Reviewer's report

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

Version: 2 Date: 28 June 2015

Reviewer: Gabriel Rojas-Ponce

Reviewer's report:

Reviewer: Gabriel Rojas-Ponce (GRP)

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)

GRP: it is ok with me

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.

GRP: it is ok with me

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

GRP: it is ok with me

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)

GRP: it is ok with me

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

GRP: Information about how long the sediments were stored at 2-8°C before to conduct molecular tests should be included in the manuscript. This information is important so that other labs, in case they want to use any of those molecular tests, have a reference about how long as maximum the sediments can be
stored at 2-8C to get similar performance of the tests.

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)

GRP: it is ok with me

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

GRP: it is ok with me

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.

GRP: Pouring off the supernatant after centrifugation process is a big challenge to ensure that no AFB has been removed of the sediment. If the sediment was not resuspended in buffer, which can help to have a sediment more free of NaOH, I presume that this process (pouring off the supernatant) was not done properly as the operator (lab technician) had to calculate to remain 3.5 ml of the suspension for further procedure. Of course, the addition of buffer to the sediment will reduce the number of AFB/ml; however, it is part of the procedure and many labs follow this standard procedure (Kend and Kubica, 1985). Likewise, the resuspension of the sediment is recommended in the MGIT 960 manual which help to reduce the number of false MGIT positive.

Hence, it is a limitation that should be described in the discussion also

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

GRP: NaOH remains in the sediment would affect MGIT, even molecular tests. Unfortunately there is not too much information about how to neutralize (pH 7.0) the sediment to ensure that sediment is free of NaOH. For this reason, the decontamination (treatment with NaOH, resuspension with buffer, centrifugation and re-resuspension of the sediment with additional buffer) process is a key procedure to get reliable results for any test, and not only culture.

Minor

172: Typing error corrected

GRP: it is ok with me

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

GRP: it is ok with me

193-195: rephrased (171-174)

GRP: it is ok with me
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.

GRP: Use the average of 5 probes

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests