



**REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON REPLACEMENT
OF THE INTERNATIONAL STANDARD BOVINE TUBERCULIN
AND REVISION OF OIE TERRESTRIAL MANUAL CHAPTER 3.4.6 BOVINE TUBERCULOSIS¹**

Paris, 5–7 November 2019

An *ad hoc* Group on Replacement of the International Standard Bovine Tuberculin (ISBT) and revision of the OIE *Terrestrial Manual* Chapter 3.4.6 Bovine Tuberculosis [1] (hereinafter referred to as ‘the Group’) was convened at the OIE Headquarters from 5 to 7 November 2019.

The Group had been convened because there was an urgent need to replace the current International Standard Bovine Tuberculin (ISBT) and establish a reference standard for use in calibration of purified protein derivative tuberculins and the development and evaluation of ‘second generation’ diagnostic tests for bovine tuberculosis.

The November 2019 meeting was the fifth in a series of Group meetings and teleconferences that had been convened by the Biological Standards Commission (BSC), in which the Groups were tasked with planning and coordinating a project to assess potential replacements for the current International Standard Bovine Tuberculin (ISBT).

The current ISBT had been established in 1986 by the WHO Expert Committee on Biological Standardization following successful completion of an International Collaborative Study [1,2,3,4]. The current ISBT is stored in the United Kingdom at the National Institute for Biological Standards and Control (NIBSC) [4] for distribution to regulatory agencies and tuberculin manufacturers where it is used as a reference standard for calibrating bovine purified protein derivative tuberculins [1,5]. The supplies of the current ISBT reference have now become depleted and must be replaced. The ISBT replacement project involved evaluation and calibration of two candidate tuberculins in comparison with the current international standard.

The Group’s main objectives were to review the results of the project to replace the current ISBT [4], and to discuss potential revisions for the *Terrestrial Manual* Chapter 3.4.6 Bovine Tuberculosis [1]. During the meeting, the Group assembled the available information and prepared a first draft report on the ISBT project, on the understanding that work would need to continue after the meeting, through communications among Group members by email, telephone, and video conference calls to (a) discuss the statistical analyses for the International Collaborative Study, (b) finalise the conclusions and recommendations for the ISBT replacement project, and (c) assemble working draft revisions for the *Terrestrial Manual* Chapter 3.4.6 *Bovine Tuberculosis* by mid-January 2020, for presentation to the Biological Standards Commission (BSC) in February 2020.

This final report for the BSC is intended to summarise the results, conclusions, and recommendations from the ISBT project - focusing on results that were compiled during and following the November 2019 *ad hoc* Group meeting. As such, this report is based on the data that was available for review at the meeting, on 5-7 November 2019, and additional information and statistical analyses that became available up until mid-February 2020.

1. Opening and Welcome

Dr Matthew Stone, Deputy Director General, International Standards and Science, welcomed the Group participants on behalf of Dr Monique Eloit, Director General of the OIE. He thanked them for taking time to work on the project prior to, and during, the Group meeting. He acknowledged the excellent work of the members of the original ISBT *ad hoc* Group in developing and implementing the protocols for preparation and validation of a replacement International Standard Bovine Tuberculin (ISBT-2), which involved contacting

¹ Note: This *ad hoc* Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the February 2020 report of the Biological Standards Commission because this report provides its considerations and comments. It is available at: <http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/laboratories-commission-reports/meetings-reports/>

manufacturers to solicit donations of prospective reference tuberculin, screening and selection of candidate tuberculin, and coordinating the subsequent laboratory testing of two candidate tuberculin in guinea pigs and cattle. The aim was to evaluate and calibrate the candidate tuberculin in comparison with the current ISBT in a preliminary evaluation (PE) and an international collaborative study (ICS).

Dr Stone reminded the Group of the importance of their engagement and upcoming tasks for a) reviewing the results of the tuberculin evaluation studies, preparing a summary report for presentation to the Biological Standards Commission and subsequent communication of the results and recommendations to OIE Member Countries, and b) preparing proposed revisions for the *Terrestrial Manual* Chapter 3.4.6 to include updated information and guidance pertaining to diagnostic tests and vaccines for bovine tuberculosis.

Dr Stone noted that OIE Member Countries rely on intradermal tuberculin testing for domestic control and eradication programs, as well as for testing to meet international trade requirements. As such, the ISBT serves as an essential international reference standard for use in calibrating Member Countries' national reference standards as well as for standardising the working reagents that are used by tuberculin manufacturers for internal quality control.

2. Appointment of the chair and rapporteur

The meeting was chaired by Dr Steven Edwards, and Prof Glyn Hewinson was designated as rapporteur, supported by the OIE secretariat.

3. Adoption of the agenda

The Group endorsed the proposed agenda that focussed on the ISBT replacement project, analysis and interpretation of the results, together with identification of revisions required and preparation of draft updates for the text of the *Terrestrial Manual* chapter on bovine tuberculosis.

4. Introduction

Dr Edwards thanked the members of the current and previous *ad hoc* Groups for their valuable contributions. He noted that the current Group is proceeding with completion of work that was initiated by a Group that met in November 2015 to plan the studies and draft the testing protocols, and two subsequent Groups that had taken those plans to implementation. He observed that continuity is provided by some members of the current Group having also participated in the previous *ad hoc* Groups, plus three new members of the Group who were involved in the ICS. These three new Group members and will also be contributing to upcoming discussions about potential revisions for the *Terrestrial Manual* chapter on bovine tuberculosis.

The [List of Participants](#), [Terms of Reference](#), and [Agenda](#) for the Group are presented as [Appendices I, II and III](#), respectively.

5. Finalisation of the Project to Replace the International Standard Bovine Tuberculin, and Preparation of a Draft Report for Biological Standards Commission.

Dr Glen Gifford briefly reviewed the history and objectives of the ISBT replacement project and updated the members on the current status of the animal studies and analysis of the data. He noted that the work began with an *ad hoc* Group that met in [November 2015](#). Additional *ad hoc* Group meetings were scheduled in May 2017 (teleconference), [June 2017](#), December 2017 (teleconference) and November 2019 to plan, implement, and analyse the various phases of the project. The individual archived ISBT *ad hoc* Group meeting reports are also accessible online, as annexes to the biannual OIE [BSC meeting reports](#) or as separate OIE [ad hoc Group meeting reports](#).

Dr Gifford also reviewed the validation and approval processes that were followed when the original ISBT was adopted in 1986. Briefly, at the request of the World Health Organisation Expert Committee on Biological Standardization, an international collaborative study was conducted to calibrate a bovine PPD tuberculin reference standard. The studies were conducted in guinea pigs and cattle, with a candidate tuberculin that had been donated by the Centraal Diergeneskundig Instituut, The Netherlands [2]. On the basis of the results of the collaborative study, the WHO Expert Committee on Biological Standardization established the proposed international standard as the International Standard for Purified Protein Derivative (PPD) of Bovine Tuberculin and defined the activity of the contents of each ampoule as 58 500 International Units of Purified Protein Derivative (PPD) of Bovine Tuberculin [3].

In 1986, the OIE had not yet developed the concept of Reference Laboratories, or designated standard reference materials, so WHO filled the gap by establishing certain veterinary reference materials, some of which continue to be managed by the WHO. With the strengthening of the OIE in recent years, and the stronger focus by WHO on human diseases (including One Health issues) the November 2015 *ad hoc* Group had agreed that it seemed appropriate that any new International Standard Bovine Tuberculin should be evaluated and calibrated through an OIE-led international collaborative study guided by a study expert panel that could be formed from the members of the November 2015 *ad hoc* Group. This approach was supported by the WHO representative Dr David Wood, who served on this original Group.

An OIE *ad hoc* Group of bovine tuberculosis experts, working under the OIE Biological Standards Commission (BSC), coordinated a project to assess potential replacements for the current International Standard Bovine Tuberculin (ISBT). This reference standard was established in 1986, and it has now become depleted. The project involved evaluation and calibration of two candidate tuberculin in comparison with the current international standard.

The ISBT replacement project included participants from OIE Headquarters; an *ad hoc* Group of internationally recognised bovine tuberculosis (bTB) experts, including scientists from the OIE bTB Reference Laboratories from France, Argentina and the United Kingdom, who planned and coordinated the project; the United Kingdom National Institute for Biological Standards and Control (NIBSC) which was responsible for production of lyophilised candidate preparations using bulk material donated by the manufacturers, storage and distribution of the tuberculins; and collaborating scientists from three OIE bTB Reference Laboratories and 15 other national laboratories, research institutes and a manufacturer, who conducted the testing in guinea pigs and cattle. Collaborators were located in Argentina, Brazil, Canada, Ethiopia, France, India, Italy, Republic of Ireland, The Netherlands, New Zealand, Russia, South Africa, Spain, United Kingdom (England and Northern Ireland), and United States.

The validation of the candidates was carried out in three phases, namely: (1) an initial screening to identify potential tuberculin candidates, based on an examination of manufacturers' documentation and test results, (2) a Preliminary Evaluation (PE) that was conducted in two OIE Reference Laboratories for Bovine Tuberculosis using a small 'test fill' that was tested in guinea pigs to evaluate potency and specificity and (3) a larger scale International Collaborative Study (ICS) where samples from a larger 'final fill' that produced an inventory of approximately 5000 vials of each candidate were tested in laboratories with the necessary animal care/biocontainment facilities and expertise to conduct potency and specificity tests in experimentally sensitised guinea-pigs, or 'fitness for purpose' tests in experimentally sensitised cattle or naturally exposed 'reactor' cattle.

