Author’s response to reviews

Title: Performance of Loop-Mediated Isothermal Amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda

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Author’s response to reviews:

The Editor,

BMC Infectious Diseases

Dear Sir/Madam,

Re: INFD-D-17-01139: Performance of Loop-Mediated Isothermal Amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda

On behalf of the co-authors, I wish to thank you and the academic editor for allowing revision and resubmission of this manuscript titled, “Performance of Loop-Mediated Isothermal Amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda”. We are also highly indebted to the reviewers for their excellent comments and suggestions on how to improve this manuscript. We have carefully considered each of the reviewers’ comments and have revised the manuscript to address their concerns. This
process has strengthened the manuscript, and we look forward to your team’s further consideration of our work for publication in BMC Infectious Diseases.

One of the main concerns from the editor was the incorrect use of the term ‘accuracy’ in the manuscript and the missing sub-analysis on bacillary burden especially from smear microscopy. We have addressed this and the sub-analysis results have been included and discussed in the revised version of the manuscript.

Kindly find a point by point response to each of the reviewers’ comments highlighted blue and the revised manuscript. We believe that we have submitted a better version following revision. I will be happy to answer promptly any issues concerning this revision.

Sincerely,

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POINT BY POINT RESPONSE TO REVIEWERS COMMENTS

Editor Comments:

Although a comparison of diagnostic screening performance is essential in evaluating new laboratory tests. The authors fail to understand the term "accuracy" which when used as a measure of screening performance means the weighted average of the sensitivity and specificity. [See Alberg AJ et al. J Gen Intern Med 2004 May 19(5 Pt 1) 460-465]. The authors use the term "accuracy" often but incorrectly throughout the manuscript which takes away from the impact of the manuscript.

Response: Thank you for the guidance. The incorrect use of the term ‘accuracy’ has been noted and the manuscript has been revised to correct the use of the term. The revised manuscript specifies the performance measures i.e. sensitivity, specificity or predictive values being referred to and these are now used appropriately throughout the manuscript.

In addition, there are multiple sub-analyses which were not done or overlooked including bacillary burden (also mentioned by Reviewer 1). This data should be available as smear microscopy (as stated) is the fundamental diagnostic TB screening test used by the rural laboratory in question.
It is hard for the readers to discern what the significance of the hospital setting means in terms of culture status. As this is a significant finding in the characteristics of comparing culture positive from culture negative is should be expanded on in greater detail. Although culture is a composite between liquid and or solid media growth it may be informative from a bacillary load stand-point and as such the authors may want to conduct a sub-analyses of this differentiation also. The smear results were positive in only 59% of the culture positive specimens (22/37) and 21/89 (24%) of the culture negative specimens, this relates to bacillary burden which should be mention and discussed.

Response

Thank you for this suggestion. The sub-analysis on bacillary burden has been performed and the revised manuscript includes results on bacillary load from smear microscopy and LJ culture (colonies) shown in Table 1 and Table 2.

Furthermore, a differentiation in settings (hospital vs health center) with regards to bacillary load has been detailed in Table 2. From this sub-analysis, we did not find a significant difference in bacillary load between the two settings but found a significant difference in bacillary load between TB LAMP positives and negatives, also shown in Table 2.

The discussion has been revised and included bacillary burden as an explanation for the low sensitivity of TB-LAMP found in our study as follows: ‘…. our findings showed that majority of the TB-LAMP positives also had high bacillary load on sputum smear (AFB 2+ and 3+) and LJ culture (greater than 20 colonies). It is therefore possible that TB-LAMP will only detect TB in specimens with high bacillary load but not low bacillary load specimens, thus the low sensitivity found in our study’ (Lines 338-342, discussion section).

Line 159: The PURE acronym means little to the reader unless explained or the acronym is defined.

Response: The PURE procedure for DNA extraction in TB-LAMP testing has been defined and referenced (Ref # 17). The manuscript section has been revised for clarity as follows: ‘The modified Eiken TB-LAMP assay which has a procedure for ultra-rapid DNA extraction (PURE-LAMP) has a simplified specimen processing methodology which simplifies the procedure….’ (Lines 164-166, TB-LAMP procedure section)

Line 210: This methodology should be referenced.

