IFN test option exceeded the total cost of the status quo option over all values of IFN test sensitivity and specificity investigated (Table 15). The loss from applying the IFN test option to the postulated year 2000 population of 305 MTR herds varied from £964,942 at an IFN test sensitivity of 75% and specificity of 99% to £1,559,385 at a test sensitivity of 95% and a specificity of 94%.

<u>Cost of testing</u>

The total cost of applying the SICCT test to 305 MTR herds between the disclosure test and the fifth test after the disclosure test under the status quo option was £494,076. The cost of SICCT testing fell to £427,282 under the IFN test option because more herds had movement restriction orders lifted before the fifth test after the disclosure test. However, this decrease in cost was small compared with the cost of applying the IFN test to 305 MTR herds, which came to £998,559 (Table 16).

TABLE 16: Cost (£) of testing MTR herds between the disclosure test and the fifth test after the disclosure test (SQ option) or IFN test (IFN test option) by herd size category and for all MTR herds

Herd size	No. of herds	**IFN test option**			**Status quo option**		
		IFN test	SICCT tests	Total cost	IFN test	SICCT tests	Total cost
50	71	63,630	34,911	98,541	n/a	41,991	41,991
150	113	296,896	125,394	422,290	n/a	148,136	148,136
250	57	248,404	103,228	351,632	n/a	119,896	119,896
350	64	389,629	163,749	553,378	n/a	184,053	184,053
All herds	**305**	**998,559**	**427,282**	**1,425,841**	**n/a**	**494,076**	**494,076**

The effect of changing the price of the IFN test on the cost of carrying out the IFN test in 305 MTR herds is shown in Table 17. When the price of the laboratory component of the IFN test was dropped from its current price of £15.00 to £1.00, the total cost of the IFN test option fell from £2,339,98 to £1,539,889. However, this latter cost was still higher than the total cost of applying the status quo option to the 305 MTR herds. In small herds, the IFN test option was more cost-effective than the status quo option at an IFN test price of around £2.00. However, in herds with 150 or more cattle, the status quo option was more cost-effective than the IFN test option even when the price of the IFN test was dropped to £1.00. The reason is that as herd size increases, the probability that the movement restriction order will be lifted on a herd decreases, even when the herd is free from tuberculous animals. Therefore, use of the IFN test in large herds offers little advantage over the status quo option in reducing the number of tests required to meet the criteria for lifting the movement restriction order.

TABLE 17: Effect of changing the price of the laboratory component of the IFN test on the cost (£) of the first IFN test carried out in MTR herds (IFNT1) and on the total cost (£) of the IFN test option in MTR herds. The cost of the status quo (SQ) option is shown at the bottom of the table for comparison.

IFN test option	Herd size								All herds in 2000 population (n=305)	
	50 (n=71)		150 (n=113)		250 (n=57)		350 (n=64)			
Price of IFN test	Cost of IFNT1	Total cost of option	Cost of IFNT1	Total cost of option	Cost of IFNT1	Total cost of option	Cost of IFNT1	Total cost of option	Cost of IFNT1	Total cost of option
£15.00	63,630	232,009	296,896	736,861	248,404	551,094	389,629	820,025	998,559	2,339,989
£10.00	45,880	214,259	212,146	652,111	177,154	479,844	277,629	708,025	712,809	2,054,239
£5.00	28,130	196,509	127,396	567,361	105,904	408,594	165,629	596,025	427,059	1,768,489
£2.50	19,255	187,634	85,021	524,986	70,279	372,969	109,629	540,025	284,184	1,625,614
£2.00	17,480	185,859	76,546	516,511	63,154	365,844	98,429	528,825	255,609	1,597,039
£1.00	13,930	182,309	59,596	499,561	48,904	351,594	76,029	506,425	198,459	1,539,889
SQ option	0	186,249	0	459,913	0	306,185	0	418,786	0	1,371,133

Cost of restricting herds

Under the IFN test option, the average length of a herd restriction order in the 305 MTR herds was reduced by 29 days up to the fifth test after the disclosure test (Table 18). The total cost to farmers of restricting herds under the status quo option was £576,027 compared with £509,360 under the IFN test option (Table 18). This resulted in a saving to farmers of around £220 per MTR herd in the year 2000 population.

