Author's response to reviews

Title: High background rates of positive tuberculosis-specific interferon-gamma release assays in a low prevalence region of UK: a surveillance study

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Author's response to reviews: see over
Dear Tonilynn Manibo,

We would like to thank you, and the referees, for your thorough review of this manuscript. Please find attached a revised manuscript taking account of each of the comments and below a point-by-point response to the reviewer’s comments. We feel the manuscript has been much improved by this process, and we look forward to the outcome of your review.

Yours sincerely,

Dr Timothy Hinks

**Editorial comment:**

*Please add method section to abstract*

A methods section has been added to the abstract as requested.

**Referee 1**

**Major comments:**

Page 4, Background, first para: “Such data would be valuable for interpreting the significance of a positive IGRA result, and guiding cost-benefit analyses of new diagnostics. [1] ”

This sentence needs some improvement because “significance” of a positive IGRA result has already been shown e.g. for exposed contacts of infectious TB source cases. Further, some cost-benefit analyses have been performed with respect to the topic of implementing the IGRA tools.

We thank the reviewer for these comments, and have rephrased the sentence as follows (inserted text underlined) [page 4, line 13]

Such data would be valuable for interpreting the likely clinical significance of a positive IGRA result in low risk populations, and further informing cost-benefit analyses of new diagnostics.

The rationale for this revised phraseology is that the “significance” of a positive IGRA – i.e. the positive predictive value of a such a test for future development of active TB disease – is population specific and whilst it has indeed been shown for exposed contacts, it has not been characterised in the population in question in this paper: those without known recent TB exposure. Its positive predictive value in such individuals is likely to be very much lower due to a number of factors such as increased likelihood the IGRA represents other MTB-complex bacteria other than MTB and the likelihood that any immune sensitisation in these individuals occurred much more remotely in the past: falling rates of disease reactivation are well documented in remote versus recent exposure. It is for these reasons that the current investigation was conceived.
In response to the reviewer's second comment, we believe the revised phrase “further informing” is more helpful as it implicitly acknowledges prior work on cost-benefit analyses.

Nonetheless, the situation in UK is that the national guidelines, referenced in the paper, (Excellence NIfC. In: Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. edn. London: Royal College of Physicians of London; 2011: 1-325.) are based on 10 cost-efficacy studies, all of which looked at participants from high prevalence countries, and of which none were performed in UK populations. These guidelines specifically raise the generalizability of these data to the UK population as a concern (p57), and stress that some of the main conclusions of the technology appraisals were based in many cases on assumptions (p48) and were sensitive to relatively small differences in estimates of prevalence (p57).

Page 5, Results, first para: “Testing was not performed in 168 exposed patients because they had died (n=66), or testing was declined or considered inappropriate (n=102).” If the 102 individuals were real contact persons how could testing considered to be inappropriate? Please clarify!

In the majority of these 102 instances we believe this was patient preference. Where individuals declined screening, or could not be contacted directly, contacts were approached through primary care physicians. In some instances it was the opinion of the attending primary care physicians, who bore the responsibility of duty of care, that, for example, as the subject was receiving end of life care, the risk / benefit balance did not favour further investigation. The details of such judgements were not always available to the study investigators, and were beyond their remit to pursue. Furthermore the investigators did not have access to the exact breakdown of numbers where screening was declined because of patient preference, versus a combined agreement between the patient and the physician.

To reflect this situation concisely the sentence in question has been rephrased as follows: [page 7, line 5]

Testing was not performed in 168 exposed patients because they had died (n=66), or (n=102) testing was declined due to patient preference or considered inappropriate, for example due to instigation of end of life care.

Page 8, Methods, first para: “Contact tracing identified 445 potential contacts of the index case, comprising 142 staff and 303 patients.”

What about the progression rate within the 15 months of the contacts scored IGRA positive?