In addition to conducting a preliminary assessment of potency and specificity, the PE also included an evaluation of a standardised formulation of heat-inactivated *M. bovis* in mineral oil for potential use as an alternative to live *M. bovis* AN5 for sensitisation of guinea pigs.

In the Group members' discussions during and following the November 2019 meeting, it was decided that, at the February 2020 BSC meeting, the results, conclusions, and recommendations would be presented to the BSC. However, the BSC would be informed later of the results from two remaining 'fitness for purpose' studies in experimentally infected cattle, which were temporarily delayed while awaiting access to biocontainment facilities, as well as shelf-life/stability studies in guinea pigs that are ongoing and will not be completed until spring 2021. Consequently, this additional data from the cattle 'fitness for purpose' testing in experimentally infected cattle, and guinea pig shelf-life/stability testing would not be available in time to be included in the report for presentation to the BSC in February 2020. However, when the additional data becomes available, it will be added to [Appendix VI](#) and made available through a manuscript for publication in a peer-reviewed paper.

Results, Conclusions and Recommendations

The Group noted that, when the performance of the candidates A and B was evaluated in comparison with the current ISBT in guinea pigs sensitised with a standardised formulation of heat-inactivated *M. bovis* in mineral oil or live *M. bovis*, both candidates performed satisfactorily. The test results and statistical analyses are shown in the summary tables listed in [Appendix VI](#).

The Group recognised that the responses elicited in guinea pigs that have been sensitised with heat-inactivated *M. bovis* in mineral oil would not be fully representative of the spectrum of responses that develop in naturally sensitised 'reactor' cattle or experimentally infected guinea pigs. Nevertheless, in light of the laboratory biosafety and animal welfare considerations associated with use of live *M. bovis* for sensitisation of guinea pigs, and in the interest of standardising the test methods as much as possible, when conducting potency tests, if animal testing is necessary for calibration of tuberculins, the Group considered that there were advantages in employing a standardised inactivated sensitisation reagent, such as the reagent that was assessed in this study.

In 'fitness for purpose' studies that were conducted in cattle, both candidates also performed satisfactorily when tested in experimentally infected or naturally sensitised 'reactor' cattle. In the cattle 'fitness for purpose' studies that have been completed to date, both candidate A and candidate B were found to reliably detect 'reactor' cattle that had been experimentally challenged with live *M. bovis* when the candidate tuberculins were administered at the recommended dose. The responses to the ISBT and candidate tuberculins were moderately diminished when cattle were tested with a 1:5 the recommended dose, which resulted in a larger proportion of reactions that would be classified as 'suspect' or 'negative'.

Since both candidates were found to perform satisfactorily in comparison with the ISBT in guinea pig testing and cattle 'fitness for purpose' testing, as noted above, the Group concluded that both candidates could be considered appropriate candidates to replace the current ISBT. However, since candidate B performed satisfactorily in all bioassays and its biological activity and physical-chemical properties most closely resemble those of the current ISBT, the Group considered that candidate B should be selected as the preferred candidate to serve as a new ISBT-2. In addition, candidate B was formulated to a higher potency than candidate A, and candidate B exhibited a correspondingly higher biological activity than candidate A.

The Group, therefore, recommended that, subject to satisfactory completion of two remaining 'fitness for purpose' studies in experimentally infected cattle which will be completed in spring 2020, and satisfactory completion of ongoing stability/shelf-life studies which will be completed in 2021, candidate B should be conditionally designated as the replacement for the ISBT.

Although the Group recommends that candidate B be designated as the future replacement for the ISBT, the Group also recommends that candidate A be retained as an alternate reference standard, for use in case the supplies of candidate B become depleted or unusable for some reason in the future.

Since the current ISBT also performed satisfactorily in these studies, the Group recommends that the current ISBT be retained and used in the meantime, until the ongoing 'fitness for purpose' and shelf-life/stability studies have been satisfactorily completed. Once these studies have been satisfactorily completed, the current ISBT inventory which is stored at the NIBSC should be replaced by candidate B.

The Group proposed that, if the recommendations regarding replacement of the ISBT are endorsed by the BSC, a brief summary and overview of the results of the supporting validation studies could be presented in the BSC report to the OIE Delegates at the 88th OIE General Session, to support a *Resolution* recommending conditional approval of a new ISBT and adoption of the new ISBT through a vote by OIE Delegates at the 88th OIE General Session in May 2020, subject to satisfactory completion of the scheduled two year shelf-life / stability studies that will be concluded in spring 2021.

As such, if the Group's conclusions and recommendations are endorsed by the BSC, once the final 'fitness for purpose' and shelf-life/stability studies are satisfactorily concluded later in 2020 and in 2021 respectively, the new ISBT-2 could be presented for official adoption at the OIE General Session in May 2021. The new ISBT-2 could then potentially be made available for distribution to national regulatory agencies and tuberculin manufacturers for use in standardising national references, and quality control of commercially manufactured bovine tuberculins.

Project funding and in-kind support.

The Group acknowledged the support that the ISBT replacement project received from several sources, including generous donations of candidate tuberculins from manufacturers; in-kind contributions from participating countries and testing laboratories and NIBSC which were funded through their national governments, the Bill & Melinda Gates Foundation, and the European Commission; as well as targeted funding appropriations from the OIE World Fund Unit and the Government of Switzerland.

6. Revision of the Bovine tuberculosis chapter in the *Terrestrial Manual*

During the November 2019 meeting and in follow-up discussions among Group members, the Group reviewed the current chapter text and prepared a working draft chapter with proposed revisions, focusing on the following areas:

- a. Epidemiology;
- b. Available diagnostic tests and vaccines, and their fitness for various purposes;
- c. Guidance for manufacturing and quality control of bovine tuberculosis diagnostic tests and vaccines;
- d. Expanded guidance for diagnostic tests for use in camelids and goats.

During these discussions, it was evident there have been significant scientific developments in diagnostic methods and vaccines since the chapter was last updated. In addition to preparing draft revisions in the above areas, the Group recommended that the chapter title should be changed from 'Bovine tuberculosis' to 'Mammalian tuberculosis', and the chapter should be moved to the 'Multiple species' section of the *Terrestrial Manual*. The Group also advised that the BSC should consider hyperlinks to videos of key test procedures in future online versions of the *Terrestrial Manual* chapter.

It was agreed that a working draft showing the proposed revisions to the chapter would be presented to the BSC at the February 2020 meeting, to solicit the BSC's review and preliminary endorsement of the proposed text. After incorporating comments from the BSC and further editing, a final version with proposed revisions would be submitted to the BSC in advance of the September 2020 BSC meeting. The draft revised chapter would then be taken forward by the OIE *Terrestrial Manual* editorial team for further revisions and added to the 2020/2021 review cycle to seek Member Country comments, with a view to adoption by the World Assembly in May 2021.

7. Selected references

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Paris, 5–7 November 2019

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Appendix II

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Paris, 5–7 November 2019

Terms of Reference

1. To finalise the ISBT project and prepare a draft summary report.
 2. To review the current text of the OIE *Terrestrial Manual Chapter 3.4.6, Bovine tuberculosis*, and prepare a draft updated text. The revisions would provide updated information in the following areas, including guidance for diagnostic tests for camelids and goats (a specific request of the BSC):
 - a. Epidemiology.
 - b. Available diagnostic tests and vaccines, and their fitness for various purposes.
 - c. Guidance for manufacturing and quality control of bovine tuberculosis diagnostic tests and vaccines.
 - d. Expanded guidance for diagnostic tests for use in camelids and goats.
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Agenda

1. Appointment of the chair and rapporteur.
 2. Adoption of the agenda.
 3. Finalise the ISBT project and prepare a draft report for Biological Standards Commission.
 4. Draft the revised version of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 3.4.6 Bovine tuberculosis.
 5. Any other issues.
 6. Adoption of the report.
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Appendix IV**Materials and Candidate Tuberculin Preparations**

Bulk material of bovine tuberculin purified protein derivative (PPD) was sourced from selected manufacturers². The materials received was formulated at low concentration by the manufacturers and is stable when stored at 2° – 8°C. The information of the bulk material provided by the manufacturers is stated as following.

	Candidate A	Candidate B
Manufacturing date	30 Nov 2017	12 Jun 2017
Appearance	Light yellow aqueous solution	Light yellow aqueous solution
Volume received	~ 7 litres (10 bottles)	12 litres (1 bottle)
Buffer	PBS	Glucose phosphate buffer
Phenol content	0%	0.04% w/v
Protein concentration	1.37 mg/ ml	1.0 mg/ ml
Estimated potency	23 108 IU/ ml	34 630 IU/ ml
pH	6.89	7.0

Test/trial fills were performed on both candidates to determine the optimal freeze-drying process/ conditions for the specific formulation of each candidate. No additional buffer, bulking agent or stabilizer was used prior the lyophilization process. The definitive fills of candidate material were performed in August 2018, within the Standards Processing Division of NIBSC. The liquid bulk of each candidate was stirred constantly during filling to ensure homogeneity of fill and the temperature was maintained between 4° – 8°C; clear glass ampoules with capacity of 5 ml volume were used. Freeze-drying was performed under clean controlled conditions (under laminar air flow) though the processed material cannot be guaranteed sterile.

