Author's response to reviews

Title: Contribution of two laboratory-developed PCR methods for the diagnosis of Pulmonary Tuberculosis in Brazilian patients with and without HIV infection

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R1

This is a covering letter with a point-by-point description of the changes made in manuscript

Contribution of two revelation systems of in house PCR (colorimetric and non colorimetric) in the diagnosis of Pulmonary Tuberculosis in Brazilian patients with and without HIV infection MS: 1037585323765168

Manuscript revised with point-by-point description of the changes made.

\textbf{Reviewer 1:}

\textbf{Reviewer's report}

\textbf{Title:} Contribution of two revelation systems of in house PCR (colorimetric and non colorimetric) in the diagnosis of Pulmonary Tuberculosis in Brazilian patients with and without HIV infection

\textbf{Version:} 1 \textbf{Date:} 31 July 2010
Reviewer: Moses Joloba

Reviewer's report:

Major Compulsory Revisions:

1) The title should be improved for clarity, such as “Diagnosis of Pulmonary Tuberculosis in Brazilian patients with and without HIV: Evaluation of Colorimetric and non-Colorimetric detection for IS6110 Amplicons following In-House PCR”. The manuscript was revised and changed in accordance to suggestions by reviewers.

2) The way I understood the manuscript, the authors evaluated two methods (PCR-AG and PCR-dot blot) for detection of amplicons following in-house PCR; so amplification was similar for both PCRs –PCR-dot blot and PCR-AG. If so, then this should be clarified that you are addressing detection of amplicons following in house PCR. The manuscript was revised and changed.

Minor Essential Revision:

Generally the manuscript is comprehensible; however, there are several grammatical errors that occur throughout the text, such as missing full stops, spaces between words, etc. The occurrence of these should be reduced by authors to the bare minimum. The manuscript was revised and changed.

Discretionary Revisions:

The subtopics in results may be rephrased for clarity as below:

Lines 221-222: “Performance of AFB smear, culture and two in house PCR methods in comparison to the history of anti-TB treatment”
The manuscript was revised and changed in lines 238-240

Lines 236-237: “Performance of AFB smear, culture and two in house PCR methods in comparison to HIV status”

The manuscript was revised and changed in lines 255-255

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
Additional material submitted by Referee 1:

1. The authors address TB diagnostics, a field that is very important for tuberculosis control; globally reports reveal that the efficiency of methods for diagnosis of TB vary from setting to setting. Hence the importance of determining what works best for a particular setting.

The research question is generally well defined, but it needs some clarification or improvement:

In the background, the authors should mention or review the drawbacks for in-house PCR; such as relatively low sensitivity. Authors should pose that this could be due to the conventional method of detection/analysis used following PCR, which is usually agarose gel electrophoresis. Indeed, there is a minimum amount of DNA that can be detected or visualized through agarose gel electrophoresis (usually from 20ng and above, <20ng is not visualizeable with UV); yet dot blot methods can detect amounts less than these. This is the importance of colorimetric detection (PCR-dot blot), and the gist of this manuscript (at least in my opinion).

The way I understood the manuscript, the authors evaluated two methods (PCR-AG and PCR-dot blot) for detection of amplicons following in-house PCR; so amplification was similar for both PCRs –PCR-dot blot and PCR-AG. If so, then this should be clarified that you are addressing detection of amplicons following in house PCR.

The manuscript was revised and changed in according to reviewer.
2. The study was well designed and controlled. The methods are appropriate and relatively well described. However, the authors should clarify/include the following:

- The nature of template used for PCRs: Was the DNA extracted from cultures or sputum? If so, what method was employed for the DNA extraction? Did you use crude DNA extract or pure DNA?

The manuscript was revised and changed in line 175.

- How did you control cross contamination especially for PCR? How far is the culture facility from the laboratory where molecular assays were done? Does the design/layout of the PCR facility ensure minimal contamination upon PCR?

The manuscript was revised and changed in lines 198-202.

- For the INS primers which were used to amplify the probe: were these primers designed by the authors or the authors used primers described in literature? This should be clarified.

The manuscript was revised and changed in lines 179-180.

- What was the source (company) of BCP/NBT? It should be indicated

The manuscript was revised and changed in lines 191-193.
• Purity (for instance, presence of oligonucleotides, etc) of the amplicons is known to influence sensitivity. So, prior to transfer of PCR amplicons for PCR-dot blot, were the PCR products purified (say, using phenol/chloroform or commercial purification kits)? Please clarify.

The manuscript was revised and changed in lines 174-180.

• Which method of transfer was used? Vacuum vs. Capillary? Transfer method can influence sensitivity of detection.

The manuscript was revised and changed in lines 185-188

• For PCR-AG, which buffer was used during agarose electrophoresis? TAE or TBE?

The manuscript was revised and changed in line 182.

• Line 166: please rephrase as “the presence of the amplified fragment of IS6110 insertion sequence in positive PCRs was checked

The manuscript was revised and changed in lines 170-172.

• Lines 134-135 and 194-195 are redundant (repetition); one of the two should be deleted.

The manuscript was revised and changed in line 208
3. Generally the data are sound and well represented. However, the subtopics in results should be rephrased for clarity as below:

- Lines 221-222: “Performance of AFB smear, culture and two in house PCR methods in comparison to the history of anti-TB treatment”

The manuscript was revised and changed in lines 238-240.

- Lines 236-237: “Performance of AFB smear, culture and two in house PCR methods in comparison to HIV status”

The manuscript was revised and changed in lines 255-256

4. Yes

5. Yes. However line 316 should be rephrased as “-----due to certain strains of MTB lacking this element-------

The manuscript was revised and changed in lines 338-340.

