Author's response to reviews

Title: QuantiFERON-TB-Gold In-Tube test conversions and reversions among Tuberculosis patients and their household contacts in Addis Ababa: a one year follow-up study

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Author's response to reviews: see over
Dear Editor,

On behalf of the authors of this manuscript, I would like to thank you for getting our manuscript reviewed in such a short time span. I would like to extend my appreciation to the reviewers for their critical comments that improved our manuscript. We have addressed their comments and concerns point-by-point below (reviewers’ comments in light blue color is followed by responses in black color).

We hope the manuscript has been improved as suggested and we look forward for your response.

Reviewer #1 (Melissa Nyendak)

Minor revisions

1. Line 80: Authors might refer to the work done by Hill et al. in the Gambia; although a different population, they did study conversions/reversions in 2006 (I believe Aiken et al.)

   Response: This has been included (reference 25)

2. Line 112: I believe it is McNemar - not MacNemar - also might state that this was a univariate analysis only

   Response: This error has been corrected

3. Results: 1st paragraph: if a table is not included given baseline characteristics would separate the patients and the contacts (eg mean age for patients and gender and then mean age for contacts and gender)- might also be interesting to know on average how long was patient symptomatic before coming to care

   Response: This is now corrected as suggested

4. Lines 142/143, same comment on McNemar vs MacNemar

   Response: This error has been corrected
5. Line 169, typo - change Fore to For (same comment for line 199)
Response: This error has been corrected

6. Might consider in the discussion talking about the large # of QFT positive contacts at baseline which gets at prior exposure in a TB endemic country. However, your study did show that among qft negative persons, there was conversions, suggesting that this test may be of use in this subgroup. You did have two qft negative people that progressed - you might have caught them in the window period.
Response: Prior exposure and its contribution towards high prevalence of latent TB among contacts has now been high lightened (Lines 191-198).

7. Line 162 Also some recent publications to suggest significant amount of exposure may occur outside the house (andrews JID 2014)
Response: We have unpublished data revealing a high prevalence of latent TB infection among adults with no history of household contacts suggesting exposure outside the house has in fact major contribution to the prevalence of latent TB infection. However, the additional prevalence beyond the background in the community is probably due to recent infection as a result of household contact. This has been included in the discussion section. (Lines 191-198)

Reviewer #2 (Lisa Pascopella)

Major compulsory revisions:

1. Can the authors provide the numeric test results (i.e. IU/ml of interferon gamma) for each study subject that demonstrated QFT-GIT conversion or reversion? Although these are presented in Figures 1, 2 in aggregate, it is important to see how each value changed from baseline to the one year follow-up time point. (Figure 3 shows these in graph form for only the subset of subjects with negative QFT-GIT at baseline. This figure could be improved by labeling each point (because it is difficult to differentiate values between 0 and 1, e.g. around the 0.35 cut off point), or, could be replaced with a table that provides values for each subject at baseline and at one year. If many of the conversions are around the 0.35 cut off point, how would this finding impact the authors’ interpretation of these conversions?
Response: Figure 3 was replaced with Table 2. Table 2 contains IFN-γ levels at baseline and at 12 months for both converters and non-converters. As can be seen, 9/11 converters had IFNg above 1 IU/ml) whereas two had just above 0.5 indicating they are most likely positives and not related to the sources of variability...

2. Can the authors provide further methodological detail, specifically related to potential sources of known variability of the QFT-GIT test? (e.g. how was the process from phlebotomy to readout quality-controlled? Was blood volume standardized or could it have differed by at least 0.2 ml from subject to subject and/or time to time?

Response: The blood collection, sample transport and processing, incubation, supernatant storage and ELISA were all done following pre-prepared standard operating procedure. The tubes have marks indicating 1 ml and blood volume was checked for sample to make sure the recommended amount is added in each tube. All samples were incubated within 6 hours of collection and the incubation time was at 24 hours consistently although the company recommends 16-24 hours of incubation. Supernatants were stored at -80oc until ELISA was done. ELISA was done following the company’s recommendations. We assess the negative and positive controls (PHA stimulated samples); besides, the software provided by the company checks the validity of each run and invalid results were repeated (This was further explained in the methods part (Lines 121-124).

3. If the authors can address above-mentioned questions and points, the conclusions need a bit of softening, because the study findings are suggestive, but not definitive. For example, line 52, “repeated screening of QFT negative contacts may be (rather than is) needed to diagnose TB infection in a TB endemic setting.”

Response: We have revised the conclusions as suggested.