This is an interesting question, but the study is not able to give a robust answer for several reasons. Firstly numbers are relatively small, so there is not adequate power in the sample size to give an accurate estimate of progression rate. Secondly there are several reasons specific to the unique nature of this outbreak. The outbreak evolved gradually with 8 secondary cases being diagnosed over a 15 month period. Due to the methodology of a “stone in the pond” approach, and the limited resources of a small district hospital in a low incidence area, initial screening was limited and protracted, so the maximum extent of the contact tracing was not achieved until 1 year after the initial exposure, during which time 5 secondary cases had already occurred, and a 6th individual had developed TB secondary to exposure to one of the 5 secondary cases. Therefore prospective TSpot data were not available on most of the incident cases. Furthermore TSpot positive individuals generally received chemoprophylaxis. We feel discussion of these complexities would detract from clarity in delivering the main message of this paper.

Instead, and also in response to referee 2’s comment (4), we suggest that the following text is inserted in the section on limitations in the discussion: [page 11 line 3]

due to the time-course of the outbreak, sample sizes and use of chemoprophylaxis, the study was not designed to provide data on rates of progression amongst IGRA positive individuals.

In addition, to clarify the sequence of events, the following sentence has been moved to the end of the paragraph it was in originally: [page 5 line 9]

Contact tracing identified 445 potential contacts of the index case, comprising 142 staff and 303 patients.

Page 8, Methods, second para: “These comprised a further 191 individuals with a similar age distribution recruited from staff and adult patients on the same respiratory and general medical wards where exposure had previously occurred, but who had not been exposed to any of the 9 cases.”
Why was only a total of 191 individuals taken as control group of the 445 contacts? Was the reason, if the matching took place in the same time frame, that only 191 individuals were available? Was the matching performed by random? Please clarify!

Our original intention was to recruit 200 individuals as a control group. This was based on three considerations.  
1) at the time of sample size calculations the number of contacts needing screening was believed to be 150-200. This circle was subsequently widened as the outbreak evolved.  
2) As the referee correctly surmises, there were a limited number of staff available on the same ward who had not been present at the time of the exposure.  
3) Funding for these control subjects was limited.  
4) The following sample size considerations suggested that a sample of approximately 200 would provide a sufficiently narrow confidence interval width to be of value.

“Without recent direct estimates of background prevalence of LTBI in U.K. extrapolations must be made from the incidences of active disease. Thus amongst hospital workers Khanna et al found prevalence of IGRA positivity amongst U.K. born new nursing entrants to be 2.7%. [1] If it were assumed that rates of LTBI varied in the same way with age as do cases of active TB [2] we would estimate the following age specific prevalence rates: 20-40 years 2.7%, 40-60 years 5.4%, ≥60 years 16.2%, although this is probably an overestimate. If the overall LTBI prevalence were 10% amongst 200 controls then the confidence interval width would be 4.15%, whilst for an LTBI prevalence of 2.5% the C.I.W. would be only 2.17%, making these useful estimates of point prevalence.”

As it transpires our initial estimates proved fairly accurate.

Consecutive consenting, control subjects were randomly selected from the same wards, except for some selection according to age only to ensure overall matching of the group’s age distribution to the exposed cases.

In response to the referees question we have inserted the following sentence in the methods section. [page 6 line 6]

This sample size was predicted to give a confidence interval width for overall LTBI prevalence of <4.5%.

Figure 1: A total of 34 contacts were T-Spot positive and 4 were “borderline”
Do the authors add the contacts scored “borderline” to the IGRA-positive or to the IGRA-negative contacts?

The handling of borderline results was specified in methods section (page 5), and is in accordance with US CDC guidelines. Amongst all staff, borderline results were repeated and definitive results obtained. Amongst other groups, where repeat results were not available borderline results were excluded from subsequent analysis. For completeness borderline results are presented in full in table 1 to enable readers with an interest in these values to derive their own calculations.

Given 34 positive contacts, at least 6 out them progressed to active TB, i.e. the progression rate was 6/34 (17.6%). It may be helpful to add the progression rate to the text because it reflects that the selection of contacts was valid.

