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: 1 Date: 7 May 2015

Reviewer: Gabriel Rojas-Ponce

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Major Compulsory Revisions:

1. Authors should comment about the complexity of each test. For example, HAIN test require area for DNA extraction, pre and post PCR. GeneXpert is an instrument for performing the full PCR procedure. What about Anyplex™ plus MTB/NTM/DR-TB? For example what is the meaning of "It requires manipulation of amplicons from the first PCR which may increase cross contamination" (155-156); is this procedure performed in any room/area? A table to show differences/similarities among Xpert, HAIN and Anyplex could help a lot to understand differences among those tests. Likewise, how long take the full procedure for each test.

2. Authors should detail the procedure to rule out contaminated MGIT culture as only ZN smear, and not Blood agar test, was performed from those tubes.

3. Authors should give a clear information about how was done the extraction of DNA for HAIN. Was Genolyse used for HAIN?

4. Others:

   - 171 - 172 and 185 - 186: Was there any difference in the results between first and second culture? That information would tell us how harsh was the second decontamination for treating the sample and killing AFB and how it would explains false positive in some molecular tests.

   - 172: how long those sediments were stored and what temperature (8ª or -20º or -80ºC or another)?

   - 178 and 183: Centrifugation of sample (twice) would affect the load of AFB in the sample. It should be another limitation that should be included in the discussion.

   - 181: "...decontaminated with 4% sodium hydroxide solution..." was it a initial or final NaOH concentration?

   - 183: "The remanent sediment was split in three aliquots". Authors should provide details about the resuspension of the sediment in buffer (volume) or distilled water (volume)?

   - 270 - 272: "False positive results on molecular assays have been attributed to the presence of non-viable organisms in patients on treatment, thus, these
assays are not recommended for monitoring response to therapy.." Other reasons are the load of AFB in sputum, and decontamination method using a high concentration of NaOH. If NaOH is killing AFB during the decontamination process, the isolation of M.tb into MGIT will be affected (probably negative).

Minor:
- 172 culturewere (missing space)
- 183 neutralize (it should be replace by "dilute" in case that final pH after adding buffer has not been measured)
- 193-195. Its not clear how was done the extraction of DNA extraction; however, it is clear when I read discussion (292-294). I suggest to rephrase 193-195.
- 217 - 221: Xpert Ct values could give additional information to TTP, authors should include this value in the results.

**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