The finished products were coded 18/148 (Candidate A) and 18/150 (Candidate B) and stored in the dark at -20°C. About five thousand ampoules were lyophilized into a single batch for each candidate. Here are some key parameters of the definitive filled ampoules.

	Candidate A	Candidate B
Definitive fill date	24 Aug 2018	30 Aug 2018
Number of ampoules filled	4471	5706
Appearance	White cake	White cake
Mean fill mass (g)	2.0143	2.0174
Mean dry weight (g)	0.0213	0.0639
Mean residual moisture (%)	1.39	5.09
Mean oxygen head space (%)	0.17	0.27

The ampoules have an average actual fill mass of 2.0 g per ampoule with a target CV of precision of filling for fill mass of <0.25% (of at least 150 randomly selected ampoules, weights being measured). Nitrogen was used to back fill the freeze drier chamber at the end of the cycle and hence provide the headspace gas for the ampoules. The purity of the boiled off nitrogen is certified at 99.99%. Microbiological assessment was made on the product pre- and post-processing and the bacterial, mould/yeast colony counts were undetectable at pre- and post-filling stages. NIBSC does not routinely perform an automated 100% post sealing check on container integrity. Visual checks on seal quality and general container integrity are made at the time of sealing, whilst labeling and again at the time of picking material ready for dispatch to customers. NIBSC will act as custodian of the preparation which is stored under assured temperature-controlled conditions within the Institute's Centre for Biological Reference Materials, at Potters Bar, Hertfordshire, UK.

² The candidate tuberculins that were selected for laboratory testing and validation were donated by two commercial manufacturers, CZ Veterinaria (Porrino, Spain) and Thermo Fisher Scientific (Lissieu, France). The candidate tuberculins were coded as candidate A and candidate B during testing and data analysis. The collaborating scientists who were involved in the laboratory testing were blinded regarding the identity of the suppliers.

Appendix V

Protocol

Protocol for the Evaluation and Adoption of a Replacement International Standard Bovine Tuberculin

Appendix VI

**Test Results and Statistical Analyses:
Preliminary Evaluation and International Collaborative Study**

Preliminary Evaluation (PE) Tabulated Results.

Table PE 1. Preliminary Evaluation, Guinea Pig Testing: Sensitising Reagents and Adjuvants

Table PE 2. Preliminary Evaluation, Guinea Pig Testing: Lyophilisation

International Collaborative Study (ICS) Tabulated Results

[Table ICS 1b. International Collaborative Study \(ICS\) Guinea Pig Potency Test Results: Average Induration Response Following Sensitisation with a Standardised formulation of Heat-Inactivated *M. bovis* in Mineral Oil](#)

[Table ICS 2. ICS Guinea Pig Test Results – Induration Response Following Sensitisation with Live *M. bovis*](#)

[Table ICS 3. Cattle ‘Fitness for Purpose’ Testing: Tuberculin Reactions in Experimentally Infected Cattle](#)

[Table ICS 4. Cattle ‘Fitness for Purpose’ Testing: Tuberculin Reactions in Naturally Sensitised ‘Reactor’ Cattle](#)

Statistical Analysis and Potency Estimates for Candidates A and B.

[Table S1. Potency estimates for Candidate A: Heat-inactivated *M. bovis* in mineral oil sensitisation](#)

[Table S2. Potency estimates for Candidate B: Heat-inactivated *M. bovis* in mineral oil sensitisation](#)

[Table S3. Potency estimates for Candidate A: Live *M. bovis* sensitisation](#)

[Table S4. Potency estimates for Candidate B: Live *M. bovis* sensitisation](#)

Table ICS 1b. International Collaborative Study (ICS) Guinea Pig Potency Test Results: Average Induration Responses Following Sensitisation with a Heat-Inactivated *M. bovis* in Mineral Oil

ICS Table 1b.		International Collaborative Study: Guinea Pig Potency Test Results - Induration Response							
Lab	Average Induration Diameter (mm) at each Dilution: ISBT (R) and Candidate A (CA) *								
	ISBT				Candidate A				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
A (P1)	19.5	15.9	12.9	9.3	18.7	14.7	11.2	7.7	
B (P2)	20.4	16.9	14.9	12.2	17.7	14.7	12.0	9.1	
C (P4)	17.6	15.7	13.8	11.3	16.6	14.3	12.1	9.9	
D (P5)	17.8	15.3	13.4	11.1	16.0	14.5	12.0	8.7	
E (P10)	16.2	14.3	12.6	9.3	14.6	12.9	11.4	6.7	
F (P11)	19.4	15.4	12.1	8.1	17.5	14.0	9.9	5.9	
G (P13)	19.7	15.7	11.5	5.5	19.3	15.5	11.6	6.0	
H (P16)	17.1	15.0	12.1	9.5	14.8	12.4	9.6	6.1	
I (P17)	16.3	14.0	11.2	8.5	15.4	12.5	9.9	8.0	
J (P18)	17.9	16.0	14.2	11.9	17.3	15.0	12.5	9.6	
AVG	18.2	15.4	12.9	9.7	16.8	14.1	11.2	7.8	
STDEV	1.5	0.8	1.2	2.0	1.6	1.1	1.0	1.5	
SE	0.5	0.3	0.4	0.6	0.5	0.3	0.3	0.5	
AVG + SE	18.7	15.7	13.2	10.3	17.3	14.4	11.6	8.3	
AVG	18.2	15.4	12.9	9.7	16.8	14.1	11.2	7.8	
AVG - SE	17.7	15.2	12.5	9.0	16.3	13.7	10.9	7.3	

Lab	Average Induration Diameter (mm) at each Dilution: ISBT (R) and Candidate B (CB) *							
	ISBT				Candidate B			
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500
	R1	R2	R3	R4	CB1	CB2	CB3	CB4
A (P1)	19.4	15.9	12.5	9.4	19.2	15.5	12.4	8.7
B (P2)	19.5	16.9	14.2	11.7	18.7	16.2	13.6	10.6
C (P4)	18.5	15.7	14.0	10.8	17.2	15.3	12.5	9.5
D (P5)	17.4	15.3	13.2	9.9	17.0	15.0	12.6	9.5
E (P10)	16.8	14.3	12.6	9.8	15.9	13.9	12.1	8.6
F (P11)	19.3	15.4	11.7	7.9	18.2	15.1	12.1	7.7
G (P13)	19.2	15.7	12.1	7.6	18.8	15.9	11.4	6.3
H (P16)	16.1	15.0	12.3	9.7	15.3	13.7	9.9	5.8
I (P17)	16.2	14.0	11.5	8.6	16.2	13.6	11.6	9.0
J (P18)	18.0	16.0	14.5	12.3	17.6	15.6	13.7	11.1
AVG	18.0	15.4	12.9	9.8	17.4	15.0	12.2	8.7
STDEV	1.3	0.8	1.1	1.5	1.3	0.9	1.1	1.7
SE	0.4	0.3	0.3	0.5	0.4	0.3	0.3	0.5
AVG + SE	18.4	15.7	13.2	10.3	17.8	15.3	12.5	9.2
AVG	18.0	15.4	12.9	9.8	17.4	15.0	12.2	8.7
AVG - SE	17.6	15.2	12.5	9.3	17.0	14.7	11.8	8.1

* Based on average of results from three studies at each site to compare candidate A and candidate B versus ISBT.

Note: In these 'heat-map' tables included in this report, the intensity of the colour noted in each cell is proportional to the diameter of the induration response, on a graduated scale from dark red to white. Darker shades indicate larger induration diameters.

Table ICS 2. Guinea Pig Test Results – Induration Response Following Sensitisation with Live *M. bovis*

ICS Table 2.		Guinea pig assay with live <i>M. bovis</i> sensitisation P4							
Experiment 1A	Induration Diameter (mm) at each Dilution for ISBT (R), Candidate A (CA), and Candidate B (CB)								
		1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500
P4	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	15.2	16.2	14.1	12.2	15.2	14.4	13.6	10.0	
Animal A2	16.3	13.9	14.7	13.3	15.2	15.7	14.5	13.2	
Animal A3	15.6	14.9	11.3	11.2	16.5	15.4	10.6	9.8	
Animal A4	15.1	13.4	11.6	12.4	15.3	15.3	13.6	9.1	
Animal A5	ND	ND	ND	ND	ND	ND	ND	ND	
Animal A6	16.6	12.2	11.3	10.9	16.1	14.7	12.8	11.2	
Animal A7	16.4	14.2	8.2	9.7	15.6	13.9	10.1	8.7	
Animal A8	14.2	13.7	13.3	10.0	12.7	12.0	10.4	8.1	
Experiment 1B	Induration diameter (mm)								
P4	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	18.7	17.1	14.2	13.6	15.1	17.1	15.3	17.4	
Animal B2	ND	ND	ND	ND	ND	ND	ND	ND	
Animal B3	18.1	11.7	11.6	10.5	13.5	12.4	10.8	10.6	
Animal B4	16.3	14.0	12.7	12.2	15.8	14.7	10.4	10.0	
Animal B5	16.5	14.4	14.7	14.3	16.9	17.1	17.2	13.0	
Animal B6	16.1	11.4	11.1	11.0	14.8	14.5	14.1	13.0	
Animal B7	16.1	14.3	12.5	9.4	15.9	14.4	12.5	9.6	
Animal B8	16.1	15.2	14.6	9.4	13.9	11.5	13.4	13.3	
Experiment 2A	Induration diameter (mm)								
P4	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	17.6	14.5	13.8	12.7	15.1	13.6	14.0	8.8	
Animal A2	12.6	18.5	16.6	11.2	13.3	13.4	14.3	14.4	
Animal A3	15.4	11.5	12.1	11.0	16.0	12.9	9.9	9.3	
Animal A4	13.8	12.6	10.7	12.3	11.8	11.2	10.2	9.6	
Animal A5	14.8	15.7	13.4	13.0	14.0	12.4	12.4	8.7	
Animal A6	14.9	15.8	15.0	14.9	14.6	13.7	12.3	11.3	
Animal A7	13.8	12.1	13.5	9.0	14.2	11.5	12.6	9.8	
Animal A8	14.9	14.3	12.9	10.2	15.2	11.9	11.0	13.6	
Experiment 2B	Induration diameter (mm)								
P4	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	15.4	13.7	13.4	14.2	13.6	12.9	10.8	7.9	
Animal B2	16.8	14.1	11.5	9.8	13.5	11.6	9.3	7.1	
Animal B3	14.2	14.0	13.5	11.4	14.8	12.6	11.1	9.6	
Animal B4	15.7	14.9	12.6	8.4	12.9	11.9	8.7	6.9	
Animal B5	16.5	16.0	15.2	13.4	16.6	15.4	14.6	12.8	
Animal B6	18.5	14.7	13.4	11.1	15.5	12.4	10.7	9.0	
Animal B7	19.2	17.8	16.6	13.8	16.2	13.5	11.8	15.2	
Animal B8	17.2	15.2	13.5	11.1	ND	14.1	12.5	10.9	
Experiment 3A	Induration diameter (mm)								
P4	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	14.1	13.3	13.4	11.9	11.7	12.1	10.5	10.3	
Animal A2	13.8	16.0	14.0	14.5	15.7	15.5	13.8	12.7	
Animal A3	16.9	17.6	15.2	14.3	16.9	16.1	12.1	12.8	
Animal A4	14.7	14.4	14.7	12.2	14.2	13.8	12.2	11.2	
Animal A5	15.0	15.8	13.5	13.6	15.5	14.5	13.8	12.8	
Animal A6	17.8	12.0	13.0	13.2	14.5	13.2	14.2	13.2	
Animal A7	13.5	11.8	10.2	9.3	15.6	14.1	10.1	10.4	
Animal A8	15.9	13.9	12.8	11.2	16.4	14.2	13.9	11.7	
Experiment 3B	Induration diameter (mm)								
P4	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	16.3	16.4	13.9	12.7	17.3	16.3	15.3	12.6	
Animal B2	15.7	15.2	14.9	14.0	17.4	15.5	14.1	13.5	
Animal B3	17.7	14.0	13.3	13.6	16.4	14.7	13.0	10.7	
Animal B4	17.5	16.7	15.2	11.4	17.6	17.3	14.6	11.0	
Animal B5	15.8	16.7	15.7	14.7	16.9	16.8	15.5	14.3	
Animal B6	18.4	16.9	15.3	13.3	16.0	15.5	15.4	15.1	
Animal B7	17.7	15.4	12.6	10.0	15.5	11.8	13.7	9.2	
Animal B8	15.4	16.7	14.4	13.9	19.2	14.3	14.6	12.0	
Candidate A vs ISBT	Induration Diameter								
P4	R				CA				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
AVG	15.2	14.3	13.0	11.9	14.8	13.7	12.3	10.9	
STDEV	1.3	1.9	1.9	1.7	1.4	1.4	1.6	1.9	
SE	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.4	
Relative Reaction Diameter (%) at Each Dilution (Candidate A vs ISBT)					97.9%	95.9%	94.5%	91.4%	
Candidate B vs ISBT	Induration Diameter								
P4	R				CB				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
AVG	16.8	15.1	13.8	12.1	15.7	14.3	13.0	11.5	
STDEV	1.3	1.6	1.4	1.9	1.6	1.9	2.2	2.7	
SE	0.3	0.3	0.3	0.4	0.3	0.4	0.5	0.6	
Relative Reaction Diameter (%) at Each Dilution (Candidate B vs ISBT)					93.5%	94.8%	94.6%	95.5%	

Table ICS 2. Guinea Pig Test Results – Induration Response Following Sensitisation with Live *M. bovis*

ICS Table 2.		Guinea pig assay with live <i>M. bovis</i> sensitisation P4							
Experiment 1A	Induration Diameter (mm) at each Dilution for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
P4	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	15.2	16.2	14.1	12.2	15.2	14.4	13.6	10.0	
Animal A2	16.3	13.9	14.7	13.3	15.2	15.7	14.5	13.2	
Animal A3	15.6	14.9	11.3	11.2	16.5	15.4	10.6	9.8	
Animal A4	15.1	13.4	11.6	12.4	15.3	15.3	13.6	9.1	
Animal A5	ND	ND	ND	ND	ND	ND	ND	ND	
Animal A6	16.6	12.2	11.3	10.9	16.1	14.7	12.8	11.2	
Animal A7	16.4	14.2	8.2	9.7	15.6	13.9	10.1	8.7	
Animal A8	14.2	13.7	13.3	10.0	12.7	12.0	10.4	8.1	
Experiment 1B	Induration diameter (mm)								
P4	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	18.7	17.1	14.2	13.6	15.1	17.1	15.3	17.4	
Animal B2	ND	ND	ND	ND	ND	ND	ND	ND	
Animal B3	18.1	11.7	11.6	10.5	13.5	12.4	10.8	10.6	
Animal B4	16.3	14.0	12.7	12.2	15.8	14.7	10.4	10.0	
Animal B5	16.5	14.4	14.7	14.3	16.9	17.1	17.2	13.0	
Animal B6	16.1	11.4	11.1	11.0	14.8	14.5	14.1	13.0	
Animal B7	16.1	14.3	12.5	9.4	15.9	14.4	12.5	9.6	
Animal B8	16.1	15.2	14.6	9.4	13.9	11.5	13.4	13.3	
Experiment 2A	Induration diameter (mm)								
P4	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	17.6	14.5	13.8	12.7	15.1	13.6	14.0	8.8	
Animal A2	12.6	18.5	16.6	11.2	13.3	13.4	14.3	14.4	
Animal A3	15.4	11.5	12.1	11.0	16.0	12.9	9.9	9.3	
Animal A4	13.8	12.6	10.7	12.3	11.8	11.2	10.2	9.6	
Animal A5	14.8	15.7	13.4	13.0	14.0	12.4	12.4	8.7	
Animal A6	14.9	15.8	15.0	14.9	14.6	13.7	12.3	11.3	
Animal A7	13.8	12.1	13.5	9.0	14.2	11.5	12.6	9.8	
Animal A8	14.9	14.3	12.9	10.2	15.2	11.9	11.0	13.6	
Experiment 2B	Induration diameter (mm)								
P4	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	15.4	13.7	13.4	14.2	13.6	12.9	10.8	7.9	
Animal B2	16.8	14.1	11.5	9.8	13.5	11.6	9.3	7.1	
Animal B3	14.2	14.0	13.5	11.4	14.8	12.6	11.1	9.6	
Animal B4	15.7	14.9	12.6	8.4	12.9	11.9	8.7	6.9	
Animal B5	16.5	16.0	15.2	13.4	16.6	15.4	14.6	12.8	
Animal B6	18.5	14.7	13.4	11.1	15.5	12.4	10.7	9.0	
Animal B7	19.2	17.8	16.6	13.8	16.2	13.5	11.8	15.2	
Animal B8	17.2	15.2	13.5	11.1	ND	14.1	12.5	10.9	
Experiment 3A	Induration diameter (mm)								
P4	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	14.1	13.3	13.4	11.9	11.7	12.1	10.5	10.3	
Animal A2	13.8	16.0	14.0	14.5	15.7	15.5	13.8	12.7	
Animal A3	16.9	17.6	15.2	14.3	16.9	16.1	12.1	12.8	
Animal A4	14.7	14.4	14.7	12.2	14.2	13.8	12.2	11.2	
Animal A5	15.0	15.8	13.5	13.6	15.5	14.5	13.8	12.8	
Animal A6	17.8	12.0	13.0	13.2	14.5	13.2	14.2	13.2	
Animal A7	13.5	11.8	10.2	9.3	15.6	14.1	10.1	10.4	
Animal A8	15.9	13.9	12.8	11.2	16.4	14.2	13.9	11.7	
Experiment 3B	Induration diameter (mm)								
P4	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	16.3	16.4	13.9	12.7	17.3	16.3	15.3	12.6	
Animal B2	15.7	15.2	14.9	14.0	17.4	15.5	14.1	13.5	
Animal B3	17.7	14.0	13.3	13.6	16.4	14.7	13.0	10.7	
Animal B4	17.5	16.7	15.2	11.4	17.6	17.3	14.6	11.0	
Animal B5	15.8	16.7	15.7	14.7	16.9	16.8	15.5	14.3	
Animal B6	18.4	16.9	15.3	13.3	16.0	15.5	15.4	15.1	
Animal B7	17.7	15.4	12.6	10.0	15.5	11.8	13.7	9.2	
Animal B8	15.4	16.7	14.4	13.9	19.2	14.3	14.6	12.0	
Candidate A vs ISBT	Induration Diameter								
P4	R				CA				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
AVG	15.2	14.3	13.0	11.9	14.8	13.7	12.3	10.9	
STDEV	1.3	1.9	1.9	1.7	1.4	1.4	1.6	1.9	
SE	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.4	
Relative Reaction Diameter (%) at Each Dilution (Candidate A vs ISBT)					97.9%	95.9%	94.5%	91.4%	
Candidate B vs ISBT	Induration Diameter								
P4	R				CB				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
AVG	16.8	15.1	13.8	12.1	15.7	14.3	13.0	11.5	
STDEV	1.3	1.6	1.4	1.9	1.6	1.9	2.2	2.7	
SE	0.3	0.3	0.3	0.4	0.3	0.4	0.5	0.6	
Relative Reaction Diameter (%) at Each Dilution (Candidate B vs ISBT)					93.5%	94.8%	94.6%	95.5%	