As explained above this study is not designed to address the issue of progression rate and it would be misleading for us to attempt to present data. Not all of these 6 subjects had been tested prior to progression to active TB so the referee’s calculations are not valid. Timecourse of events during the outbreak preclude meaningful estimates of progression rates.

Minor Comments:
Page 4, Background, first para: “Rates of 6.7-9.9% IGRA-positivity have been observed in healthcare workers in low prevalence countries.[4]”

The information referenced lacks any significance, thus I suggest qualifying this statement, perhaps as follows “The few studies that have reported on IGRA positivity in healthcare workers place the figure in the 6.7-9.9% range.”

The sentence has been rephrased along the lines suggested by the referee, as follows: [page 4 line 8]

The few studies which have reported on IGRA-positivity in healthcare workers place rates in the range of 6.7-9.9% in low prevalence countries.

Page 4/page 8, general comment: It is common practice that the Method section
The methods section has been moved as requested.

Page 5, Results, second para: “The rates of IGRA positivity were: unexposed patients 8.7% (95%CI, 4.2-13, n=149), unexposed staff 9.5%(3.0-22, n=21), exposed patients 22%(14-29, n=130), exposed staff 11%(6.1-16, n=142). Please add the number of positives, not only the number of tested persons, and the term “95%CI” into each bracket!

The numerators and the term “95%CI” have been inserted as requested. [page 7 line 12]

Page 5, Discussion: “All these unexposed individuals were white-Caucasians…”. The sentence should read “presumably not recently exposed individuals…” because they must have been exposed to TB patients at least once in the past to acquired MTB infection (as evidenced by their IGRA positivity)!

The sentence has been rephrased to take account of this suggestion, as follows: [page 9 line5]

All these “unexposed” individuals, without known recent TB exposure, were white-Caucasians,

Page 9, Acknowledgements: The name of the company correctly spells “Oxford Immunotec” instead of “Oxford Immunotech.”

This typographic error has been corrected. [page 13 line 13]

Referee 2

1. The question posed by the authors is well defined and the methods are appropriate and well describe. But in the abstract the methods are missing and in the main manuscript the method section is at the end of the paper. Please add methods in the abstract… a methods section has been added to the abstract as requested. …and change the method section in the main manuscript after the background section.

The methods section has been moved as requested.

2. The data are sound and the discussion and conclusions are balanced and adequately to the data.

3. The writing of the manuscript is acceptable

Major Compulsory Revisions

4. Limitations are missing in the manuscript. Please give a shorts statement about possible limitations of your study for example selection bias (e.g. hospital control groups).

We thank the reviewer for this suggestion and include the following text at the end of the discussion section: [page 11 line 1]

This study has several potential limitations: sample sizes are relatively small, particularly amongst younger age-groups; data may not be directly generalizable to other regions or non-hospital communities; due to the time-course of the outbreak, sample sizes and use of chemoprophylaxis, the study was not designed to provide data on rates of progression amongst IGRA positive individuals.

5. Also you use different classifications in figure 2 (e.g. age groups) and the in the text. Please adapt this.

We have made the suggested change to figure 2, now as revised figure 2a, as this provides consistency with the text and also the fewer age groups have narrower confidence interval widths, providing greater precision. However in addition we strongly wish to retain the original figure 2 as a lower panel (figure 2b), because this has been designed to mirror the figure presented in the paper by Syed et al (reference 2 in the text). The mirroring draws attention graphically to the remarkably similar bimodal distribution amongst unexposed controls, despite a difference of diagnostic test and the passage of two decades of time.
The description of the study population is not easy to follow. The different groups exposed and unexposed patients and members of staff should be described in more detail.

As requested we have added the following text to the methods section. In particular this text now draws attention to the demographic data presented in table 1, and the study group numbers presented in Figure 1.