TABLE 18: Cost (£) of restricting MTR herds between the disclosure test and the fifth test after the disclosure test (SQ option) or IFN test (IFN test option) by herd size category and for all MTR herds

Herd size	No. of herds	**IFN test option**				**Status quo option**			
		Mean no. of days restricted	Farmer cost	DEFRA admin. cost	Total cost	Mean no. of days restricted	Farmer cost	DEFRA admin. cost	Total cost
50	71	169	93,185	418	93,603	202	110,967	418	111,385
150	113	214	184,918	664	185,582	246	211,767	664	212,431
250	57	245	104,947	335	105,282	272	117,051	335	117,386
350	64	265	126,310	376	126,686	284	136,242	376	136,618
All herds	**305**	**220**	**509,360**	**1,793**	**511,153**	**249**	**576,027**	**1,793**	**577,820**

Costs associated with reactor animals

Under the IFN test option, 1,827 cattle were taken as reactor animals from the 305 MTR herds compared with 1,356 cattle under the status quo option (Table 19). The loss to the country from the premature slaughter of these cattle was estimated at £74,900 under the IFN test option and £55,615 under the status quo option (Table 19). There was also an increased DEFRA administration cost associated with the reactor animals under the IFN test option of nearly £6,000. However, the biggest cost associated with reactor animals was the procedures required to confirm TB in reactor cattle at the slaughterhouse and laboratory. Under the IFN test option, this cost was £305,078 compared with £226,530 under the status quo option (Table 19).

TABLE 19: Costs (£) associated with reactor animals between the disclosure test and the fifth test after the disclosure test (SQ option) or IFN test (IFN test option) by herd size category and for all MTR herds

Herd size	No. of herds	**IFN test option**					**Status quo option**				
		No. of reactors	Reactor depreciation	DEFRA admin	PM and lab exam	Total cost	No. of reactors	Reactor depreciation	DEFRA admin	PM and lab exam	Total cost
50	71	181	7,409	2,277	30,179	39,865	149	6,110	1,878	24,885	32,873
150	113	585	23,974	7,367	97,648	128,989	450	18,464	5,674	75,208	99,346
250	57	427	17,504	5,379	71,297	94,180	312	12,806	3,936	52,161	68,903
350	64	634	26,013	7,994	105,954	139,961	445	18,235	5,604	74,276	98,115
All herds	**305**	**1,827**	**74,900**	**23,017**	**305,078**	**402,995**	**1,356**	**55,615**	**17,092**	**226,530**	**299,237**

Because no diagnostic test is 100% sensitive and specific, the more tests carried out over the same time period, the greater the number of animals that will give a positive test over that time period. As six herd tests were applied under the IFN test option compared with five herd tests under the status quo option, there were more reactor animals under the IFN test option. This effect on the number of reactors was offset to some extent by more herds having the movement restriction order lifted under the IFN test option than under the status quo option over the same time period. However, this effect was not sufficient to reduce the total number of herd tests under the IFN test option to less than under the status quo option, especially in large herds. As the costs associated with reactor animals were linked to the number of reactor animals, changes in the unit prices of the various components did not affect the cost differential between the two options.

Discussion and conclusions

This study investigated the impact of introducing a new test – the IFN test – into the testing regime currently used to identify tuberculosis cattle in multiple tuberculin reactor herds. To isolate the effects of test sensitivity and specificity and the change in the testing regime on the clearance rate of tuberculous cattle from MTR herds and the length of herd movement restrictions, it was assumed that there was no spread of infection within, or new introductions of TB into, the herds after the disclosure test. To give a realistic picture of the outcomes and costs of the two options, the decision tree models were run on a population of MTR herds constituted to mirror that expected in Great Britain in the year 2000 with regard to herd numbers, number of cattle tested and number of tuberculous cattle in the herds. Therefore, the results presented in the report are the average expected outcomes and costs from using the status quo or IFN test options in a defined population of 305 MTR herds, given the properties of the tests, the number of cattle tested, the prevalence of tuberculous cattle in the herd at the time of the test and the testing rules or regime applied.

