Author's response to reviews

Title: Comparison of Two Interferon Gamma Release Assays in the diagnosis of Mycobacterium tuberculosis infection and disease in The Gambia

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Author's response to reviews: see over
Dr. J.A. Le Good  
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Dear Dr J. A. Le Good,

**Response to reviewers’ reports: Comparison of Two Interferon Gamma Release Assays in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia**

Thank you for considering our manuscript for publication and the feedback by way of reviewers’ comments.

Please find below, a description of our responses to the reviewers’ comments and revisions we have made to the manuscript.

**Reviewer Jae-Joon Yim**

This study performed in-house ELISPOT instead of commercialized T.SPOT.TB and QTF-in tube rather than QTF-TB Gold assay. Given that large body of data is available from T.SPOT TB assay kit and QTF-TB Gold assay rather than QTF-in tube, the data in this manuscript could be varied because of the tests method.

The In-tube test format is actually the 3rd generation test compared to the 2nd generation QFT-TB Gold. Most ELISPOT papers from the Oxford group, linked to the T-spot commercial assay, have utilised their in-house assay and we have published extensively using our in-house assay too. More recently studies are being published using the T-spot commercial assay. Indeed, we are currently assessing the two commercial kits as our next study. We mention this in the discussion towards the end of the 2nd paragraph.

The observation that ELISPOT is more accurate than QTF-TB assay in terms of diagnosis of TB disease. It is different from previous publication (Kang YA et al. Chest 2007, In press) The authors can refer this paper and may discuss about the previous reports on the comparison of two IGRAS.

We are aware of the findings in the Kang YA, et al paper. We have included it in our discussion (paragraph 2 of our discussion, reference 23) along with other references.
In results part of abstract, the authors mention that ‘significant discordance between the IGRAs and TST, mostly from IGRA positive and TST negative combinations’. However, 24 had TST positive / ELISPOT negative and 40 had TST negative / ELISPOT positive. I don’t think, the author can say ‘mostly’.

We agree and have struck out the word ‘mostly’.

It could be much easier for readers to understand this manuscript, if the authors present the results of two IGRA and TST in a table format.

It is our understanding that table 2 already shows what is suggested. We do note that selection of subjects by Mantoux status in the sampling process is described in the methods- we show why this is primarily a comparison of the two T cell assays with each other.

Figure 2 and table 2 contains same information, essentially. The authors can omit one of them.

Figure 2 has been removed.

Reviewer Andrew Vernon

Abstract:
The statement that IGRA/TST discordance was “mostly from IGRA positive and TST negative combinations” seems a bit misleading, since every type of discordance occurred in significant numbers. I would remove this phrase.

We have removed this phrase
The abstract might include a statement that significant discordance among the two IGRAs and the TST remain largely unexplained.

The abstract has been revised to incorporate this suggestion.

Methods:
The reason for limiting contacts to those over 15 years of age is unclear. Why was this done?

This was in interlocking study. The overarching study looking at surrogate markers of treatment efficacy was approved for household contacts 15 years and over. Therefore this study was necessarily restricted to this age group. This is now made clear in the methods section: paragraph 2.

Persons with TB diagnosed within one month of recruitment were excluded. How many were these? Were they included as TB cases?

There were none. Only 2 contacts progressed to TB after recruitment. These contacts are not included as TB cases. This information is now found in the results section, paragraph 1.

IGRA testing was done one month after the TST. The discussion incorrectly states that IGRAs are not subject to boosting. The IGRA will not cause boosting,
because no antigen is exhibited to the patient by the IGRA. However, an earlier TST might affect subsequent IGRA results, and the cited CDC guidelines state this as a possibility for which no conclusive data are yet available. There is at least one recent report on this issue (Naseer, Europ Resp J 2007; 29(6): 1282-3).

We agree there was a theoretical possibility of boosting at the time the study was conducted. There have since been the reports by Igari at al (ref 36), Leyten et al( ref 37) and Naseer et al (ref 38) suggesting this can happen. We think the evidence regarding boosting of IGRAs by TST is still inconclusive and will require more work. We have amended the discussion (paragraph 6) to make this point.

The Elispot is an in-house test. How do the peptides used compare to the commercial product of Oxford Immunotec? How does the spot counting criteria compare to what is done with the commercial product?

Our understanding is that the peptides are essentially the same as those used by the in-house and commercial Oxford assay, made up by SSI (Denmark) as theirs are. Indeed, our assay was established by a former Oxford employee who worked with ELISPOT in Oxford. The spot counting criteria we used are based on our published study where we used mathematical tools to identify a cut-off (Jeffries et al Int J Tuberc Lung Dis 2006; 10:192-198). We are not aware of any publication justifying the cut-offs that have been used for the Oxford assays. Note, there is in reality very little difference in the results if one does use the 6 spot cut-off (T-spot).

We have not made any changes to the manuscript in relation to these points.

The QFT-GIT had no positive control. I believe that the manufacturer now includes a positive control with the test. This should be noted if correct.

The lack of a positive control and its likely effect on interpreting our data is acknowledged in the discussion. The provision of a positive control tube by the manufacturers and its use in our current studies is stated in the discussion (paragraph 2).

How was household clustering taken into account in the analysis?

We performed random effects logistic regression to account for clustering by household.

Results:
The percentages in the 2nd paragraph seem in error. They should be corrected or explained.
This has been corrected.