Response: The methodology on HIV testing in Uganda from the recommended National guidelines has been referenced (Ref # 19) as suggested. (Line 206)

Reviewer reports:

Paul Drain (Reviewer 1): Review for BMC Infectious Diseases
Title: Performance of Loop-Mediated Isothermal Amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda

The authors conducted a diagnostic accuracy study of TB-LAMP, a novel isothermal amplification assay of TB genomes, for accessible point-of-need testing for TB. Overall, among patients with TB symptoms, the diagnostic sensitivity and specificity of the test was limited, when compared to Tb culture. Overall, the test showed rather low sensitivity among those with smear-negative disease, which is often the majority of people who would benefit from Tb testing. The study is helpful by including a comparison to the GeneXpert, but is limited by not comparing TB-LAMP to the urine LAM assay.

Response: Thank you for this comment. We agree with the reviewer on the importance of comparison of TB-LAMP with the recently endorsed urine TB-LAM assay by the World Health Organization. Regrettably, at the time of this study, studies on performance of urine TB LAM had not been concluded and therefore, urine LAM was not performed for this study. We acknowledge this limitation and the limitation section has been revised as follows: ‘A strength to our study is the comparison of the TB-LAMP test with the routine tests such as smear microscopy and Xpert MTB/Rif test, however, we acknowledge the limitation that we did not compare the TB-LAMP to urine TB Lipoarabinomannan (LAM) test as at the time of the TB-LAMP diagnostic evaluation study, urine TB LAM test was only available in research settings’ (Lines 348-351, Discussion section).

The authors concluded that sample transportation reduced the sensitivity of the test. However, the sensitivity may be related to the bacillary burden of disease, which does not appear to have been measured in this study. I would suggest the authors remove this conclusion from their abstract.

Response: We agree with the reviewer that the sensitivity obtained in our study could have been influenced by the bacillary burden. A sub-analysis on bacillary burden quantified from LJ cultures and smear microscopy has been included (Table 1 and Table 2). The results have also been discussed as mentioned in editor’s comments above.

The conclusion that sample transportation reduced sensitivity has been revised in the resubmitted version of the manuscript.

The methodology was to use 'left-over expectorated sputum' which may have limited the sensitivity of the test. In addition, it appears that only 1 sputum samples was obtained. These limitations should be discussed in more detail in the Discussion.

Response: We agree with the aspects highlighted by the reviewer, which could have influenced the sensitivity of TB-LAMP test in our study. These have been noted and included as study limitations in the discussion section. We have revised the manuscript to include: ‘However, use of left-over expectorated sputum specimens could have reduced the quality of sputum tested thereby affecting our study results.’ (Lines 354-355, Discussion section).

Overall, this is a nice manuscript and I have no major suggested changes.
Response: Thank you

Vineel Reddy, Ph.D (Reviewer 2):

1. Earlier clinical studies and metaanalysis (Nagai et al 2016 Sci Rep) shows that LAMP test has a sensitivity close to 75-80%. Why this study has a sensitivity close to 62% even though the sample collection and testing is done in a Hospital (N=126) is unknown and the overall sensitivity has dropped to 55% (N=233). The low sensitivity of TB-LAMP results shown in this study are a bit concern. In addition the sensitivity of LAMP in smear negative (N=190) is also very low (24.4%) compared to previous studies.

Response: Thank you for pointing out the issue of low sensitivity. We also noted the lower sensitivity of the LAMP test in our study compared to what has been reported in some of the earlier studies. We attributed this to a number of factors that we have highlighted in the discussion. Among these factors are: The quality of sputum used, i.e. our study used left-over sputum for testing which could have affected the quality of sputum; secondly, transportation of samples from the health centers to the hospital for testing could have caused delays and affected the quality of sputum, resulting into low sensitivity; lastly, the low sensitivity could be attributed to low bacillary load in the samples used as this was largely an HIV-infected population.

All these possible explanations have been included in the discussion section which has also been edited to include those suggested by the reviewer. (Lines 320-342, Discussion section).

2. It is unclear as to whether using the TB-LAMP test has an advantage over smear microscopy in diagnosing TB in patients who are already infected with HIV? Do the authors check the comparison of LAMP with smear microscopy in HIV-TB patients also? HIV TB co-infection seems to be major concern in the area.

Response: Table 2 has summary results for the performance of TB-LAMP among HIV co-infected participants with sensitivity of TB LAMP among HIV co-infected of 52.3%, which is only slightly higher than that reported with smear microscopy (less than 50%).

However, based on our results, we would not recommend use of TB-LAMP test for diagnosis of TB among HIV co-infected individuals given the low sensitivity. The discussion has been revised and includes a discussion on use of TB-LAMP among HIV co-infected individuals as follows: ‘Given the low sensitivity, TB-LAMP may have limited use in the diagnosis of TB among HIV co-infected individuals’. (Lines 366-367, Recommendation section).