ICS Table 2.		Guinea pig assay with live <i>M. bovis</i> sensitisation P18							
Experiment 1A	Induration Diameter (mm) at each Dilution for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	15.8	16.0	15.3	12.9	15.3	12.4	11.6	11.5	
Animal A2	16.0	16.0	14.7	13.1	15.7	14.7	12.6	11.6	
Animal A3	16.6	15.3	13.5	13.1	16.0	14.5	14.3	11.6	
Animal A4	18.0	15.9	13.2	13.2	16.9	15.3	14.5	12.9	
Animal A5	18.0	16.5	15.1	13.2	15.2	13.7	12.8	12.4	
Animal A6	19.9	17.6	15.7	15.5	14.2	14.4	13.8	13.1	
Animal A7	16.9	14.8	12.4	11.2	16.0	13.5	11.1	9.0	
Animal A8	17.0	15.6	14.1	12.2	17.8	15.6	13.6	13.7	
Experiment 1B	Induration diameter (mm)								
P18	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	17.9	16.1	15.3	14.3	17.5	16.2	14.6	13.3	
Animal B2	17.1	16.4	14.6	12.7	17.8	15.2	13.3	11.4	
Animal B3	16.5	14.9	14.2	13.4	16.6	13.7	14.5	13.2	
Animal B4	16.3	14.5	12.7	11.4	16.0	13.8	12.5	12.0	
Animal B5	17.6	15.4	13.6	12.6	16.7	14.3	12.6	11.4	
Animal B6	17.1	15.5	14.1	13.0	17.0	16.1	14.9	13.8	
Animal B7	15.6	14.8	14.7	13.7	16.2	13.2	12.3	10.5	
Animal B8	16.8	16.3	14.9	13.0	17.1	15.1	14.2	11.0	
Experiment 2A	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	17.9	15.3	14.3	12.6	17.6	15.0	14.4	11.7	
Animal A2	16.0	13.6	12.2	10.9	15.5	14.7	13.7	11.5	
Animal A3	16.2	13.7	12.0	11.2	15.3	12.8	12.8	10.8	
Animal A4	16.9	14.7	13.2	12.8	16.1	14.4	12.8	11.3	
Animal A5	17.2	15.6	13.9	12.7	16.4	15.1	13.6	11.6	
Animal A6	16.9	15.9	14.3	12.4	15.9	14.2	13.0	10.7	
Animal A7	17.2	15.8	15.0	13.8	16.4	13.6	13.4	11.4	
Animal A8	16.6	14.3	12.6	9.7	17.2	15.7	13.7	12.4	
Experiment 2B	Induration diameter (mm)								
P18	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	17.8	15.9	13.9	12.8	16.0	14.2	12.4	10.8	
Animal B2	17.1	15.4	14.4	13.2	17.2	15.2	13.4	13.1	
Animal B3	17.3	14.9	14.6	13.9	16.8	14.8	13.9	12.6	
Animal B4	16.6	14.9	12.9	11.1	16.6	15.4	12.7	10.2	
Animal B5	18.0	16.9	14.5	12.2	17.2	16.2	14.9	13.1	
Animal B6	17.8	16.4	15.4	12.5	16.0	15.6	15.0	14.2	
Animal B7	17.3	15.2	15.3	13.8	16.3	14.6	13.5	12.9	
Animal B8	17.4	16.5	14.9	14.9	17.4	15.7	14.6	13.3	
Experiment 3A	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	16.2	15.5	14.4	12.6	16.4	15.3	13.1	11.6	
Animal A2	15.5	14.5	13.4	13.1	15.3	15.0	13.9	11.8	
Animal A3	15.7	14.8	14.0	12.3	16.2	14.3	13.5	12.2	
Animal A4	16.8	15.3	13.6	12.6	16.3	14.9	13.1	12.2	
Animal A5	17.3	15.2	13.5	11.7	16.0	14.8	14.0	12.9	
Animal A6	16.2	15.7	13.9	12.6	15.9	15.2	14.8	13.8	
Animal A7	16.5	15.3	13.8	12.5	16.2	14.9	13.6	12.2	
Animal A8	17.5	16.2	13.8	12.4	17.1	14.9	12.6	11.3	
Experiment 3B	Induration diameter (mm)								
P18	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
Animal B1	17.3	16.3	14.9	13.3	18.9	16.6	14.7	12.9	
Animal B2	17.8	15.4	14.6	13.2	17.2	15.2	14.3	13.0	
Animal B3	17.2	14.8	14.0	13.1	17.4	16.1	14.2	12.4	
Animal B4	17.7	16.3	14.6	13.3	16.8	15.3	14.1	13.3	
Animal B5	17.6	16.2	14.6	13.9	17.5	15.6	14.0	12.8	
Animal B6	17.5	15.9	14.9	13.5	17.5	15.7	14.9	13.9	
Animal B7	16.9	15.5	17.0	15.1	17.3	13.6	13.7	12.6	
Animal B8	17.5	15.8	13.8	13.8	16.6	14.9	13.4	12.8	
Candidate A vs ISBT	Induration Diameter								
P18	R				CA				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
AVG	16.9	15.4	13.8	12.5	16.1	14.5	13.3	11.9	
STDEV	1.0	0.9	1.0	1.1	0.8	0.8	0.9	1.0	
SE	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
Relative Reaction Diameter (%) at Each Dilution (Candidate A vs ISBT)					95.4%	94.5%	96.4%	94.9%	
Candidate B vs ISBT	Induration Diameter								
P18	R				CB				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
AVG	17.2	15.7	14.5	13.2	17.0	15.1	13.9	12.5	
STDEV	0.6	0.7	0.9	0.9	0.7	0.9	0.9	1.1	
SE	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2	
Relative Reaction Diameter (%) at Each Dilution (Candidate B vs ISBT)					98.6%	96.3%	95.5%	94.6%	