The different types of discordance among the contacts is of considerable interest. Tables should compare the results of each IGRA with the TST, and compare the results of the two IGRAs. These could be provided as an appendix or an online data supplement for interested readers.

We have chosen to present the different types of discordance as a figure. Figure 2 shows the discordance in contacts.
Discussion:
The senior author published in 2004 a paper with a figure very similar to Figure However, the absolute values of % positive were in the 40’s rather than the 60’s. That study included children, I believe. The authors should explain why their results now differ in absolute value (although the trends are similar).

The absolute figures here are higher because the study population was restricted to contacts aged 15 years and more. This group tends to have higher positive rates compared to children <15 years with rates from 21%-32% in our population. We have not made any changes to the manuscript on this point.

The authors comment on the absence of an association with BCG. They could note that the exclusion of children meant that no person had received BCG less than 15 years earlier. This time differential, and the lack of prior skin testing as an anamnestic, might be one explanation for the lack of association of discordance with BCG. Another reason might be that scar is not a robust differentiator between persons with and without response to or receipt of BCG.

We agree that there are various possibilities for this lack of association. We have consistently reproduced this absence of an association with BCG in all age groups including those <15 years (Hill et al. Pediatrics 2006:117; 1542-1548). Therefore we have made no additional changes to the manuscript.

Tables and Figures:
Why does Figure 2 not show the TST results? These should be added.

As suggested by the other reviewer, we have removed figure 2 and retained table 2.

MINOR:
Typo page 10, line 4: “may not BE as sensitive....”
Typo page 11, line 22: “…once AN individual is infected....”

Thank you for pointing these out. They have been corrected.
Reviewer Claudio Fortis

Page 5, 1st paragraph: from "Negative control wells..." the cut off reported is very high and unusual, how has been calculated?

This cut-off for the negative control wells and in fact our in-house ELISPOT technique is the same as in Scarpellini P, et al (J Clin Microbiol 2004;42: 3469-3474). We reference our paper describing our cut-offs. (ref.12)

Page 7, 2nd and 3rd paragraphs: should be better to report the data in a 2x2 table or in a flowchart.

We have produced 2 flow charts (figures 1 and 2) to report most of these data.

Page 7, 3rd paragraph: from "there was no significant discordance". I am not convinced of this sentence (25% QFT-GIT positive/ELISPOT negative and 24% ELISPOT positive/QFT-GIT negative)?

The figures for discordance quoted are correct but the statistical test for discordance is not significant. We have recalculate this using STATA and confirm that our result is indeed correct.

We have not made any additional changes to the manuscript with respect to this point.

Figure 2 is redundant respect to table 2 and should be eliminated.

Figure 2 has been eliminated.

The lack of a positive control for QFT-GIT could effectively lead to false negative results.

We have stated this as a possible limitation (discussion paragraph 2)

The discordant results found with the two IGRAs are a new and unknown observation that should be better discussed. Technical problems like an unusual cut off for positive ELISPOT results or the absence of a positive control for QFT-GIT should be considered.

The phenomenon of discordant results with the 2 IGRAs has also been reported with the commercial assays (Ferrara et al in Lancet 2007; 367: 1328-1334; Lee et al in Eur Respir J 2006; 28: 24-30; Arend et al in Am J Respir Crit Care Med. 2007 Mar 15;175(6):618-27). This discordance may be possibly be related to test formats and inclusion of an additional antigen in QFT. Unfortunately, this phenomenon is still poorly understood.

We have expanded our discussion on discordant results in the manuscript (discussion, paragraphs 4 and 5)

Page 11, 1st paragraph: from "IGRAs are not subject to boosting", however some recent reports (Naseer et al., Eur Respir J 2007, 29:1282 and Igari et al., Int J Tuberc Lung Dis 2007,11: 788) reported of a booster phenomenon of QFT-G after prior TST administration (6 months and one month before, respectively).

We agree there was a theoretical possibility of boosting at the time the study was conducted. There have since been the reports by Igari at al (ref 36), Leyten et al( ref
36) and Naseer et al (ref 37) suggesting this can happen. We think the evidence regarding boosting of IGRAs by TST is still inconclusive and will require more work. We have amended the discussion (paragraph 6) to make this point.

Page 3, end of the 1st paragraph: from "the need for 2 patient visit" is not a confounding factor and the sentence should be rephrased.

The sentence has been rephrased.

Page 5, 1st paragraph: from “The ELISPOT was considered...” sentence not intelligible, should be rephrased.

The sentence has been rephrased.

Page 7, 1st paragraph: from "Just over 60%...", sentence not clear, should be rephrased as "Just 60% of the household contacts were female while 60% of cases were male".

Thank you, the sentence has been rephrased.

Page 7, 2nd and 3rd paragraphs: could the authors better define “test failure”? it is referred to undetermined results or to technical problems or what?

The numbers of failed tests and the reasons for the classification have been included in the results (paragraphs 2 and 3)

Page 8, 3rd paragraph: from "When a positive TST..." is referred to all subjects or to contacts only?

The sentence has been rephrased and now makes it clear contacts were being referred to.

Page 10, 4th paragraph: for what concern the discordance between the TST and the IGRAs, the sentence "is most often due to negative TST and positive IGRAs results" should be true for active TB, but not for LTBI.

The sentence has been rephrased (discussion, paragraph 4).

Yours sincerely

Ifedayo Adetifa
Philip Hill
On behalf of the authors