



School of Medicine
North Haugh, St. Andrews, KY16 9TF, UK

23rd Aug 2019

Dear Editor

Re: Response to Review and editorial comments

We are grateful to the thorough review given to our manuscript. We have taken all the editorial and peer review comments on board and below we provide point by point response in blue coloured text. A tracked manuscript indicating the changes made has been uploaded. Figures have been formatted as requested.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
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The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]." [Cited](#)

3. Authors and affiliations: Please provide an email address for each author in the manuscript: [Provided](#).
4. Keywords: Please provide at least 6 keywords or phrases: [Added](#).
5. Please define acronyms/abbreviations (BCG, etc.) upon first use in the main text: [Defined](#).
6. Please use SI abbreviations for all units: L, mL, μ L, h, min, s, etc. Please use the micro symbol μ instead of u and abbreviate liters to L (L, mL, μ L) to avoid confusion: [Corrected as recommended](#).
7. Please include a space between all numbers and the corresponding unit: 15 mL, 5 g, 7 cm, 37 °C, 60 s, 24 h, etc: [Corrected as recommended](#).
8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols ([™]), registered symbols ([®]), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials. You may use the generic term followed by "(Table of Materials)" to draw the readers' attention to specific

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commercial names. Examples of commercial sounding language in your manuscript are: Xpert: [Xpert MTB/RIF is a name of a tuberculosis diagnostic test. This is a standard name known globally and approved by the World Health Organisation.](#), (FastPrep, MP Biomedicals UK, Eppendorf, Precellys 24, VWR, ThermoFisher scientific, Turbo DNA): [These names have been removed](#), etc. 9. Please revise the Protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "NOTE." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion: [Language updated as recommended](#).

10. 1.2: Please specify the source of sputum specimen: [Specified](#).

11. 3.5.3: 3S = 3 seconds? [Yes and the symbol has been changed to indicate s for seconds](#).

12. Lines 246-281: The Protocol should contain only action items that direct the reader to do something. Please move the solutions, materials and equipment information to the Table of Materials. [Removed](#).

13. Reference 9: Please provide journal title: [Provided](#).

14. References: Please do not abbreviate journal titles; use full journal name: [Revised](#).

15. Please remove the embedded table(s) from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text: [Tables moved to excel](#).

16. Figure 1: Please include a space between all numbers and the corresponding unit (600 nm, 80 °C, etc.). [Changed as advised](#).

17. Figures 2-4, 6: Please change the unit "ml" to "mL". [Changed as advised](#).

18. Table of Materials: Please ensure that it has information on all relevant supplies, reagents, equipment and software used, especially those mentioned in the Protocol. Please sort the materials alphabetically by material name. [All material transferred to the table of materials](#).

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The Manuscript is presenting the effect of heat inactivation of *M. tuberculosis* organism to the tuberculosis Molecular Bacterial Load Assay (TB-MBLA). The authors are part of the team that have been validating the TB-MBLA as a test for measuring response to TB treatment in several countries. The Authors are comparing the modifications to the original protocol in measuring *M. tuberculosis* bacterial load. The justifications for the modifications are well stated and relevant. The conclusions and future studies are suggested which are relevant as the LOD are needed to see the effect of heat killing to reducing *Mtb* 16srRNA (<1log10 CFU/ml).

Major Concerns:

The Manuscript title should speak to the methods and conclusion of the manuscript. Currently, it

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reads as if the authors are describing the method, yet the method is already described as stated, but the current manuscript is describing the effects of heat killing of Mtb to TB-MBLA. In this case the authors may consider revising the manuscript title. [The title has been changed to reflect the conclusions.](#)

Minor Concerns:

Line 48, the authors may need to look into the category of live Mtb, because the samples for TB-MBLA are most of the time positive until sputum conversion

Line 55, consider revising headquarters to level since not all district health facilities are at district headquarters: [Revised to levels as asked.](#)

Line 106: PROTOCOL, this may need not to be detailed here since it is only the modifications that are changed from the originally published TB-MBLA protocol. May be consider this as supplementary material: [The full protocol is needed for visualisation video capture.](#)

Line 398: does this line refer to correlation between TB-MBLA performed on heat inactivated samples and MGIT culture TTP? if yes, please clarify: [No, this is an exemplar result to illustrate how TB-MBLA measures treatment.](#)

The figures are not well reference and have no titles. [Figure are cited in body text and legends are given at the end of the title.](#)

Reviewer #2:

Manuscript Summary:

The manuscript describes the use of different temperatures to heat inactivate MTB bacilli and whether this has an impact on the results of the MBLA assay. Heat inactivation of the TB bacilli is a requirement to avoid infectious risk for the lab personnel when manipulating biological samples or infectious material outside a CL3 laboratory.

Major Concerns:

none,

Minor Concerns:

-The title The tuberculosis "Molecular Bacterial Load Assay (TB-MBLA)" does not reflect the content of this manuscript as this is about Heat inactivation and stability of RNA related to the MBLA test. [The title has been changed to reflect the conclusions.](#)

- In fig 4, the term heat killed (HK) is used were throughout the text its Heat inactivated (HI), for consistency use the same term unless it means something different. [Changed to Heat Inactivated \(HI\).](#)

- In the text its states that The limitation is that heat inactivation lyses some cells exposing the RNA to RNases, is this what is assumed or is there evidence for that, if so add the reference. [This is the hypothesis and in figure we provide electron micrographs showing intact bacilli after 20 min heating at 95°C.](#)

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- In fig 4 the preservation of sufficient RNA for TB-MBLA bacterial load measurement is depicted, is there an explanation why within the sputum with TB the result of the control and 85C is much more consistent compared to results of the other temperatures and the in vitro BCG assay. [This has been explained in the discussion to indicate that the difference is driven by heat inactivation at 95°C since 80°C and 85°C are not different.](#)

Sincerely,

Dr. Wilber Sabiti
Senior Research fellow in Medicine

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