These two groups of “exposed staff” and “exposed patients” were defined by having received treatment or worked regularly on the same wards at the same time that the index case was present, and were therefore considered to have had known, recent exposure to MTB. “Exposed staff” had a median age of 34 (range 18 to 69) and 84% were BCG vaccinated. “Exposed patients” were older with a median age of 73 (range 27 to 95) and only 41% were known to be BCG vaccinated. See Table 1 and Figure 1.

And to further describe and clarify the remaining two groups of “unexposed staff” and “unexposed patients” we have largely rewritten the following methods paragraph as follows.

Uniquely, to inform interpretation of positive results obtained amongst the exposed contacts, two additional comparator groups of “unexposed” individuals were recruited. These comprised a further 191 individuals with a similar age distribution recruited from staff (“unexposed staff”, n=22) and adult patients (“unexposed patients”, n=169), see Figure 1. These individuals were recruited from the same respiratory and general medical wards where exposure had previously occurred, but who had not been exposed to any of the 9 cases at the time of their admissions. Subjects with any suspected TB exposure within the last 2 years were excluded. Demographics of the unexposed individuals are shown in Table 2. “Unexposed staff” had a median age of 44 (range 23 to 95) and 82% were BCG vaccinated. “Unexposed patients” were older with a median age of 71 (range 25 to 93) and only 48% were known to be BCG vaccinated. This sample size was predicted to give a confidence interval width for overall LTBI prevalence of <4.5%.

In the manuscript you did not differed clearly between unexposed and exposed participants. Please define this in more detail.

The rest of the text has been revised to ensure consistent differentiation between the groups, in the following way:

Results paragraph 1 the following text has been inserted: amongst the group of exposed patients,

We have replaced the term “unexposed individuals” with the term “unexposed patients” in Results paragraph 3 first sentence, results paragraph 3 last sentence, discussion paragraph 1 second sentence. [page 7 line 9, page 7 line 12, page 7 line 19, page 7 line 22, page 8 line 1]

We have replaced the term “unexposed controls” with the term “unexposed control patients” in discussion [page 9. Line 11]

We have inserted the phrase “amongst unexposed controls” in discussion [page 10 line 3].

Include see referee 2 point 9 response

In the results sections you write: …data were available from 465 staff and patients. Are these the unexposed group? In your flow chart there are only 445 unexposed staff and patient, please check this carefully.
This number has been checked carefully and is correct. The number of 465 comprises all subjects from either “exposed” or “unexposed” groups on whom clinical data were available and on whom an assay was performed. It therefore comprises 442 definite TSpot results plus 12 borderline results, plus 11 indeterminate assays. To make this clearer to the reader the sentence has been rephrased as follows: [page 7 line 2]

Full clinical data were available and IGRA performed on 465 staff and patients.

8. In the next passage of the results you report a number of 34 positive IGRA results, please report also a proportion. This number of 34 is extrapolated from the observed data to estimate the total excess number of positive IGRA results would be observed amongst the 379 live individuals (142 staff, 237 patients) we identified as having known exposure as a result of the outbreak. As requested we provide the numerator to complete the proportion, but have also rephrased the sentence to clarify explicitly what this proportion represents, as follows. Furthermore in correcting this sentence we have observed a rounding error in our calculation. The proportion is more accurately described as 35/379 rather than 34/379 [page 7 line 15]

From these data we estimate an additional 35/379 living staff (n=142) and patients (n=237) known to be exposed during this outbreak, would test positive by IGRA, if full data were available. These excess positive IGRAs probably reflect recently-acquired LTBI.

9. Also in the next passage you write “unexposed individuals”. Are these both staff and patients? Please describe this in more detail. These are patients only. Staff were excluded as they would decrease the generalizability of the findings. Therefore we have replaced the term “unexposed individuals” with the term “unexposed patients” in the first and last sentences of this paragraph. [page 7 line 19]

10. Please check Table 2 carefully. In the line “Country of birth” you report that 136 participants were TSpot negative but UK born are 126 and other are 9 (n=135).
We thank the reviewer for spotting this error. The correct numbers are UK born 126 and other 10 (n=136). The table including the relevant percentage has been corrected. [page 19 line 12]

11. The most interesting part of the paper is the description of the prevalence of positive IGRA in an unexposed population as those data are sparse so far. We agree, and hope this will be communicated as the primary focus of the paper.