Over the range of IFN test values used, the IFN test option cost between £1.0 million and £1.5 million more than the status quo option up to the fifth test after the disclosure test. The main reason for the cost differential was the high cost of carrying out the IFN test in a population comprising many large herds. As all herds still had to have two consecutive clear SITs 60 days apart before movement restriction orders could be lifted, use of the IFN test had only a small effect on reducing the average length of the restriction period. The specificity of the IFN test had a much greater impact on the total cost of the IFN test option than its sensitivity.

Ideally, the tests and testing regime used should not restrict herds past the time when they no longer constitute a threat to other herds. In the study, the cost of a non-tuberculous herd being declared a reactor or IR only herd (false positive herd) was reflected in the cost of unnecessary testing, slaughter and restriction of the herd. However, it is also important that a movement restriction order is not lifted on a herd that still contains tuberculous cattle. The cost of failing to identify a tuberculous herd was not calculated in the study but it does carry a potential cost if TB spreads within the herd and from the missed herd to other TB-free herds. By the fourth test after the disclosure test, movement restriction orders had been lifted on six herds that still contained tuberculous cattle under the status quo option compared with two herds under the IFN test option. Use of the IFN test at 10 days after the disclosure test also resulted in tuberculous cattle being removed from herds more quickly. By the time of the SIT1, only 165 herds still contained tuberculous cattle under the IFN test option compared with 210 herds under the status quo option. Without knowledge of the likely reduction in the spread of TB from these herds, it was difficult to value the benefits from using the IFN test to reduce the number of false negative herds and the weight of infection in tuberculous herds. However, to be indifferent between the two options, this benefit would have to be worth between £1 million and £1.5 million. If it were possible to reduce the cost of the laboratory component of the IFN test from £15 per test to £5 per test, the difference in total costs between the two options would fall to around £0.5 million if the specificity of the IFN test was 99%.

The study highlighted the significant impact that herd size has on the herd outcomes under both options because of the use of imperfect tests. In herds in which 250 or 350 cattle in a herd were given the IFN test, nearly all herds had at least one IFN test positive animal even though there were no tuberculous cattle in the herds. Similarly, none of the herds that were free from tuberculous cattle at the SIT1 and SIT2 and which had 350 cattle met the criteria for the lifting of the movement restriction order. In herds with 250 cattle, the percentage of false positive herds at the SIT2 was 98% and in herds with 150 cattle, it was nearly 90% under both options. By the fourth test after the disclosure test, most large herds remained under movement restriction even though the herds had been free from tuberculous cattle at their last two tests.

Recommendations

- The study showed that use of the IFN test at around 10 days after the disclosure test reduced the number of tuberculous cattle in multiple tuberculin reactor herds more quickly than the current testing regime. Movement restriction orders were also lifted on fewer herds that still contained tuberculous cattle. However, the IFN test option cost between £1.0 million and £1.5 million more than the status quo option over the period evaluated, that is, up to the fifth test after the disclosure test. Therefore, **wholesale use of the IFN test in multiple tuberculin reactor herds should be approached with caution**.

- One benefit from use of the IFN test that the economic analysis did not take into account was the savings associated with the likely reduction in the spread of TB within and between herds by removing tuberculous cattle from multiple tuberculin reactor herds more quickly and releasing fewer tuberculous herds from movement restriction. However, to make use of the IFN test option cost-effective, this benefit would have to exceed £1.0 million to £1.5 million. As it seems unlikely that this level of benefit would be realised from use of the IFN test as described in this study, **priority should be given to conceiving alternative ways of using the IFN test in multiple tuberculin reactor herds and then evaluating their cost-effectiveness.** The models developed in this study could be used for this purpose.

- To improve the decision-making value of the economic analysis, **information on the magnitude of the losses caused by the spread of TB within and between herds should be sought from current projects modelling the spread of TB in cattle herds**.

- As the cost of the IFN test comprised over 40% of the total cost of the IFN test option, **attention should be focussed on improving the properties of the IFN test, especially its specificity, and on reducing its cost**. Therefore, support should continue to be given to the development of more specific antigens for use in the IFN test and to the cost-saving measures recommended in Part B of the project.

- The study showed that the number of cattle tested in a herd has a significant impact on herd outcomes that are based on imperfect tests applied to individuals in herds. As the number of cattle in a herd increased, the probability that the movement restriction order would be lifted on the herd decreased although the herd contained no tuberculous cattle. The problem is accentuated in multiple tuberculin reactor herds as the proportion of large herds falling into this category is greater than in the general population of herds. **It is recommended that the models developed in this study are used to explore what conditions would be necessary to improve the accuracy of herd outcomes in large herds**, for example, by changing the interpretation of the SICCT test and the rules governing the lifting of movement restriction orders.

PART B: OPERATIONAL FEASIBILITY OF USING THE IFN TEST IN MULTIPLE REACTOR HERDS

Aim

The objective of the trial was to investigate the *feasibility* of performing the gamma-interferon test:

- Specifically, how feasible was the routine use of the test in the control programme for bovine TB in Great Britain? ***The trial was not intended to generate precise information on the efficiency of the test for diagnosis, incident resolution or disease control.***

Trial herds and tests

- Brief descriptions of the five herds appear in Annex 3, Table 1. Because of the need for all herds to commence the trial within the space of about a month, the requirement that about half the herds should be beef herds was waived. Four herds were dairy herds (with an average of 80 cows and 80 other cattle), and one fattened purchased calves for beef. All of the herds had multiple reactors (3 to 10) at their first skin test.
- The tests were timed as intended, except that the epidemic of Foot and Mouth disease curtailed testing in the three herds (B, C and D) in Devon. The IFN-γ test following the disclosing test was performed between 15 and 29 days after the first skin test, almost within the target range of 10 – 28 days. As one would expect, the number of animals tested tended to diminish with time in the herds with the largest numbers of reactors slaughtered (A and E; Annex 3, Table 2).

Description of the test

- ***On day 1***, blood is collected in anticoagulant tubes from cattle at least 6 weeks of age and sent to the laboratory by courier;
  - It is **essential** that the blood samples are not chilled below 10°C or warmed above 26 °C. Since the risk that this might occur was detected by the feasibility trial, the viability of blood cells in an adequate proportion of the samples should be checked in the laboratory using a superantigen. Re-usable and appropriate transport containers that maintain the temperature within the acceptable range are now commercially available.
- ***On day 2 (i.e. 24-30 hours after taking the samples)***, blood is pipetted into wells in empty multi-well plates, antigens are added to duplicate wells, and the culture is incubated overnight (16 to 24 hours). The gamma interferon (IFN-γ) produced during stimulation of the blood cells in this incubation is measured *later* in the ELISA part of the test;
  - It is **essential** that the incubation begins within about 24-30 hours of taking the blood sample, thus the scheduling of sampling is important to avoid overwhelming the laboratory;
  - The antigens have traditionally been diluent only (control), avian tuberculin, and bovine tuberculin. *In the trial, we also included ESAT6 (a purified antigen), a mixture of peptides (from ESAT6 and CFP10), and a superantigen (to monitor the viability of the lymphocytes). Thus there were control + 5 antigens, which occupied 12 wells of a BOVIGAM(TM) ELISA plate per sample.*
- ***On day 3***, the incubation plates are centrifuged in a plate centrifuge and the plasma is transferred to small tubes for freezing at –20 to –80 °C, for testing later. *Freezing the plasma was essential in case the test on any sample had to be repeated, but not all of the plasma had to be frozen if work could be completed on day 3.*
- ***On day 3 (if there is time) or later (plasma can be stored frozen for several weeks to months)***, fresh or frozen plasma is transferred to BOVIGAM ELISA plates and processed. Finally, the optical density (OD) of each well of the plates is read colorimetrically to obtain an estimate of IFN-γ activity. *If there was a large difference between readings from the duplicate sub-samples, this stage of the test was repeated. Thirteen of the 1527 IFN-γ tests were repeated for this reason.*

