Author's response to reviews

Title: Higher blood volumes improve the sensitivity of direct PCR diagnosis of Mycobacterium tuberculosis sepsis among HIV-AIDS Patients: An observation study

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Author's response to reviews: see over
RESPONSE TO REVIEWER’S COMMENTS

Date: 7th January 2015

Respondent/Submitter: Freddie Bwanga

RESPONSE TO COMMENTS FROM REVIEWER 1: COLLEEN KRAFT

Major Compulsory Revisions

Overall

Comment: Overall: Bwanga et al describe their study in which they used increased blood volumes in PCR to diagnose bloodstream infection with MTB in HIV-infected patients in Uganda. To begin with, this article is framed in a way that is very confusing to the reader. In general, HIV-infected patients can have “mycobacteremia” from several different types of Mycobacterium species. I think that this term should be stricken from this article, because it is confusing. It has nothing to do with mycobacteremia, but it has to do with MTB sepsis or blood stream infection.

Response: The writing in the entire manuscript has now been presented in simple non-confusing language. The term “mycobacteremia” has been replaced with the term Mycobacterium tuberculosis (MTB) sepsis throughout the manuscript.

Comment: Also, it seems as if both studies were essentially quality improvement type studies in a diagnostic test, and I think that the manuscript over-reaches what is a simple technique of enhancement of PCR sensitivity.

Response: Well, the theme of the study was actually to improve the sensitivity of PCR for diagnosis of MTB sepsis on whole blood. This could sound simple but in light of how many HIV/AIDS people die of MTB Sepsis in Africa (30-days mortality rate of 50%), and many before a diagnosis is even made, the impact of these study findings on future early diagnosis and timely initiation of therapy is potentially big.

Abstract

Abstract Comment 1: It takes until the conclusion to understand that this only has to do with MTB and nothing else. This is very confusing. Also, I think the term Mycobacteremia should be removed, but if it is used, it should not be capitalized.

Response: The abstract has been re-written and the term "mycobacteremia" has been replaced with the term Mycobacterium tuberculosis (MTB) sepsis throughout the manuscript.

Abstract Comment 2: In the methods, it should say whether this is retrospective or prospective.

Response: The study was prospective, and this has been indicated in the revised abstract of the manuscript.

Abstract Comment 3: In the results, it does not say what the diagnostic sensitivities refer.

Response: Diagnostic sensitivities refer to the FluoroType® MTB PCR assay when applied on DNA extracts from small and large blood volumes. This now corrected in the results section of the abstract.
**Abstract Comment 4:** Again, the conclusion seems to come from nowhere, and if this is the conclusion of the paper, it needs to be set up less like a clinical study, and really just as a diagnostic paper.

**Response:** The conclusion has been re-stated to emphasize the main finding of the study, and to show it comes from the results obtained.

**Background:**

**Comment** Line 69: I think the term fast growing is too colloquial-I think just bacteria or standard bacteria

**Response:** Sentence has been deleted from the manuscript.

**Comment** Line 74: instead of mycobacteremia, I would use MTB sepsis as the term throughout the manuscript.

**Response:** The term “mycobacteremia” has been replaced with the term *Mycobacterium tuberculosis* (MTB) sepsis throughout the manuscript.

**Comment** Paragraph starting with line 77: This really sets up the whole article, and I would just stick to this aspect, instead of trying to also have a clinical question.

**Response:** Thank you, and this has been adopted.

**Methods:**

**Comment** Line 99: cross-sectional studies are not conducted over a 2 year period. Is this a convenient sample? Is this prospective? I believe this is a prospective study with batched samples at the end?

**Response:** The study design and the study periods for each of the sub studies A and B are now clearly shown under study design. It was a prospective and this has been indicated too

**Comment** Line 106: would say infection instead of infected.

**Response:** Corrected to HIV-infection

I do not understand the value of the MTB sputum except to place the bloodstream infections in context.

**Response:** Initially we thought it would strengthen the diagnosis of MTB sepsis, but we have now opted to remove from the entire manuscript all information on sputum.