Minor Essential Revisions:

1. Grammar and spelling need to be reviewed and corrected/made consistent throughout the document.

Response: Manuscript check for grammar and spelling errors.
2. Further detail is needed to describe the timing of QFT-GIT testing in relation to TB diagnosis and assessment of contacts (e.g. rule-out of active TB disease). Following diagnosis of TB among patients, contacts were invited and included in the study. It took 1 to 7 days to collect sample from contacts following diagnosis of TB among patients (Lines 104-106). Clinical assessment and chest x-ray were used for all contacts to screen for TB. Sputum smear microscopy and culture were done for those contacts with productive cough.

   How was active TB disease confirmed (with culture or only with smear and clinical signs/symptoms?)

   Diagnosis of TB among patients was further explained in the methods section: Clinical assessment, sputum smear microscopy and culture were used for TB diagnosis in all patients (Lines 98-101).

3. How were contacts assessed for exposure- was it self-reported exposure to the index case living within same household?

   Yes, it was self-report. We didn’t have any other means to assess their exposure. However, both patients and contacts had confirmed that contacts were living in the same household while the patient was sick.

   Did the contact report sleeping in same room as index case?

   Not all contacts shared the same bed but in other preliminary analysis of the mother project data, sleeping together doesn’t seem to influence infection contrary to previous reports.

   Were number of hours of exposure to the index case within the home determined? Yes, we have determined the average number of hours contacts had been with the patient every day. The median number of hours contacts had been exposed in the house was 12 (IQR: 9-12 hours).

4. Were all study subjects tested for HIV infection, or, was HIV-negative status based on self-report?

   We have tested all participants for HIV infection and this is further explained in the methods section (Lines 107-108).

5. Please provide further detail about what occurred during the one year follow up (in addition to blood draw for QFT-GIT test). Were subjects assessed for TB/TB medication side effects during the interim?
Patients were collecting their anti-TB medications every day for the first 2 months and then every week for one month and every 2 weeks in the fourth month but thereafter, they were collecting medications every month for 2 months. During these visits, patients were clinically assessed for any serious side effects as well as other concurrent illnesses. None of the patients had either serious side effects or major illness. Some with minor medical illnesses were treated.

On the other hand, contacts were informed to report any illness in between their appointments. They were assessed at baseline, 6 and 12 months for any infection including TB. (Line 107-109)

6. Was one year actually 12 months, or, was it 12 months plus or minus 2 weeks, or a month or?
   It was at 12 months+ a few days (< 1 week)

7. Line 149. The data in Table 1 shows that 10 contacts had conversions, but the sentence says “11.” Which number is correct?
   The number of contacts who converted is 11 and Table 1 is also right. We now have explained Table 1 to avoid confusion. One contact who was positive at baseline became negative at 12 months and therefore, instead of 61 positives, we have 60 at 12 months. Please see the legend for Table 1.

8. Consider summarizing the findings with numerical data (e.g. lines 156 and 157, add percentages of reversions and conversions in parentheses)
   We now have included these points in the results section (Lines 166-169).

9. Line 159 -160. Consider providing the range of prevalences/percentages of latent TB from similar studies.
   Response: This was included in the manuscript as suggested (Line 195)

10. Lines 175-178. Consider adding the estimate of the TB rate in Ethiopia to further bolster the point that multiple exposures to TB were expected during the study timeframe.
    Response: we have included the estimated prevalence of smear positive pulmonary TB from a recent national survey in Ethiopia (Lines 217-219).

11. Lines 182-183. But the specific antigens in QFT-GIT exclude most environmental mycobacteria. Are there data that suggest that M. marinum,
which cross-reacts, is the main environmental bacterium to which Ethiopians would be exposed?

**Response:** Although we have previously found a significant proportion of strains isolated from Afar Region to be environmental mycobacteria (data submitted), further genetic studies are not done yet and we are not sure if *M. marinum* or other environmental mycobacteria are the predominant ones. Therefore, we can’t rule out such possibilities although we don’t have strong evidence in favor of our hypothesis. We have removed the statement to avoid speculative explanations.

12. Line 188. Suggest caution when using term “immunity.” QFT-GIT measures one component of the immune response; interferon gamma’s role in “protective immunity” is not clear.

**Response:** This is now replaced with IFNg-γ to avoid confusion

13. Lines 205-212. Study limitations will need to be expanded to include how the known sources of variability in QFT-GIT may impact the study findings (see above).

**Response:** This has now been included although we believe the impacts of such sources of variability are minimal as discussed in number 12.

Kind regards,

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