6. Yes. However, the authors did not mention that Real-Time PCR-based methods may be more sensitive than the conventional PCR; and these can also be an alternative to PCR-AG.

7. Yes

8. The abstract is ok.
However, the title may be improved for clarity, such as “Diagnosis of Pulmonary Tuberculosis in Brazilian patients with and without HIV: Evaluation of Colorimetric and non-Colorimetric detection for IS6110 Amplicons following In-House PCR”.

The manuscript was revised and changed.

9. Generally the manuscript is comprehensible; however, there are several grammatical errors that occur throughout the text, such as missing full stops, spaces between words, etc. These should be addressed by the authors.

Please note:

It looks like the referencing used is not the BMC style

The tables lack bottom borders

Legend for Figure 1: this appears on the top of the figure: figure legends should be at the end of the main text

The manuscript was revised and changed.
Reviewer: Kim Musser

Reviewer's report:

This report describes a well designed comparison of two in-house/ laboratory developed PCR methods for the diagnosis of pulmonary TB including a comparison of HIV status and history of past TB treatment. As would be expected PCR with the additional dot blot detection was a more sensitive test. There are a number of minor essential revision needed in my opinion:

Title- Perhaps a better more clear title- Comparison of two laboratory-developed PCR methods for the diagnosis of pulmonary tuberculosis in Brazilian patients with and without HIV infection.

general: Consider switching to laboratory-developed instead of in house throughout

The manuscript was revised.

Throughout "+" and "-" symbols after HIV are not consistent.

The manuscript was revised and changed in lines 338-340.

refs sometimes are in ( ) and sometime sin [ ].

The manuscript was revised and changed.
Consider switching language of "non-previously treated for TB" to "with no history of prior TB treatment"

The manuscript was revised and changed in line 225.

Not necessary to add in colorimetric and noncolorimetric as part of description. Pulmonary Tuberculosis and Tuberculosis do not need to be capitalized throughout.

Titles are not consistent in their capitalization of some words

The manuscript was revised and changed.

Abstract- line 55 remove "the"

The manuscript was revised and changed in lines 53-57.

line 59 write out polymerase chain reaction (PCR)- short name comes second at first use

The manuscript was revised and changed in line 59.

line 66 culture should not be capitalized
The manuscript was revised and changed in line 66.

line 67- "The" before Gold, remove "the" before culture, change positive to positivity

The manuscript was rephrased in lines 67-68.

line 68 add "meeting" before the/

The manuscript was rephrased in lines 68.

Better sentence: The gold standard was culture positivity combined with meeting the definition of clinical pulmonary TB.

The manuscript was rephrased in lines 67-68.

line 69 Results should be bolded and on separate line like other subtitles, no colon.

The manuscript was rephrased in lines 69.

Throughout "+" and "-" symbols after HIV are not consistent.
The manuscript was revised and changed.

line 76 need period at end

The manuscript was revised and changed in line 76.

Background: line 88 add "with" after present

The manuscript was rephrased in lines 92-93.

line 101 HIV- was already defined as seronegative so that word can be removed.

The manuscript was revised and changed.

Methods:

line 199 need space between at and public

The manuscript was revised and changed in line 128.

line 127 typo in ineligible

The manuscript was revised and changed.
line 136 Logistics

The manuscript was revised and changed in line 144.

line 151 - sentence needs to be rewritten

The manuscript was rewritten.

line 153 up to and less than are redundant, space needed before 14

The manuscript was revised and changed.

line 154 typo in enrollment

The manuscript was revised and changed in line 162.

line 161 need space in Ziehl Neelsen

The manuscript was revised and changed in line 168.

Line 164 Lowenstein missing "n"

The manuscript was revised and changed in line 169.
lines 168, 177 ultraviolet different spellings

The manuscript was revised and changed in lines 174 and 185.

line 178 need period
line 182 extra period

The manuscript was revised and changed.

Lines 184-188- need more description of what was used for controls and the amount of DNA used for inhibition test.

The manuscript was revised and changed in lines 198-200.

Was a PCR flow utilized to avoid cross- contamination?

The manuscript was revised and changed in lines 200-204.

Results

line 210 "non-previously treatment" is not good english structure

The manuscript was revised and changed.

line 277 need space
The manuscript was revised and changed.

Lines 221-222
Better title needed "according the history of anti-TB treatment" is not good sentence structure

The manuscript was revised and changed.

PPV and NPV values should have %.

The manuscript was revised and changed in all text and in the tables.

Line 226 need period

The manuscript was revised and changed.

line 236-237 "two" should not be italic

The manuscript was revised and changed in line 241.

Line 250 extra t after )
The manuscript was revised and changed.

line 255 Title should be bolded

The manuscript was revised and changed in line 258.

Line 256
Figura should be figure

The manuscript was revised and changed in line 291.

Discussion
line 316 The IS6110 target is often a multi-target element and is known to increase sensitivity compared to other PCR targets. Even though there are rare reports of the absence of this target this should not explain overall sensitivity decreases.

The manuscript was revised and changed in lines 341-343.

Conclusions: Although the dot blot test is a more sensitive test it does require additional steps post PCR that is not performed properly could lead to contamination and incorrect diagnosis. Perhaps some commentary on the care that needs to be taken to avoid cross contamination when using a test such as this.
The manuscript was revised and changed in lines 363-367..

References: periods after year throughout and at end- is this the correct format? Reference 34 Perhaps a better representation of the API Consensus Expert Committee should be used. ref 36 missing period at end.

The manuscript was revised and changed.

Tables inconsistent use of capitalization and PPV and NPV values should have %

The tables were revised and changed.

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

**Declaration of competing interests:**

I declare that I have no competing interests.
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