**Comment** Line 119: what is MBN?

**Response:** MBN is a noun, *i.e.* a name of a laboratory entity in Kampala. See www.mbnlab.com

**Results:**

**Comment** Line 175: statements like these belong in the discussion, and makes no sense unless it has been tested- inferences do not belong in the results, and should be tempered with references in the discussion.

**Response:** the statement “It should be put into consideration that *M. tuberculosis* hosts in general multiple copies of IS 1610 detected in the FT MTB test. With TB instead of *M. bovis BCG* an even higher analytical sensitivity could be expected” has been removed from the results section.

**Comment** Line 193: Are A10 and B10 the same individual? I am not sure how someone can be in both study A and B.

**Response:** A10 and B10 were NOT the same individual. The study code 10 is just coincidental. A correction in that sentence has been done.
Comment Line 222: I do not understand why this is not mentioned in the results, and only in the discussion?
Response: the diagnostic sensitivity and specificity of the FluoroType® MTB PCR assay in studies A and B have now been included in the results section.

Comment Line 228: same, why is this in the discussion, and is this mixed infection diagnosed by culture?
Response: A correction in information about the SepsiTest has been done. Since this test was not our primary target, only one sentence has been left in the text to emphasize the point of higher blood volumes being necessary for obtaining adequate template DNA. Mixed infection was not diagnosed with culture but with the SepsiTest.

Comment Line 256: I think it is important to clarify, that while this takes 4.5 hours, it was performed in Germany, and in this case, not in a timeframe to make clinical decisions, since they were batched and stored?
Response: That is true; and we have included this statement about FT testing in Germany, and also added that these real-time PCR assays are now available in Uganda and being used for similar assays here, thus it is possible to have the results in the 4.5 hours even in Uganda.

Comment Table 3: what is the diagnostic sensitivity compared to? Culture? These tables need to stand alone.
Response: Corrections effected, see the new table 3.

Level of interest: An article of limited interest
Response: It can be very difficult to appreciate the interest in this article unless one works in HIV prevalent settings where MTB sepsis patients die at 50% rate within 30 days of making a diagnosis, which definitely comes too late!

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests
RESPONSE TO REVIEWER 2: David Holland

Title: Higher Blood Volumes Improve the Sensitivity of Direct PCR Diagnosis of Mycobacteraemia among HIV-AIDS Patients: An Observation Study

Comment: The article by Bwanga et al basically compares two blood volumes for detection of MTB in whole blood using PCR. While the methods and conclusion are of some interest, the article needs some work. In general the analysis was appropriate, but the authors could improve the article significantly by consulting with a clinical trials expert for a description of the methods used.
Response: We have markedly improved on the description of the methods used.

Abstract
Comment 1. Abstract: This needs major reworking, as it is not at all clear from the methods section what was done.
Response: The abstract has been re-written in a more clearer and easy to read format.

Comment: 2. It appears that the two "studies" were in fact two convenience samples as part of a QA project. If indeed this is what occurred, it would be fine, but it should be specifically stated. If subjects were, in fact, recruited prospectively for a comparative trial, why were not both methods tested on the same subjects (which would have strengthened the results)?
Response: The study prospectively conducted. It was preceded by development of a clear study protocol, which was IRB approved on using 1 ml of blood. After getting low sensitivity, we decided to explore how sensitivity would change if we increased the blood volume to 9 mls. We again resubmitted the new protocol for IRB approval. Therefore, it was not possible to use the same patient subjects in the two studies, but we acknowledge that this would have made the study stronger.

Comment: 3. Lines 170-174: Should be in methods
Response: Corrections were done. See new manuscript

Comment: 4. Lines 175: Should be in discussion.
Response: Corrections effected.

5. Line 228: Should be in results
Response: On that line, the sensitivity of only 21 % is referring to the Cepheid Xpert MTB/RIF testing brought in here for the purpose of comparative discussion. Cepheid Xpert MTB/RIF was not used in the current study; would it be good to place its results in the results section of the manuscript?

Level of interest: An article of limited interest
Response: It can be very difficult to appreciate the interest in this article unless one works in HIV prevalent settings where MTB sepsis patients die at 50% rate within 30 days of making a diagnosis, which definitely comes too late!

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: 'I declare that I have no competing interests.