Referee 3

Hinks and coworkers try to investigate a relevant clinical and epidemiological problem in the TB field: the background rate of positive INF-gamma assays in a low prevalence country.

The manuscript is well written, concise and report very interesting results.

Major compulsory Revisions

1. Figure 1: my personal suggestion is to add only borderline in the figure; it sounds little be confusing write 3+ 1-, you might be discussed this in the results session.
We appreciate this suggestion and have simplified the figure to state only the total number of borderline cases in each group. The breakdown of borderline + and borderline – has been moved to footnotes in the figure legend. [page 16 line 10]

2. Table 1: it seems better if you add the ethnicity for the Exposed Staff group, why not?
Unfortunately staff data were obtained through the occupational health department who would not release these ethnicity data to us to ensure staff confidentiality.

3. Pag 5 line 5-6: It seems reasonable if you add a possible explanation for the 10 indeterminate results with IFN-gamma assays in the unexposed patients group, e.g. association with immunosuppressive conditions or treatment or whatever else?

The reason was predominantly due to low sample volume in these subjects due to inexperienced phlebotomists not ensuring the tubes were adequately filled. That is to say this is an operational problem with the test method, rather than due to any immunological difference between groups. This has been clarified by rephrasing the sentence as follows: [page 7 line 9]
Overall 11 assays were indeterminate because of insufficient cells (n=9) – predominantly due to under-filled tubes – or high background in the wells (n=2).

4. Pag 5 line 9: why you estimate 34 positive IGRA results attributable to exposure to the index case and not 44 as I understand in Table 1 or I confused data?

This was discussed above in answer to referee 2 point 8, and the necessary clarification has been made.

5. Pag. 5 line 14: check OR for IGRA-positivity associated with prior TB treatment in individuals without a recent exposure, you report OR 12 in Table 2.

As stated in the text, the odds ratios presented in the text are positive results from multivariate analyses, as these provide the most robust estimates of the effect size. The Table 2 presents univariate analyses, as stated in the table header, which have the advantage that data on each individual variable can be presented in isolation, along with a relevant specific P value, even for the non-significant factors.

6. The results should be discussed with some criticism; particularly, what about 9.5% positive IFN-gamma test in the group of unexposed Staff? Please, add in the discussion your explanation for this relevant data.

As suggested, we have inserted the following additional paragraph of discussion. [page 9 line 16]

The 9.5% rate of IGRA-positivity amongst the unexposed staff is consistent with a rate of 9.9% observed by Schablon et al in their large study of healthcare workers in Germany. [4] These authors observed associations between IGRA positivity and older age, foreign birth and prior personal history of TB. In our study the IGRA positive, unexposed staff were neither foreign born nor had a personal history of TB, but were both in their 6th decade. It seems likely that relatively high rates of IGRA-positivity in healthcare workers may be due to unrecognised occupational exposure to MTB, particularly in older workers whose exposure may predate current infection control procedures.

About a hypothetical positive IFN-gamma results caused by M. marinum or M. kansasii or M. szulgai, it seems to me not so arguable, you might to be discussed better.

