Author's response to reviews

Title: Use of Real time Polymerase Chain Reaction for detection of M. tuberculosis, M. avium and M. kansasii from clinical specimen

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Version: 5
Date: 25 January 2015

Author's response to reviews: see over
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Title: Use of Real Time Polymerase Chain Reaction for Detection of
M. tuberculosis, M. avium and M. kansasii From Clinical Specimens
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Version: 3
Date: 25/01/2015
Authors response: Appear in red below
Reviewer’s report 1
Title: Use of Real Time Polymerase Chain Reaction For Detection Of M. tuberculosis, M. avium and M. kansasii From Clinical Specimens
Version:2
Date: 4 June 2014
Reviewer: Satoshi Mitarai
Reviewer’s report:
General comment:
The authors evaluated the real-time PCR system for the detection of M. tuberculosis and M. avium complex using clinical specimens. The objective and methodology will be relevant and the analyses are comprehensive. However, the quality of standard laboratory procedures, including smear microscopy and culture examination, is somewhat ambiguous.
Major Compulsory Revisions;
1. “Lack of rapid diagnosis” is just one of the bottlenecks for the quick reduction of TB in the world, but not “a critical stumbling block”. Revised the statement removing ‘stumbling block’ on page 3 of the introduction
2. What is “month 2 specimen”? Please clarify. If it is the specimen of after 2moths treatment, culture examination will never be the gold standard. So the analysis will not make any sense.
Month 2 specimens were removed from the analysis
3. Reference #2 is not relevant for this statistic description Changed the reference to ‘WHO report 2014’ on page 3
4. The first sentence of the results should be in the methodology section. Rephrazed the statement to ‘A total of 241 baseline sputum specimens collected…’ on page 9
5. The recovery of M. avium by MGIT culture is much less than real-time PCR. It seems strange. The authors should provide comprehensive explanation.
This real time pcr assay seems to be so sensitive for identification of m.avium since the primers and probes are designed based on 16S SNPs. On the other hand, the M. avium cells in culture may not be viable.
6. The authors should provide the results of solid culture and smear microscopy to verify the quality of culture examinations. In addition, the culture positivity among smear-positive and negative specimens should be shown. 
Solid culture and smear results were eliminated from the analysis because the methodology done routinely could not differentiate the three species. So mention of smear and solid culture was removed from the manuscript.
7. The 2 x 2 analysis should be performed on two different categories; smear positive and negative specimens. The 2x2 table shown indicates both positive and negative specimens from two different assays.

Minor Essential Revisions:
8. The manuscript has no line number and it makes reviewing very difficult.
Numbering put on each page
9. Ethics: The reviewer is wondering if the laboratory procedure described in this text is exactly same as the routine one. If the authors increased the suspension volume after digestion/decontamination for this study, it might reduce the sensitivity of other tests (smear and culture). It might affect the routine examination result, and would need an informed consent. Please clarify this point.
The method described in this study is the routine protocol used and it’s aimed at concentrating the organisms for culture than diluting. So the sample volumes are measured before decontamination process to ensure each volume of the decontaminating solution is used.
10. Centrifugation power should be indicated by rcf (xg), not by rpm. Changed that to centrifugal force.
11. Page 4: Why is the contamination chance limited? Please explain. Contamination chance is limited with this real time PCR because the amplification and detection is all done by the ROCHE instrument than other assays like HAIN which involve other manipulations after amplification.
Reviewer’s report 2
Title: Use of Real Time Polymerase Chain Reaction For Detection Of M. tuberculosis, M. avium and M. kansasii From Clinical Specimens
Version: 2
Date: 12 June 2014
Reviewer: Sylvia Costa
Reviewer's report:
Major Compulsory Revision
1) In the discussion section, please clarify "real time PCR assay should not be considered as a replacement for culture of MTB given the observation that it was unable to identify 1 culture positive specimen." As per table 1, it appears that PCR detected 95 MTB where as Capilia confirmed 94. This suggests that the PCR was positive and not the culture, and it is at odds with the statement above. Rephased the statement to show that 1 specimen was pcr positive yet culture indicated it as negative (false positive). This could be due have been a technical culture problem but this sample was re-cultured (MGIT-Liquid) and it showed the same result. So maybe it was non-viable cells picked up by PCR (This is on page 7 of the manuscript)
Minor Essential Revisions
1) Would suggest consistency when reporting numbers as some numbers are rounded up and others are not.
Revised that
2) Would suggest consistency with naming the different assays in the text and the tables.
Revised that to use ‘liquid culture and mycobacterium detection assay’
3) In the Methods - Mycobacterium real time PCR, is there an UNG activation step?
According to manufacturers’ instruction, the cycle is first held at 95ºC for 10 minutes to deactivate the UNG. It does not show when UNG is activated but according to literature, UNG activates at temperatures lower than 65ºC with a half life of 30 seconds. So from the time you make the mastermix, the reaction is already taking place.
4) In the Methods - Mycobacterium real time PCR, is the melt temperature for segment 3 temperature 75 or 70?
Its 75
5) Would suggest verification that numbers in the tables match those in the text. One example is table 4 unit cost per test and the unit cost stated in the Results section. Verified all the numbers used but changed the sample unit cost to '20 and 12.2 USD' because of the included cost of instruments
6) Would suggest verifying some calculations. This reviewer did not arrive at the exact same numerical results as the authors in table 3 for Specificity.
Reviewed the calculations again
7) This reviewer thinks the paper would benefit from significant language editing.
Language reviewed and improved

Level of interest:
An article whose findings are important to those with closely related research interests

Quality of written English:
Needs some language corrections before being published

Statistical review:
No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.