Logistics

- ***Scheduling of tests***. Except for the first blood sampling, most samples were taken when animals were assembled for tuberculin skin testing (necessarily on the first of the two days of the skin test). Even though only five farms provided samples for the laboratory, and the test protocol allowed for freezing plasma just before half-way through the test, it was necessary on one occasion to reschedule blood sampling to avoid overloading the laboratory. The days at present used to start the skin test avoid having to visit farms on the weekend, and are Mondays, Tuesdays and Fridays. Since the laboratory also prefers not to process samples at the weekend, only the first two of these days are suitable for blood sampling and thus for the receipt of samples by the laboratory.
- ***Taking blood samples.*** The macro format six-antigen test used required slightly more than 9 ml of blood from each animal. For this reason, we insisted on 2 tubes of blood (of either 10-ml or 7-ml capacity). Animal movement causing the needle to slip out of the vein when tubes were changed, blood clotting in the needle, keeping track of duplicate samples, etc, made the taking of two tubes cumbersome and sometimes impossible. We estimate that two or three times as many blood samples could be taken in the same time if only one blood tube is taken. *We recommend the use of a micro-format test, possibly in conjunction with fewer antigens, to allow a significantly smaller (< 3 ml) volume of blood to be taken.*
- ***Transporting samples to the laboratory.*** Problems were encountered that would probably diminish as IFN-γ testing becomes more widespread.
    - In Staffordshire, but not in Devon or Carmarthen, the contracted couriers (TNT) were reluctant to carry samples associated with TB. It appears that each TNT office has considerable freedom to interpret their company's safety rules. The chosen alternative courier (ParcelForce) was unable to find Luddington, with the result that the samples were stranded in an unsuitable environment on the night of 29/1/2001. Of the 16 batches of samples received by the laboratory, two (both sent on the same date) yielded a very low IFN-γ yield from the superantigen (Table 1), implying that lymphocytes in the samples had lost most of their ability to produce IFN-γ at the time of incubation. *Superantigen was therefore essential for monitoring the status of the samples, and should be used with at least one in four of the samples.*

Table 1 - Mean values of reactions to superantigen, by herd and sampling date

Herd A		Herd B		Herd C		Herd D		Herd E	
14/11/00	2.86	5/12/00	2.62	6/12/00	3.00	14/12/00	0.86	18/12/00	1.19
15/11/00	1.83	14/12/00	1.92	14/12/00	1.46	2/1/01	1.96	3/1/01	1.76
29/1/01	0.15	6/2/01	2.50	29/1/01	0.20	15/1/01	2.65	20/2/01	1.85
								24/4/01	1.52
								25/6/01	0.74
								3/9/01	1.56

Costs

Blood sampling

- The cost of taking samples depends on the volume of blood taken. The laboratory cost depends on the number of ELISA wells per blood sample (a minimum of two wells are used for each antigen), the cost of antigens, depreciation of capital equipment, and staff requirements.
- The participating farmers themselves incurred costs when taking blood samples. We received invoices for the actual time of farmers and farm staff: £1.19 per sample on one farm with 156 or 153 cattle (27 or 25.5 h @ £6.90) and £0.74 per sample (18.5 h @ £6.00 for 158 cattle) on another. Although farmers were not paid for the labour they contributed after March 2001, they were willing to continue without payment. The cost of National Insurance and hire of equipment (e.g. to bleed more than one group of cattle simultaneously) must be added to this.
- Up to 32 animals that were negative to the skin test were positive to the IFN-γ test (Table 3, below). These animals were not reactors under the European Union directive as it stands at present. The Ministry

accepted responsibility for compensation, treating them all as dangerous in-contact animals (DCs), since a positive IFN-γ test strongly suggests that the animal has been in contact with infection. Two or three of the five farmers also had insurance against consequential losses.

- The cost of veterinary staff (SVS or LVIs, assuming a micro format in which only 0.4 ml blood is incubated with each of the antigens) may be estimated as 1.25 x the amount paid to LVIs for blood sampling for routine *B. abortus* serology, i.e. £3.00 per sample. *This increased fee would be justified in ensuring that samples reach the laboratory in time and in optimum condition.* Since non-veterinarian staff are allowed to take blood samples it may be possible to reduce the cost of sampling.