3. Request authors to include significant "p" values wherever required in text as well as tables.

Response: Thank you for the guidance, this has been noted and significant p values have been included as suggested by reviewer.
4. Line 50-51. Can rephrase the sentence for the sake of clarity. "TB-LAMP was performed by a technician after a week of training in the district hospital who had no prior experience in the technology".

Response: The sentence has been rephrased as suggested.

5. Authors need to mention about TB-LAMP specificity and sensitivity at the end of abstract rather than smear microscopy data.

Response: This has been noted and abstract revised as suggested by the reviewer. (Lines 56-64, Abstract section).

6. Methods section is written too elaborated. Especially TB-LAMP section. Authors can consider reducing the length. Authors did not mention about the primers used in TB-LAMP.

Response: The TB-LAMP procedure section has been revised and summarized as suggested (Lines 162-186, TB-LAMP procedure)

The primers used in TB-LAMP have also been included in the revised manuscript in the background section as follows: ‘The fundamental reaction requires four types of primer which are complementary to six regions of the target gene: the F3c,F2c and F1c regions at the 3'side and the B1,B2 and B3 regions at the 5'side’ (Lines 90-92, Background section).

7. Authors need to mention about MGIT/LJ in the laboratory procedures.

Response: The MGIT/LJ procedures are described in the procedures section, under sub-section ‘Xpert MTB/Rif and MTB Culture testing’ (Lines 192-200)

8. Line 178. What does powder means? Authors can explain clearly.

Response: The methods section on TB-LAMP procedure has been revised and the TB-LAMP procedure referenced for clarity (Ref # 17).

9. Authors can move declaration (ethics approval and consent to participate) to the methods section.

Response: Thank you. Manuscript submission guidelines given in the ‘instructions to authors’ by the BMC Infectious Diseases journal were followed.

10. Line 255. Could not find 49 (21%) in the tables. Need to check.
Response: Thank you. 49 (21%) is the total TB LAMP positives out of the 233 analyzed participants and includes the ‘true’ positives (culture positive-TB LAMP positive N=46) and false positives (Culture negative-TB-LAMP positive=3). TB LAMP results summary have been included in Table 1.

11. Lines 288-269. Smear positive (AFB) and smear negative numbers need to be added in table 1 for the sake of clarity. What was the reason for that low sputum smear positive for AFB in 18.5% (43/233) participants.

Response: Thank you. The smear microscopy results summary has been included in Table 1 as suggested. Low smear positivity in our population could possibly be due to the high HIV prevalence in our study population as highlighted in the discussion section (Lines 321, Discussion section)

12. Line 289. Authors need to add TB-LAMP also in the line.

Response: TB-LAMP has been added (Line 286)

13. Lines 314-315. Is this data from reference.? If that is true then authors need to mention that Boehme's et al., showed that….

Response: The section has been revised as suggested by reviewer.

14. Line 333-335. Authors need to mention time factor in this sentence for the sake of clarity. Because single person has to setup the room and perform the tests which might cause delay in processing the samples within time.

Response: Thank you. The discussion section has been revised and time factor discussed as follows: ‘Ultimately, only a single technician performed the tests during the entire study period and the technician was responsible for setting up the test room which could have caused delays in procedure performance and could have affected the sensitivity of the test.’ (Lines 332-334, Discussion section)

15. Table 1. Authors can include females data, New TB treatment data numbers in table 1.

Response: Female data and new TB treatment data have been included in Table 1 as suggested.

16. Table 2. Authors can include smear positive data in table 2.

1. Authors did not mention about HIV-infected data in text. Can be removed from table2.
Response: Results section has a description of the TB-LAMP performance in relation to HIV (lines 260-262) and the data have also been shown in Table 3. ‘Among HIV-infected participants, TB-LAMP sensitivity and specificity were 52.3% (95% CI 36.7-67.5) and 97.1% (95% CI 89.9-99.6) respectively compared to MTB culture.’ (Lines 260-262, Results section).

Data on TB-LAMP results in relation to smear status is indicated in Table 2.

2. LJ, MGIT can be elaborated in table legend.

Response: The table foot note has been revised and includes LJ, MGIT elaboration

17. Table 3. FM/ZN can be elaborated in table legend.

Response: The table footnote has been revised and includes ZN/FM elaboration

18. Previous clinical studies that used LAMP assay have not been cited in this study. References need to be up-to-date and formatted.

Response: The references have been updated and new references from previous studies have been added. A total of 7 new references have been added.

19. Affiliation no.7 is not assigned to author.

Response: This error has been corrected in the revised manuscript.