Author's response to reviews

Title: Performance evaluation of three commercial molecular assays for the detection of Mycobacterium tuberculosis from clinical specimens in a high TB-HIV-burden setting

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Author's response to reviews: see over
Dear Editor

Re: Performance evaluation of three commercial molecular assays for the detection of *Mycobacterium tuberculosis* from clinical specimens in a high TB-HIV-burden setting

Thank you for considering our work for publication in the *BMC Infectious Diseases* journal and the valuable feedback from the reviewers. We provide below a point by point response to the reviewers. In addition we submit a track change and clean version of the manuscript.

We look forward to further feedback.

Kind regards
Dr F Ismail

**Reviewer: Lucia Barrera**

Thank you for the positive review

As requested, we provide information on the modification of the DNA extraction for Anyplex™ plus assay (line 41-44) and the specificity rates for all the three assays in the abstract (line 53-54).

Clinical decision regarding patients whose specimens were culture negative and positive on MTBDRplus or Anyplex would need to be taken on a case by case basis reviewing previous diagnosis for TB as well as repeat testing and this is explained in the text (line 245-254)

**Reviewer: Gabriel Rojas-Ponce**

1. As suggested, we have added a Table to show the similarities and differences between the three molecular assays. (Table 1)

   The sentence “It requires manipulation of amplicons from first PCR which may increase contamination” has been rephrased (line 130-132)

2. Please note that as part of the standard laboratory procedure all positive MGIT cultures receive a Ziehl Nielsen (ZN) stain and blood agar test in preparation for phenotypic DST.
However, as the objective of this study was to confirm the presence or absence of *Mycobacterium tuberculosis*, we only mentioned the use of ZN stain and MPT64 antigen.

3. As stated in the materials and methods (line 168-171), the manufacturer’s instructions were followed for MTBDRplus and we have now further stated that GenoLyse was used for DNA extraction.

4. 171-172, 185-186: We only performed one culture and this is now clarified in the text. See lines 165-167. Furthermore in the results section we mention that 8 samples were excluded from analysis due to culture contamination (lines 184-185).

172: Sediments were stored at 2-8 °C for 3 months

178 and 183: The two step centrifugation is part of our standard laboratory process and has been added as a limitation for this study (line 284-286).

181: The 4% sodium hydroxide was the initial concentration (line 155-157).

183: The aliquots were not re-suspended in any solution, and were taken directly with no buffering applied as the sediments were in a buffered solution.

270-272: Reasons for false positive molecular results have been expanded (lines 248-254)

**Minor**

172: Typing error corrected

183: neutralize replaced by dilute (line 157-158)

193-195: rephrased (171-174)

217-221: Xpert Ct values: This is a good suggestion. The Xpert MTB/RIF has Ct values for each of the 5 probes (probe A- E). Most authors report probe A, whilst others use the average of the 5 probes. Please advise on the preferred option for inclusion in the paper.