Laboratory costs

- Estimates of staff requirements have been made at Luddington Regional Laboratory, whose capacity for the macro format test (12 ELISA wells) is 168 blood samples a week. Twelve BOVIGAM ELISA wells are used per sample, and incubations are done in large-well (24-well) plates;
  - Two Band F technicians (Assistant Scientific Officers) spend 6.5 hours each on Day 2, 4.5 hours each on Day 3, and a total of 13 hours each for the final stage. This, together with the time of the supervisor, takes about 52 staff hours, or 0.31 hours per sample. With overheads, this would cost just over £10 per sample.
- Twelve wells per sample were used in the trial to compare the results from different antigens. Using superantigen was found to be essential, but it need be used for only approx. 25% of samples. A reduced set of the other antigens is possible. For example, where superantigen is used for 25% of the samples, diluent, avian ppd and bovine ppd antigens need 6.5 ELISA wells per sample, and diluent and peptide cocktail need 4.5 ELISA wells. The labour required for all activities except the logging and handling of sample tubes would be reduced *pro rata*.
- A very large demand for testing could be catered for by automated equipment, which costs around £100,000 but could operate day and night. The bottleneck in processing would then be on the day of receipt of the blood samples: to check and arrange the tubes, to log sample details it into the computer, and to prepare the tubes for the initial pipetting.
- The Bovigam kits cost about £50 per plate, on which 90 wells are potentially available for tests. Depending on whether each sample requires 12, 6.5 or 4.5 wells, the cost of the kits would be around £7.15, £3.85 or £2.50 per sample. Quantity discounts are available. The cost of tuberculin antigens for the test was negligible – even at macro scale, which used 0.03 mg/well. The cost of the peptide cocktail would be no more than £0.50 a sample for the micro format. If the test becomes extensively used, the cost of the peptides could substantially decrease.

Use of the test in practice

Feasible cut-off point

Table 2 - The mean number of "positive" results to the IFN-γ test depends on the antigen (and antigen concentration) and the cut-off criterion: 1253 blood samples

Test antigen(s)	Cut-off = 0.05	Cut-off = 0.1	Cut-off = 0.2	Cut-off = 0.3
Tuberculin (bovine – avian)	106 [1]	63	43	30
ESAT 6 (minus diluent)	96	61	39	26
Peptides (minus diluent)	64	47	30	24
ESAT 6 and / or Peptides	119	77	48	34

[1] The results are based on the *average* differences between the readings of two antigen wells and two diluent (or avian) wells. Note that the number of positives decreases as the cut-off increases.

Some of the animals testing positive to IFN-γ using tuberculin were not slaughtered because they were younger than 6 months.

- Measurement variation inherent in the ELISA test, and the need to subtract one ELISA reading from another (e.g. bovine – avian ppd, or mixed peptides – diluent), dictated that cut-off points must be large enough to give consistent results. We recommend that a cut-off of 0.1 optical density units would be the most practical option (Table 2).

Diagnostic value

- It is agreed that the only realistic rôle for the IFN-γ test in Great Britain or elsewhere was for enhancing any information gained from the skin test, i.e. it would be an ancillary serial test.
- **Specifically, a test that picks up skin test negative animals infected with *M. bovis* is needed.**
- **This result has been achieved.** The overlaps and (more importantly) the non-overlaps between the skin test and versions of the IFN-γ test are shown in Table 3. Summaries for each herd are given in Annex 3, Table 3.

Table 3 – Fraction of *slaughtered* animals from which visible lesions were seen or *Mycobacterium bovis* was isolated, for combinations of the skin test and the various gamma-interferon test results

Antigen(s) used in the test	IFN-γ test result (optical density)	Reactor status (at severe interpretation)	
		Skin test negative (Bovine – avian < 3 mm)	Reactor
Bovine ppd minus avian ppd (the decision to slaughter was based on this test)	Negative (OD < 0.1)	0 / 4 [1]	8 / 13 (plus 6 / 6) [4]
	Positive (OD >= 0.1)	4 / 38 [2]	9 / 14
ESAT 6 minus negative control	Negative (OD < 0.1)	2 / 31 [3]	10 / 16
	Positive (OD >= 0.1)	2 / 11 [2]	13 / 17
Peptide cocktail minus negative control	Negative (OD < 0.1)	1 / 32 [3]	7 / 13
	Positive (OD >= 0.1)	3 / 10 [2]	16 / 20
(ESAT 6 OR peptide cocktail) minus negative control	Negative (OD < 0.1)	0 / 28 [3]	7 / 13
	Positive (OD >= 0.1)	4 / 15 [2]	17 / 21

Five slaughtered animals do not appear in the above table: the culture results of one NVL animal are still pending, and IFN-γ tests were not complete on four other animals because of lack of blood.