The antigens on which the TSpot.TB assay is predicated, ESAT-6 and CFP-10, are well known to be expressed in a number of other mycobacteria of the mycobacterium-tuberculosis-complex including M.marinum, M. kansasii, MSzulgai, M Africanum, M.flavescens, Mbovis and M gastrii (Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Andersen P: Evidence for occurrence of the ESAT-6 protein in Mycobacterium tuberculosis and virulent Mycobacterium bovis and for its absence in Mycobacterium bovis BCG. Infect Immun 1996, 64(1):16-22.). In comparison with IGRA studies in high risk populations, where such infections would be comparatively rare compared with MTB, immune sensitisation to such rare organisms would be expected to be more common in low prevalence populations. Indeed in the Wessex region approximately 50% of the clinical workload of the TB service consists of the treatment of opportunistic mycobacteria. In the absence of any gold standard test with which to compare the TSpot assay it would currently be impossible to refute this hypothesis. We therefore maintain this opinion, but, as requested, argue it further in the following revised and expanded text, which includes insertion of the reference to the paper by Harboe et al. [page 10 line 16]

Alternatively, as early secretory antigenic target 6 and culture filtrate protein 10 are expressed by other opportunistic mycobacteria, [14] and indeed QuantIFERON responses have been observed with M.marinum, M.kansasii, and M.szulgai[12] some positive IGRA results might be attributable to other opportunistic mycobacteria,

7. References. please check more recent references.

We have checked through the references. The majority of these articles have been published within the last five years or are presenting data which have not been superseded (Syed 1996, Davies 1999, Vynnycky 1997, Harboe 1996).

The population estimates by ethnic group date from 2009, but these are based on the UK census which is only repeated every 10 years. No more recent data are available. The national survey described in Rose 2001 has also not been repeated, so no more recent data are available. As noted in the introduction, there are a paucity of data from UK on rates of latent TB infection.

Whilst the Health Protection Agency Annual report on tuberculosis surveillance in the UK dates from 2010, it is the best reference available. Since 2010 the remit of the HPA report has been reduced and it is now only a third of the length, and no longer reports on the data cited. Again, no more recent data are
available. Furthermore, as the outbreak occurred in the period 2009-2010 it is arguably the most relevant report.

Minor Essential Revisions

1. Pag. 2 line 16-17, Results:
The referee has not specified their concern but we suspect they are referring to a lack of explanation regarding the numbers in brackets. These represent 95% confidence intervals, and the line has been amended to include this. [page 8 line 1]

2. Figure 1: check box 66 Died prior to testing.
This number is correct: there were many frail, elderly patients or those with end stage COPD, lung cancer and interstitial fibrosis on the ward, and 15 months elapsed between the first exposure and the end of the screening.

3. Pag. 5 line 5-6: " in the negative wells" or simply "high background" instead of high background in the wells (n=2)
The sentence has been amended to say simply “high background”, as requested. [page 7 line 11]

Referee 4

Minor revisions:

please, explain more widely the role of steroids in determing the positivity of IGRA results, in page 5, line 14.
The following paragraph has been added to the discussion to address this suggestion. [page 9 line 12]

Rates of IGRA-positivity amongst unexposed controls were higher in subjects receiving long term iatrogenic immunosuppression (OR 5.9). This would be consistent with the known increased risk of acquiring MTB-complex infection due to impairment of cell-mediated immunity induced by therapy with corticosteroids or with anti-TNFα therapy.[8]

methods must appear before paragraph of results
The methods section has been moved as requested.

conclusions should be wider
As requested we have broadened the conclusions by inserting the following two sentences: [page 12 line 3]

As this region has the lowest UK incidence of active TB, this figure may represent a current minimum UK background prevalence of LTBI. These data will aid interpretation of future outbreak studies. They will also inform cost-benefit analyses which may be sensitive to assumed background rates of LTBI.

finally, among the references, it’s useful to include the paper:
“Prevention of tuberculosis in patients taking tumor necrosis factor-alpha blockers.”
by Bellofiore B, Matarese A, Balato N, Gaudiello F, Scarpa R, Atteno M, Bocchino M, Sanduzzi A.
J Rheumatol Suppl. 2009 Aug;83:76-7, talking about the unexposed patients , as a possible role of immunosuppression caused by tumor necrosis factor-alpha blockers.
The reviewers suggested reference has been added as requested, in the additional paragraph discussing the role of steroids. [page 9 line 15]
References
