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

Authors:

Arnold Bainomugisa (arnoldbaino@yahoo.co.uk)
Chris Muchwa (muchwachris@yahoo.com)
Joseph Akol (jpakol01@gmail.com)
Paul Mubiri (mubirip@gmail.com)
Sam Ogwang (sxo81@case.edu)
Enock Matovu (matovue@vetmed.mak.ac.ug)
Eddie Wampande (wamps@vetmed.mak.ac.ug)
Moses Joloba (mlj10@case.edu)

Version: 6 Date: 30 January 2015

Author's response to reviews: see over
Authors’ response to reviews

Title: Use of Real Time Polymerase Chain Reaction for Detection of
*M. tuberculosis, M. avium and M. kansasii* From Clinical Specimens

Authors:
* Bainomugisa Arnold²,³ (arnoldbaino@yahoo.co.uk), Muchwa Chris²,³
(muchwachris@yahoo.com), Akol Joseph²,³ (jpakol01@gmail.com), Mubiri Paul³
(mubirip@gmail.com), Ogwang Sam²,³ (sxo81@case.edu), Matovu Enock ⁴
(matovue@vetmed.mak.ac.ug), Wampande Eddie¹,⁴ (wamps@vetmed.mak.ac.ug),
Joloba Moses¹,³ (mlj10@case.edu)

*Corresponding author:
Bainomugisa Arnold, Joint Clinical Research Centre, P.O Box 10005, Lubowa-Kampala

Version: 4
Date: 30/01/2015
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 2 months 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.
Rephrased 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. Rephrased 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 doesnot 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.
Reviewer's report
Title:
Use of Real Time Polymerase Chain Reaction For Detection Of M. tuberculosis, M. avium and M. kansasii From Clinical Specimens
Version: 2
Date:
18 May 2014
Reviewer:
Prof Sarman Singh
Reviewer's report:
The manuscript aims to compare the performance of a commercial real time PCR (Roche) with conventional (Middlebrook 7H11) and automated culture (MGIT 960). The authors also analyse the cost effectiveness of two methods and turn around time.
My major comments are:
1. The authors have compared two uncomparables. While we all know that culture and molecular tests are totally different types of tests methods comparing these two for turn around time and cost does not make any sense to me.
The currently used standard method for mycobacteria speciation in this laboratory is a combination MGIT culture, Capilia immunochromatography and HAIN CM/AS (PCR based) assays. So comparing the performance of the above three assays with one new technique (Roche real time pcr) that can identify mtb, m. avium and m. kansasii is great for patient management but this validation is important for future introduction of this assay. With this RT-PCR, culture is not necessary thus faster
2. It would have been to compare the Roche test with another real time PCR and take liquid culture as gold standard.
The lab does not have another real time assay that can identify the three species to compare it with roche real time pcr but that can be work for the future though patient management needs to be improved. In this study, the gold standard constituted of a work flow first; using MGIT liquid culture, then capilia immunochromatography assay (to differentiate mtb Complex from other mycobacteria) and HAIN CM/AS to confirm the m. avium or m. kansasii.
3. The methodology and results are not matching.

3.a. In methodology they mention the use of 7H11 but in results there is no mention of this, how it performed. Removed 7H11 solid culture from the methodology thus not mention of results.

3.b. Further in methodology, there is no mention about the clinical details or category of patients, if they (all or portion) were on treatment or not; if they were drug defaulter?; what drugs were given to them during the 2 months of interval?; whether they were HIV positive or negative or unknown status.

This was a laboratory based cross sectional study. So clinical information was not availed to us though we used laboratory requisition sheets to identify patients who are being screened or starting treatment or treatment time point. But Month 2 patients were excluded.

3.c. In methodology only, they mention about sonication for 15 minute, but no details. It is expected that the sonication must have been done in several cycles. Also if after sonication, intact bacteria were counted or not to check the success of sonication.

Included the cycle frequency (37 kHz) and sonicator manufacturer. The effectiveness of this sonicator for cell lysis (H37Rv) was performed when performed on its installation and it was successful on complete lysis. So its maintained and serviced twice a year according to manufacturers’ instructions.

3.d. Regarding detection (methodology section), there is no description of the sequence this RT-PCR targets in 16srRNA HV region?

The RT-PCR target region is about 590bp but the exact region sequence and primers could not be availed to us by the manufacturer.

4. In results, the details how the cost was calculated are missing. Why 6MGltubes were taken into account for one sample. If it was done in triplicate then why cost of single RT-PCR is taken into account. The same factor should have been used there too.

When you look at table 4 it well illustrates how the calculations were done. The total cost from each assay divided by the 241 total
samples. So each sample from each patient was run on MGIT culture and Real time PCR.

5. The cost of ZN stain is shown too much and is unrealistic. In this laboratory, the ZN stains kits are procured from BD diagnostics for consistent results

6. These calculations give an impression that authors are biased toward the Roche Rt-PCR. Not at all, there is no complicating interests from any of the authors

7. The tables are confusing and must be redrawn, if the editor decides to give them opportunity of revision. Improved on table labelling for better interpretation

8. From tables this reviewer can not make out how many samples were actually culture positive irrespective of species. This denominator should have included. Bolded the positive samples for each assay in table 1

Minor comments:
1. Sample processing, the word "rotated for 20 minutes" should be changed with centrifuged for 20 min. Changed that to ‘platform shaker (Thermo scientific Inc. USA) at 60 rpm for 20 min’ on page 9

2. Several spelling mistakes and incorrect words are used. Improved that

3. More relevant references need to be included where in two or more molecular tests have been used. Used relevant references especially for studies that used molecular tests and culture tests

4. TAT for culture cannot be comparable hence this need to be addressed. We think its relevant to show this we are comparing different assays with different turn around times

Level of interest:
An article of limited interest

Quality of written English:
Needs some language corrections before being
published
Statistical review:
Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
None