[1] These animals were slaughtered after being found to be inconclusive reactors on 2 or 3 successive occasions.
[2] The cell with boldface type represents skin test negative animals positive to the respective IFN-γ test.
[3] Most of these animals were detected by the IFNγ test based on bovine and avian tuberculin but were not detected by ESAT6 or peptide cocktail. The latter tests had increased specificity and possibly reduced sensitivity.
[4] These six animals were amongst the first ten samples processed, and were reported as having large avian reactions (OD~2.0) but negligible bovine reactions (OD<0.25).

- Thus, versions of the IFN-γ test detected between 7 and 32 animals that were negative to the skin test, of which up to four contained either culturable *M. bovis* or had visible lesions. On the other hand, the IFN-γ test failed to detect between seven and ten demonstrably infected skin test reactors.

- The results suggest that tests using ESAT 6 and peptide cocktail were more specific than those using ppd (there were fewer lesion or culture-negative IFN-γ positives). ESAT 6 and peptide cocktail were less sensitive than ppd when used alone but equally sensitive when used in combination with one another, but the numbers of animals from which this statement is made is very small and no firm conclusions can be drawn from these results. *It has to be noted that this was not an objective of this trial, which was designed to assess the logistical feasibility of using IFN-γ based tests.*

- **Nevertheless, it has to be stressed that animals were identified with the IFN-γ test that would not have been identified with the skin test alone. It has also been hypothesised that the IFN-γ test detects animals earlier in the course of infection than the skin test. Thus it would be expected to detect more of the animals that had been *infected* but did not have visible evidence of *disease* (*M. bovis*-culture-negative).**
- As a standalone test, the largest proportion of evidently diseased animals per positive test was achieved by the IFN-γ test using peptide cocktail (19 *M. bovis* +ves in 29 positives), followed by concurrent peptide cocktail and ESAT 6 (21 in 36), ESAT 6 (16 in 30) and ppd (20 in 59). *Please again note that this trial was not designed to have statistical power in comparing the various implementations of the IFN-γ test.*

Recommendations

- ***Improvements in logistics***
  - Co-ordination of tests within and between Animal Health Offices, and linkage with VLA Regional Laboratories, could be achieved through a scheduling system based on an intranet or the Internet, of which VLA (Weybridge) has experience.
  - Consignments should be protected from cold environments, for example by packing them in additional insulation or in transit containers designed to maintain the temperature within the required range, and couriers should be supplied with detailed maps of where to deliver them.
- ***The micro format test***
  - The micro format IFN-γ test has been validated in Northern Ireland, has been shown to be satisfactory for research use at VLA (Weybridge) by the Department Bacterial Diseases. It offers a number of advantages:
    - Smaller blood volume: 2 ml of blood is plenty for a test using control and three antigens, so that one standard 7-ml tube will be sufficient, thus reducing the time to bleed cattle;
    - A reduced quantity of antigens is required in the incubation stage: for the peptide cocktail method, the saving would be up to £2.00 a test;
    - The transfer of plasma from the incubation plates to the ELISA plates is simpler, probably saving time and minimising the possibility of gross errors.
- ***Using a reduced set of antigens***
  - Omitting some of the antigens would reduce the cost of the test, by saving on BOVIGAM plates, pipetting, and ELISA readings. *It is not advisable to completely omit the superantigen.* The options are to reduce the test to 4.5, 6.5 or 8.5 wells from the 12 in the trial (see above).
  - The biggest saving might be obtained with the peptide cocktail – each sample would need 4.5 wells: two with diluent and two with peptides, plus two with superantigen for every four samples.
- ***Automation***
  - The maximum capacity of the Luddington Regional Laboratory has been currently estimated as just over 400 a week, or 20,000 a year, for the 6-antigen macro format test. A very large demand for the test would call for:
    - Rollout of the test to other Regional Laboratories,
    - Automated equipment (costing about £100,000), or
    - Outsourcing of the work, with its attendant headaches of quality control.

- ***Other studies needed***
  - The use of more specific antigens (ESAT6 protein, or ESAT6 and CFP10 peptides) could be useful. However, more detailed studies will be required to fully evaluate their usefulness, as the present study was not designed to do this.
  - Further trials will be needed to obtain more precise data on the sensitivity and specificity of versions of the IFN-γ test, and on the practical ability of the test to reduce the length of time that herds are under restriction.

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