Author's response to reviews

Title: Comparative evaluation of three immunochromatographic identification tests for culture confirmation of Mycobacterium tuberculosis complex

Authors:

Kinuyo Chikamatsu (chikamatsu@jata.or.jp)
Akio Aono (aono@jata.or.jp)
Hiroyuki Yamada (hyamada@jata.or.jp)
Tetsuhiro Sugamoto (tsugamoto@jatahq.org)
Tomoko Kato (dumbo22boko22@gmail.com)
Yuko Kazumi (kazumi@jata.or.jp)
Kiyoko Tamai (mml-idenshi@miroku-lab.co.jp)
Hideji Yanagisawa (h-yanagisawa@miroku-lab.co.jp)
Satoshi Mitarai (mitarai@jata.or.jp)

Version: 4 Date: 27 November 2013

Author's response to reviews: see over
28 November, 2013

Dr Juan Carlos Palomino,
Editorial Board,
BMC Infection Diseases,

Dear Dr Juan Carlos Palomino,

Thank you very much for your careful consideration of our manuscript entitled “Comparative evaluation of three immunochromatographic identification tests for culture confirmation of *Mycobacterium tuberculosis* complex” (revised title, ref. No. 1578249089106172). We have revised our manuscript consolidating our responses to the comments.

I am now sending the revised manuscript for the second review. We hope that you will consider this revised version suitable for publication in BMC Infection Disease.

Yours sincerely,

Kinuyo Chikamatsu
RESPONSE TO EDITOR:
We wish to express our appreciation to Dr Juan Carlos Palomino for his insightful comments, which have helped us to improve the paper.

Comment: Please clarify within the methods section whether isolates were collected from patients as part of standard patient.
Response: In accordance with the Reviewer's comment, we revised the manuscript lines 93 and 94 as follows.
The clinical isolates were collected from patients as a part of routine examination.

RESPONSE TO REVIEWER 1:
We wish to express our appreciation to Dr Sarman Singh for his insightful comments.

RESPONSE TO REVIEWER 2:
We wish to express our appreciation to Dr Maria Alice DaSilva Telles for her insightful comments, which have helped us to improve the paper.

Comment: My only remark is concerned to lines 150 to 153, as the authors mentioned that 99 reference strains, but then it is said that the 3 methods correctly produced negative results for 3 non- mycobacterial strains. I could not understand why this “three” if in Table 1 is showed the results of 99 strains.
Response: The 3 non-mycobacterial strains (species) also hold acid-fastness. We chose the genera closely rerated to Mycobacteria to exclude possible false positives. In accordance with the reviewer's comment, we revised the manuscript (lines 84 to 86 and 151 to 155) as follows:

- Reference strains of 96 Mycobacterium species and/or subspecies (4 MTC and 92 NTM) and 3 other genera with acid-fastness (Nocardia asteroids, Rhodococcus equi and Rhodococcus aichiense) were used for the evaluation.
Each of the three kits (Capilia TB-Neo, SD MPT64, and TBc ID) was tested using the 99 reference strains. Capilia TB-Neo correctly produced positive results for four MTC (\textit{M. tuberculosis}, \textit{M. africanum}, \textit{M. bovis}, and \textit{M. microti}) and negative results for 92 NTM and 3 non-mycobacterial species (other genera) with acid-fastness, while SD MPT64 and TBc ID generated several false positives (Table 1).

**RESPONSE TO REVIEWER 3:**
We wish to express our appreciation to Dr Lucia Barrera for her insightful comments, which have helped us significantly improve the paper.

**Comment 1:** The title does not describe the comparative evaluation performed with kits produced by different companies which is a valuable feature of this study.

**Response:** In accordance with the reviewer's comment, we revised the title to “Comparative evaluation of three immunochromatographic identification tests for culture confirmation of \textit{Mycobacterium tuberculosis} complex”.

**Comment 2:** To precise the conditions under which the kits were evaluated. It would be convenient to clarify that the clinical isolates were retrieved from a collection and subcultured. Challenges might be somewhat different when testing isolates obtained in liquid media directly from clinical specimens and immediately after being flagged as positive.

**Response:** The testing condition was clarified in the methodology section according to the reviewer's comment. The authors agree with the reviewer on the difference of sub-cultured material and MGIT positive culture from clinical specimen. We did not test other general bacteria that could contaminate MGIT, such as MRSA and \textit{Corynebacteria}. However, the sensitivity of Capilia TB-Neo is improved, and Muyoyeta et al. (2013) has reported that the specificity for mixed isolates of Capilia TB-Neo were 100%. Therefore, we consider that it is also possible to detect from liquid media directly from clinical specimens and immediately after being flagged as positive.
Comment 3: Line 71. Brands other than Capilia are available and being adopted.
Response: In accordance with the reviewer's comment, we revised the manuscript (lines 70 to 74).
In contrast, immunochromatographic species identification tests, Capilia TB (TAUNS, Izunokuni, Japan), SD BIOLINE TB Ag MPT64 rapid (Standard Diagnostics, Inc., Korea) and BD MGIT™ TBc Identification Test (Becton Dickinson and Company, USA) have been adopted as a cheap, rapid, and accurate alternative in clinical laboratories around the world.

Comment 4: Line 83: 96 Mycobacterium species or subspecies were tested.
Response: In accordance with the reviewer's comment, we revised the manuscript in line 84 as follows:
Reference strains of 96 Mycobacterium species and subspecies (4 MTC and 92 NTM) and 3 other genera with acid-fastness (Nocardia asteroids, Rhodococcus equi and Rhodococcus aichiense) were used for the evaluation.

Comment 5: Lines 43, 45, 154, 199. Revise wording.
Response: The wording of Capilia TB-Neo and Capilia TB were collected.

Comment 6: Lines 265-270: It is suggested to declare if the authors have conflict of interests in relation to any of the kits evaluated.
Response: We declared no competing interests in lines 253 to 254.

Comment 7: Some recent publications evaluating Capilia TB-Neo are not presented nor discussed.
Response: In accordance with the reviewer’s comment, we updated discussion about sensitivity of Capillia TB-Neo including other study (Muyoyeta et al. J Clin Microbiol 2013, 51:4237–4239) in line 199 to 205.
The weak false-positive reaction to M. marinum that was reported using Capilia TB (12) was not observed in this study, and resulted in better specificity. The minimum detection concentration of M. tuberculosis for
Capilia TB-Neo was $10^5$ CFU/ml (data not shown), which was one-tenth than that for the previous kit. There was a report that Capilia TB-Neo was higher sensitivity than Capilia TB (20). In summary, the overall performance of Capilia TB-Neo was better than Capilia TB in both sensitivity and specificity.