ICS Table 2.		Guinea pig assay with live <i>M. bovis</i> sensitisation P18							
Experiment 1A	Induration Diameter (mm) at each Dilution for ISBT (R), Candidate A (CA), and Candidate B (CB)								
		1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	15.8	16.0	15.3	12.9	15.3	12.4	11.6	11.5	
Animal A2	16.0	16.0	14.7	13.1	15.7	14.7	12.6	11.6	
Animal A3	16.6	15.3	13.5	13.1	16.0	14.5	14.3	11.6	
Animal A4	18.0	15.9	13.2	13.2	16.9	15.3	14.5	12.9	
Animal A5	18.0	16.5	15.1	13.2	15.2	13.7	12.8	12.4	
Animal A6	19.9	17.6	15.7	15.5	14.2	14.4	13.8	13.1	
Animal A7	16.9	14.8	12.4	11.2	16.0	13.5	11.1	9.0	
Animal A8	17.0	15.6	14.1	12.2	17.8	15.6	13.6	13.7	
Experiment 1B	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal B1	17.9	16.1	15.3	14.3	17.5	16.2	14.6	13.3	
Animal B2	17.1	16.4	14.6	12.7	17.8	15.2	13.3	11.4	
Animal B3	16.5	14.9	14.2	13.4	16.6	13.7	14.5	13.2	
Animal B4	16.3	14.5	12.7	11.4	16.0	13.8	12.5	12.0	
Animal B5	17.6	15.4	13.6	12.6	16.7	14.3	12.6	11.4	
Animal B6	17.1	15.5	14.1	13.0	17.0	16.1	14.9	13.8	
Animal B7	15.6	14.8	14.7	13.7	16.2	13.2	12.3	10.5	
Animal B8	16.8	16.3	14.9	13.0	17.1	15.1	14.2	11.0	
Experiment 2A	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	17.9	15.3	14.3	12.6	17.6	15.0	14.4	11.7	
Animal A2	16.0	13.6	12.2	10.9	15.5	14.7	13.7	11.5	
Animal A3	16.2	13.7	12.0	11.2	15.3	12.8	12.8	10.8	
Animal A4	16.9	14.7	13.2	12.8	16.1	14.4	12.8	11.3	
Animal A5	17.2	15.6	13.9	12.7	16.4	15.1	13.6	11.6	
Animal A6	16.9	15.9	14.3	12.4	15.9	14.2	13.0	10.7	
Animal A7	17.2	15.8	15.0	13.8	16.4	13.6	13.4	11.4	
Animal A8	16.6	14.3	12.6	9.7	17.2	15.7	13.7	12.4	
Experiment 2B	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal B1	17.8	15.9	13.9	12.8	16.0	14.2	12.4	10.8	
Animal B2	17.1	15.4	14.4	13.2	17.2	15.2	13.4	13.1	
Animal B3	17.3	14.9	14.6	13.9	16.8	14.8	13.9	12.6	
Animal B4	16.6	14.9	12.9	11.1	16.6	15.4	12.7	10.2	
Animal B5	18.0	16.9	14.5	12.2	17.2	16.2	14.9	13.1	
Animal B6	17.8	16.4	15.4	12.5	16.0	15.6	15.0	14.2	
Animal B7	17.3	15.2	15.3	13.8	16.3	14.6	13.5	12.9	
Animal B8	17.4	16.5	14.9	14.9	17.4	15.7	14.6	13.3	
Experiment 3A	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal A1	16.2	15.5	14.4	12.6	16.4	15.3	13.1	11.6	
Animal A2	15.5	14.5	13.4	13.1	15.3	15.0	13.9	11.8	
Animal A3	15.7	14.8	14.0	12.3	16.2	14.3	13.5	12.2	
Animal A4	16.8	15.3	13.6	12.6	16.3	14.9	13.1	12.2	
Animal A5	17.3	15.2	13.5	11.7	16.0	14.8	14.0	12.9	
Animal A6	16.2	15.7	13.9	12.6	15.9	15.2	14.8	13.8	
Animal A7	16.5	15.3	13.8	12.5	16.2	14.9	13.6	12.2	
Animal A8	17.5	16.2	13.8	12.4	17.1	14.9	12.6	11.3	
Experiment 3B	Induration diameter (mm)								
P18	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
Animal B1	17.3	16.3	14.9	13.3	18.9	16.6	14.7	12.9	
Animal B2	17.8	15.4	14.6	13.2	17.2	15.2	14.3	13.0	
Animal B3	17.2	14.8	14.0	13.1	17.4	16.1	14.2	12.4	
Animal B4	17.7	16.3	14.6	13.3	16.8	15.3	14.1	13.3	
Animal B5	17.6	16.2	14.6	13.9	17.5	15.6	14.0	12.8	
Animal B6	17.5	15.9	14.9	13.5	17.5	15.7	14.9	13.9	
Animal B7	16.9	15.5	17.0	15.1	17.3	13.6	13.7	12.6	
Animal B8	17.5	15.8	13.8	13.8	16.6	14.9	13.4	12.8	
Candidate A vs ISBT	Induration Diameter								
P18	R				CA				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CA1	CA2	CA3	CA4	
AVG	16.9	15.4	13.8	12.5	16.1	14.5	13.3	11.9	
STDEV	1.0	0.9	1.0	1.1	0.8	0.8	0.9	1.0	
SE	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
Relative Reaction Diameter (%) at Each Dilution (Candidate A vs ISBT)					95.4%	94.5%	96.4%	94.9%	
Candidate B vs ISBT	Induration Diameter								
P18	R				CB				
	1:20	1:100	1:500	1:2500	1:20	1:100	1:500	1:2500	
	R1	R2	R3	R4	CB1	CB2	CB3	CB4	
AVG	17.2	15.7	14.5	13.2	17.0	15.1	13.9	12.5	
STDEV	0.6	0.7	0.9	0.9	0.7	0.9	0.9	1.1	
SE	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2	
Relative Reaction Diameter (%) at Each Dilution (Candidate B vs ISBT)					98.6%	96.3%	95.5%	94.6%	

Table ICS 3. Tuberculin Reactions in Experimentally Infected Cattle

ICS Table 3.		Tuberculin Reactions in Experimentally Infected Cattle P6							
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P6	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	23.0	24.0	21.0	18.0	24.0	17.0			
Animal 2	12.0	9.0	11.0	14.0	10.0	4.0			
Animal 3	19.0	11.0	28.0	18.0	22.0	13.0			
Animal 4	11.0	10.0	19.0	12.0	11.0	12.0			
Animal 5	9.0	9.0	6.0	10.0	12.0	9.0			
Animal 6	24.0	23.0	30.0	18.0	17.0	15.0			
Animal 7	18.0	12.0	20.0	10.0	21.0	10.0			
AVG	16.6	14.0	19.3	14.3	16.7	11.4			
STDEV	6.0	6.6	8.6	3.7	5.8	4.3			
SE	2.3	2.5	3.2	1.4	2.2	1.6			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P6	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	19.0	22.0	21.0	12.0	14.0	11.0			
Animal 2	34.0	18.0	21.0	12.0	34.0	20.0			
Animal 3	8.0	8.0	11.0	7.0	9.0	10.0			
Animal 4	13.0	10.0	14.0	10.0	9.0	4.0			
Animal 5	16.0	10.0	13.0	11.0	16.0	12.0			
Animal 6	8.0	10.0	10.0	6.0	15.0	13.0			
AVG	16.3	13.0	15.0	9.7	16.2	11.7			
STDEV	9.7	5.6	4.9	2.6	9.2	5.2			
SE	4.0	2.3	2.0	1.1	3.8	2.1			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P6	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	19.0	12.0	22.0	11.0	11.0	10.0			
Animal 2	20.0	10.0	14.0	9.0	17.0	14.0			
Animal 3	10.0	13.0	18.0	14.0	13.0	10.0			
Animal 4	13.0	10.0	16.0	13.0	11.0	6.0			
Animal 5	17.0	10.0	10.0	14.0	17.0	13.0			
Animal 6	14.0	11.0	14.0	11.0	16.0	9.0			
AVG	15.5	11.0	15.7	12.0	14.2	10.3			
STDEV	3.8	1.3	4.1	2.0	2.9	2.9			
SE	1.6	0.5	1.7	0.8	1.2	1.2			
P6	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
	R1	R2	CA1	CA2	CB1	CB2			
AVG (3 Expts)	16.2	12.7	16.8	12.1	15.7	11.2			
STDEV	6.5	5.0	6.3	3.4	6.2	4.0			
SE	1.6	1.2	1.5	0.8	1.5	1.0			
Total 'Positive'	19	19	19	19	19	19			
Total 'Suspect'	0	0	0	0	0	0			
Total 'Negative'	0	0	0	0	0	0			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0 - 3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0 - 1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0 - 3.9 mm	'Negative' : 0.0 - 1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0 - 3.9 mm									
'Negative' : 0.0 - 1.9 mm									

Table ICS 3. Tuberculin Reactions in Experimentally Infected Cattle

ICS Table 3.		Tuberculin Reactions in Experimentally Infected Cattle P14							
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P14	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	15.0	6.0	14.5	8.0	7.0	8.5			
Animal 2	12.5	6.5	14.0	10.5	8.0	5.0			
Animal 3	12.0	11.0	13.5	11.0	14.5	9.5			
Animal 4	10.5	9.0	14.0	12.0	11.0	7.0			
Animal 5	13.0	9.5	13.5	11.0	12.0	9.0			
Animal 6	15.5	9.5	10.0	8.5	12.5	6.0			
AVG	13.1	8.6	13.3	10.2	10.8	7.5			
STDEV	1.9	1.9	1.6	1.6	2.8	1.8			
SE	0.8	0.8	0.7	0.6	1.2	0.7			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P14	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	12.5	9.5	12.5	7.5	10.0	9.0			
Animal 2	9.0	10.0	10.0	9.0	7.5	6.5			
Animal 3	13.0	10.5	14.0	10.0	16.0	7.0			
Animal 4	13.5	5.5	9.5	9.0	9.0	7.5			
Animal 5	18.0	10.5	14.5	11.5	16.5	12.5			
Animal 6	5.0	5.0	7.0	3.0	7.0	5.0			
AVG	11.8	8.5	11.3	8.3	11.0	7.9			
STDEV	4.4	2.5	2.9	2.9	4.2	2.6			
SE	1.8	1.0	1.2	1.2	1.7	1.1			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P14	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	16.0	11.0	16.0	11.5	13.0	11.0			
Animal 2	14.5	10.5	17.0	9.0	14.5	11.0			
Animal 3	10.0	8.0	13.5	11.5	11.5	7.0			
Animal 4	8.0	6.0	7.5	6.0	7.0	5.0			
Animal 5	8.0	4.5	6.0	6.0	8.0	6.0			
Animal 6	10.0	9.5	11.0	7.5	10.5	9.0			
AVG	11.1	8.3	11.8	8.6	10.8	8.2			
STDEV	3.4	2.6	4.5	2.5	2.9	2.6			
SE	1.4	1.1	1.8	1.0	1.2	1.0			
P14	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
	R1	R2	CA1	CA2	CB1	CB2			
AVG (3 Expts)	12.0	8.4	12.1	9.0	10.9	7.9			
STDEV	3.3	2.2	3.2	2.4	3.2	2.2			
SE	0.8	0.5	0.7	0.6	0.7	0.5			
Total 'Positive'	18	18	18	17	18	18			
Total 'Suspect'	0	0	0	1	0	0			
Total 'Negative'	0	0	0	0	0	0			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0 - 3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0 - 1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0 - 3.9 mm	'Negative' : 0.0 - 1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0 - 3.9 mm									
'Negative' : 0.0 - 1.9 mm									

Table ICS 4. Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle

ICS Table 4		Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle P2				
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)					
	R		CA		CB	
	1:1	1:5	1:1	1:5	1:1	1:5
P2	R1	R2	CA1	CA2	CB1	CB2
Animal 1	9.0	6.0	6.0	4.0	5.0	5.0
Animal 2	16.0	11.0	11.0	5.0	15.0	5.0
Animal 3	15.0	7.0	19.0	5.0	10.0	4.0
Animal 4	14.0	11.0	5.0	5.0	8.0	5.0
Animal 5	11.0	9.0	15.0	12.0	15.0	7.0
Animal 6	13.0	9.0	11.0	8.0	18.0	9.0
Animal 7	13.0	7.0	4.0	5.0	7.0	2.0
Animal 8	9.0	7.0	11.0	5.0	5.0	5.0
Animal 9	13.0	8.0	11.0	4.0	8.0	8.0
Animal 10	15.0	11.0	11.0	8.0	7.0	5.0
AVG	12.8	8.6	10.4	6.1	9.8	5.5
STDEV	2.4	1.9	4.6	2.5	4.6	2.0
SE	0.8	0.6	1.4	0.8	1.5	0.6

P2	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)					
	R		CA		CB	
	1:1	1:5	1:1	1:5	1:1	1:5
	R1	R2	CA1	CA2	CB1	CB2
AVG (3 Expts)	12.8	8.6	10.4	6.1	9.8	5.5
STDEV	2.4	1.9	4.6	2.5	4.6	2.0
SE	0.8	0.6	1.4	0.8	1.5	0.6
Total 'Positive'	10	10	10	10	10	9
Total 'Suspect'	0	0	0	0	0	1
Total 'Negative'	0	0	0	0	0	0

'Positive' : 4.0 mm or more
'Suspect' : 2.0-3.9 mm
'Negative' : 0.0-1.9 mm

Table ICS 4. Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle

ICS Table 4	Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle P3								
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P3	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	2.8	2.2	2.3	2.4	3.3	2.4			
Animal 2	5.8	4.9	6.2	3.6	7.3	3.8			
Animal 3	5.4	3.8	4.6	2.8	5.0	3.1			
Animal 4	13.0	9.4	11.8	7.7	5.9	6.5			
Animal 5	3.0	4.2	4.6	4.7	5.9	4.7			
Animal 6	10.0	5.6	11.9	6.5	12.6	9.3			
AVG	6.7	5.0	6.9	4.6	6.7	5.0			
STDEV	4.0	2.4	4.0	2.1	3.2	2.6			
SE	1.7	1.0	1.6	0.9	1.3	1.0			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P3	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	4.4	3.0	3.4	4.1	5.7	3.1			
Animal 2	10.0	6.9	8.8	5.5	9.4	5.4			
Animal 3	10.1	9.6	11.8	7.0	9.6	9.0			
Animal 4	7.8	5.1	7.1	4.9	5.8	2.9			
Animal 5	9.6	8.7	11.8	6.5	10.7	7.8			
Animal 6	5.4	0.6	5.4	3.7	5.1	2.0			
AVG	7.9	5.7	8.1	5.3	7.7	5.0			
STDEV	2.5	3.4	3.4	1.3	2.4	2.9			
SE	1.0	1.4	1.4	0.5	1.0	1.2			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P3	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	7.0	3.2	7.4	6.4	7.0	5.9			
Animal 2	3.5	4.7	6.2	2.4	6.6	4.7			
Animal 3	5.8	1.2	2.1	2.1	4.0	1.8			
Animal 4	6.7	5.7	4.1	5.5	5.5	1.7			
Animal 5	4.7	5.6	7.7	2.6	6.1	3.3			
Animal 6	5.5	2.6	6.0	3.1	5.8	4.6			
AVG	5.5	3.8	5.6	3.7	5.8	3.7			
STDEV	1.3	1.8	2.1	1.8	1.0	1.7			
SE	0.5	0.7	0.9	0.7	0.4	0.7			
P3	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
	R1	R2	CA1	CA2	CB1	CB2			
AVG (3 Expts)	6.7	4.8	6.8	4.5	6.7	4.6			
STDEV	3.0	2.5	3.3	1.9	2.0	2.2			
SE	0.7	0.6	0.8	0.4	0.6	0.6			
Total 'Positive'	15	11	15	10	17	9			
Total 'Suspect'	3	5	3	8	1	7			
Total 'Negative'	0	2	0	0	0	2			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0-3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0-1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0-3.9 mm	'Negative' : 0.0-1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0-3.9 mm									
'Negative' : 0.0-1.9 mm									

Table ICS 4. Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle

ICS Table 4	Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle P8								
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P8	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	9.0	7.0	12.0	7.0	9.0	3.0			
Animal 2	8.0	5.0	10.0	4.0	4.0	3.0			
Animal 3	6.0	5.0	7.0	3.0	7.0	5.0			
Animal 4	5.0	4.0	7.0	5.0	5.0	4.0			
Animal 5	6.0	0.0	4.0	4.0	4.0	3.0			
Animal 6	7.0	5.0	6.0	3.0	8.0	5.0			
AVG	6.8	4.3	7.7	4.3	6.2	3.8			
STDEV	1.5	2.3	2.9	1.5	2.1	1.0			
SE	0.6	1.0	1.2	0.6	0.9	0.4			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P8	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	8.0	4.0	8.0	5.0	7.0	2.0			
Animal 2	5.0	3.0	4.0	4.0	5.0	4.0			
Animal 3	6.0	5.0	8.0	4.0	7.0	4.0			
Animal 4	5.0	3.0	9.0	5.0	7.0	2.0			
Animal 5	13.0	6.0	11.0	7.0	13.0	6.0			
Animal 6	7.0	1.0	11.0	8.0	12.0	4.0			
AVG	7.3	3.7	8.5	5.5	8.5	3.7			
STDEV	3.0	1.8	2.6	1.6	3.2	1.5			
SE	1.2	0.7	1.1	0.7	1.3	0.6			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P8	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	8.0	4.0	15.0	7.0	5.0	4.0			
Animal 2	7.0	4.0	5.0	7.0	5.0	5.0			
Animal 3	7.0	4.0	14.0	7.0	12.0	8.0			
Animal 4	19.0	12.0	22.0	12.0	19.0	16.0			
Animal 5	4.0	3.0	5.0	2.0	5.0	2.0			
Animal 6	15.0	10.0	16.0	12.0	15.0	10.0			
AVG	10.0	6.2	12.8	7.8	10.2	7.5			
STDEV	5.7	3.8	6.7	3.8	6.1	5.0			
SE	2.3	1.6	2.7	1.5	2.5	2.1			
P8	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
	R1	R2	CA1	CA2	CB1	CB2			
AVG (3 Expts)	8.1	4.7	9.7	5.9	8.3	5.0			
STDEV	3.8	2.6	4.9	2.4	4.2	3.5			
SE	0.9	0.7	1.1	0.7	1.0	0.8			
Total 'Positive'	18	13	18	15	18	12			
Total 'Suspect'	0	3	0	3	0	6			
Total 'Negative'	0	2	0	0	0	0			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0-3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0-1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0-3.9 mm	'Negative' : 0.0-1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0-3.9 mm									
'Negative' : 0.0-1.9 mm									

Table ICS 4. Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle

ICS Table 4	Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle P9								
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P9	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	5.4	3.5	5.9	3.3	5.2	4.3			
Animal 2	11.9	7.2	9.5	7.2	9.9	6.5			
Animal 3	11.0	7.1	24.0	5.8	14.4	7.8			
Animal 4	4.1	4.1	5.0	3.2	8.2	4.7			
Animal 5	4.2	3.0	3.9	2.6	4.6	3.4			
Animal 6	8.0	2.8	7.2	5.6	6.2	5.7			
AVG	7.4	4.6	9.3	4.6	8.1	5.4			
STDEV	3.4	2.0	7.5	1.8	3.7	1.6			
SE	1.4	0.8	3.1	0.8	1.5	0.7			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P9	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	6.2	5.4	6.2	5.2	6.2	1.0			
Animal 2	2.2	1.2	4.9	0.2	1.9	1.4			
Animal 3	4.4	4.2	8.7	7.2	10.8	6.7			
Animal 4	9.3	4.2	9.4	4.5	6.4	5.6			
Animal 5	0.4	0.3	1.5	0.7	0.9	0.2			
Animal 6	8.7	7.7	6.6	5.8	12.0	6.4			
AVG	5.2	3.8	6.2	3.9	6.4	3.5			
STDEV	3.5	2.7	2.8	2.8	4.5	3.0			
SE	1.4	1.1	1.2	1.2	1.8	1.2			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P9	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	6.0	3.9	5.6	4.6	4.9	4.3			
Animal 2	7.9	7.8	6.9	5.0	9.9	5.5			
Animal 3	9.6	8.5	11.5	4.0	8.3	8.1			
Animal 4	13.3	12.7	10.9	6.6	7.9	8.8			
Animal 5	4.5	2.1	3.7	3.1	3.7	2.8			
Animal 6	6.6	6.0	9.5	6.9	8.1	5.2			
AVG	8.0	6.8	8.0	5.0	7.1	5.8			
STDEV	3.1	3.8	3.1	1.5	2.3	2.3			
SE	1.3	1.5	1.3	0.6	1.0	0.9			
P9	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
	R1	R2	CA1	CA2	CB1	CB2			
AVG (3 Expts)	6.9	5.1	7.8	4.5	7.2	4.9			
STDEV	3.7	3.2	5.3	2.1	3.6	2.6			
SE	0.8	0.7	1.1	0.5	0.8	0.6			
Total 'Positive'	16	11	15	11	15	13			
Total 'Suspect'	1	5	2	5	1	2			
Total 'Negative'	1	2	1	2	2	3			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0-3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0-1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0-3.9 mm	'Negative' : 0.0-1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0-3.9 mm									
'Negative' : 0.0-1.9 mm									

Table ICS 4. Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle

ICS Table 4	Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle P13								
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P13	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	5.0	2.0	7.0	5.0	4.0	3.0			
Animal 2	4.0	3.0	4.0	3.0	5.0	1.0			
Animal 3	0.0	0.0	2.0	2.0	2.0	2.0			
Animal 4	3.0	0.0	2.0	0.0	3.0	2.0			
Animal 5	1.5	0.5	2.5	1.5	2.5	1.5			
Animal 6	1.0	0.0	1.0	0.0	2.0	0.0			
AVG	2.4	0.9	3.1	1.9	3.1	1.6			
STDEV	1.9	1.3	2.2	1.9	1.2	1.0			
SE	0.8	0.5	0.9	0.8	0.5	0.4			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P13	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	2.0	0.0	3.0	0.0	0.0	0.0			
Animal 2	4.0	2.0	3.0	2.0	5.0	2.0			
Animal 3	4.0	3.0	3.0	3.0	3.0	3.0			
Animal 4	4.0	3.0	4.0	4.0	4.0	3.0			
Animal 5	2.5	1.5	1.5	1.0	2.5	2.0			
Animal 6	0.0	0.0	0.0	0.0	0.0	0.0			
AVG	2.8	1.6	2.4	1.7	2.4	1.7			
STDEV	1.6	1.4	1.4	1.6	2.1	1.4			
SE	0.7	0.6	0.6	0.7	0.8	0.6			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P13	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	4.0	2.0	3.0	2.0	3.0	2.0			
Animal 2	0.0	0.0	0.0	0.0	1.0	0.0			
Animal 3	12.0	8.0	10.0	8.0	13.0	11.0			
Animal 4	1.0	1.0	1.0	1.0	1.0	0.5			
Animal 5	3.0	2.0	1.0	1.0	3.0	1.0			
Animal 6	6.0	5.0	7.0	7.0	6.0	0.0			
AVG	4.3	3.0	3.7	3.2	4.5	2.4			
STDEV	4.3	3.0	4.0	3.4	4.5	4.3			
SE	1.8	1.2	1.6	1.4	1.9	1.7			
P13	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
	R1	R2	CA1	CA2	CB1	CB2			
AVG (3 Expts)	3.2	1.8	3.1	2.3	3.3	1.9			
STDEV	2.9	2.0	2.5	2.2	3.0	2.6			
SE	0.7	0.5	0.6	0.6	0.7	0.6			
Total 'Positive'	8	2	5	4	6	1			
Total 'Suspect'	4	7	7	5	8	8			
Total 'Negative'	6	9	6	9	4	9			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0-3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0-1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0-3.9 mm	'Negative' : 0.0-1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0-3.9 mm									
'Negative' : 0.0-1.9 mm									
<p>Note: Animals did not conform to selection criteria for testing. Data not included in summary calculations.</p>									

Table ICS 4. Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle

ICS Table 4		Tuberculin Reactions in Naturally Sensitised 'Reactor' Cattle P15							
Experiment 1	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P15	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	0.5	-0.7	-0.6	-0.2	2.0	-0.3			
Animal 2	4.6	4.6	5.5	5.0	4.8	5.5			
Animal 3	6.8	4.8	6.9	3.5	6.4	5.0			
Animal 4	4.3	3.1	2.4	0.2	2.4	2.9			
Animal 5	8.8	9.8	10.3	11.7	13.6	16.9			
Animal 6	4.8	2.8	5.7	2.6	6.0	1.7			
AVG	5.0	4.1	5.0	3.8	5.9	5.3			
STDEV	2.8	3.4	3.8	4.3	4.2	6.1			
SE	1.1	1.4	1.5	1.8	1.7	2.5			
Experiment 2	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P15	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	3.8	3.1	3.9	1.7	3.8	2.1			
Animal 2	4.4	3.2	4.3	2.7	4.0	3.1			
Animal 3	0.9	6.7	12.4	5.9	9.3	7.0			
Animal 4	10.7	7.2	10.8	6.3	12.7	8.4			
Animal 5	-0.1	1.4	3.1	2.0	3.5	1.9			
Animal 6	1.6	1.7	3.8	2.4	2.6	2.9			
AVG	3.6	3.9	6.4	3.5	6.0	4.2			
STDEV	3.9	2.5	4.1	2.0	4.1	2.8			
SE	1.6	1.0	1.7	0.8	1.7	1.1			
Experiment 3	Summary Data: Skin Thickness Increase (mm)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
P15	R1	R2	CA1	CA2	CB1	CB2			
Animal 1	11.8	6.4	11.7	3.9	8.9	7.4			
Animal 2	2.8	4.0	3.8	3.5	3.9	5.2			
Animal 3	2.3	0.8	2.8	3.0	1.5	3.9			
Animal 4	6.3	3.6	5.6	3.2	17.0	4.5			
Animal 5	-0.3	0.6	0.3	-0.7	0.0	0.1			
Animal 6	5.1	2.4	4.4	2.6	6.0	2.9			
AVG	4.7	3.0	4.8	2.6	6.2	4.0			
STDEV	4.2	2.2	3.8	1.7	6.2	2.4			
SE	1.7	0.9	1.6	0.7	2.5	1.0			
P15	Skin Thickness Increase (mm) at each Dilution: Undilute (1:1) or Diluted (1:5) for ISBT (R), Candidate A (CA), and Candidate B (CB)								
	R		CA		CB				
	1:1	1:5	1:1	1:5	1:1	1:5			
AVG (3 Expts)	R1	R2	CA1	CA2	CB1	CB2			
STDEV	4.4	3.6	5.4	3.3	6.0	4.5			
SE	3.8	2.8	4.1	3.1	5.0	4.2			
SE	0.8	0.6	0.9	0.7	1.1	0.9			
Total 'Positive'	10	7	10	4	10	8			
Total 'Suspect'	3	6	6	10	6	6			
Total 'Negative'	5	5	2	4	2	4			
<table border="1"> <tr> <td>'Positive' : 4.0 mm or more</td> </tr> <tr> <td>'Suspect' : 2.0-3.9 mm</td> </tr> <tr> <td>'Negative' : 0.0-1.9 mm</td> </tr> </table>							'Positive' : 4.0 mm or more	'Suspect' : 2.0-3.9 mm	'Negative' : 0.0-1.9 mm
'Positive' : 4.0 mm or more									
'Suspect' : 2.0-3.9 mm									
'Negative' : 0.0-1.9 mm									

Table S1. Potency estimates for candidate A (IU/ampoule): Heat-inactivated *M. bovis* in mineral oil sensitisation

Lab code	Assay 1	Assay 2	Assay 3	GM
A	34275	36442	36656	35775
B	19910	9129	11763	12883
C	Pr	34026	27317	30488
D	19400	Pr	50047	31159
E	16510	19260	15893	17161
F	35801	22875	28803	28679
G	86530	46861	NP	63678
H	Pr	Pr	Pr	-
I	32741	31810	34667	33051
J	NP	22394	NP	22394
GM (all assays)				27136
GM (all assays; weighted)				26705
GM (of lab GM)				27873
Inter-lab GCV				58%

Table S2: Potency estimates for candidate B (IU/ampoule): Heat-inactivated *M. bovis* in mineral oil sensitisation

Lab code	Assay 1	Assay 2	Assay 3	GM
A	42309	70746	55745	55053
B	31964	48282	48308	42088
C	22295	38885	32848	30537
D	30178	46576	77918	47845
E	26911	35445	41708	34138
F	NL	51312	50205	50755
G	43297	48703	55930	49040
H	NP	Pr	NP	-
I	106436	55760	55184	68930
J	44374	NP	32493	37971
GM (all assays)				45062
GM (all assays; weighted)				44722
GM (of lab GM)				44975
Inter-lab GCV				29%

NL: Non-Linearity (p<0.01)
 NP: Non-Parallelism (p<0.01)
 Pr: Assay precision requirement not met

GM: Geometric Mean
 GCV: Geometric Coefficient of Variation

Table S3. Potency estimates for candidate A (IU/ampoule): Live *M. bovis* sensitisation

Lab code	Assay 1	Assay 2	Assay 3	GM
C	53088	Pr	Pr	-
J	13210	42916	45152	29471
GM (all assays)				-
GM (all assays; weighted)				-
GM (of lab GM)				-
Inter-lab GCV				-

Table S4. Potency estimates for sample B (candidate B) (IU/ampoule): Live *M. bovis* sensitisation

Lab code	Assay 1	Assay 2	Assay 3	GM
C	Pr	Pr	47804	-
J	32904	32156	38898	34526
GM (all assays)				-
GM (all assays; weighted)				-
GM (of lab GM)				-
Inter-lab GCV				-

NL: Non-Linearity (p<0.01)

NP: Non-Parallelism (p<0.01)

Pr: Assay precision requirement not met

GM: Geometric Mean

GCV: Geometric Coefficient of Variation