Author's response to reviews

Title: Evaluation of Microscopic Observation Drug Susceptibility assay for diagnosis of multidrug-resistant tuberculosis in Viet Nam

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Author's response to reviews: see over
Reviewer 1: Ruth McNerney

The conclusion of the abstract should be modified to take into account the low sensitivity as discussed in the results section.

The conclusion has been modified to reflect the low sensitivity as discussed in the results section (lines 54-56).
Reviewer 2: Lucia Barrera

A. Major compulsory revisions

Recommendation #3 and 12 made by reviewer 3 should be follow:

The result and discussion sections have been modified as requested by the reviewers.

Abstract

1. Conclusion is not correct. MODS specificity for M. tuberculosis detection (92.3%) is low. MODS sensitivity to detect INH and RIF resistant isolates reported (72%) is not acceptable according to international standards

We have modified the conclusion. Please see response to reviewer 1 (lines 54-56).

Introduction

2. Line 81 Direct DST by liquid culture systems are not standardized nor included in WHO guidelines.

We have corrected this sentence as requested by the reviewer (line 82).

3. Line 91. NRA can be performed directly on specimens

We have modified the sentence as requested by the reviewer (lines 87-89).

Results

4. Line 273-280. *M. fortuitum* and *M. chelonae* were erroneously identified as M. tuberculosis by biochemical tests? It is not understandable.
The identification of non-tuberculosis mycobacteria (NTM) by biochemical tests is not reliable. Evaluation of molecular tests has shown them more accurate than conventional biochemical identification.


5. Table 1 is unnecessary

We have removed table 1 and replaced with a summary table titled “Sensitivity, specificity, positive predictive value and negative predictive value of MODS in detection of M. tuberculosis and resistant isolates”, as requested by the reviewer (lines 650-656).

6. Tables 2 and 3 are poorly presented. In table 2, specific mutations (results of sequencing) required by reviewers 2 and 3 were not presented. No detection of mutation does not mean that the isolate is “susceptible”, particularly to INH

The sequencing result is in “remarks” column of table 2. We agree with the reviewer that no mutation does not exclude resistance and have modified the MAS_PCR results in table 2 accordingly. (lines 662-663).

Discussion

7. Line 473. The plates must be opened for M. tuberculosis identification; otherwise results of DST should not be reported.
We agree with the reviewer that this is a limitation of the technique, however in settings where smear is currently the only available diagnostic test, NTM differentiation is not available. Initial development of the MODS technique in Peru indicated that *M. tuberculosis* could be identified reliably by cording morphology in MODS culture. Recently, data from other settings has shown that cording cannot reliably identify non-tuberculous mycobacteria and our data supports this, although only 5/393 samples were NTM, reflecting the high-burden setting. A modification to the MODS technique has recently been proposed to allow NTM identification without opening the plates. In this modification, one control well contains PNB so that *M. tuberculosis* can be easily differentiated from NTM by observing growth in the PNB containing well. The accuracy of this modification requires further evaluation.


**B. Minor Essential revisions**


The word *M. Cheloneae* has been corrected to *M. cheloneae* as requested by the reviewer (line 281).

**Discussion**

9. The authors do not comment that LJ were inoculated with 0.1 ml of processed samples while MODS control wells were inoculated with 0.5 ml of this material each. This might contribute to difference in *M. tuberculosis* detection rates.
The volumes of processed samples used for each well of MODS culture and LJ culture are identical (100µl). 0.5ml processed sample was used to prepare the 4.5ml suspension for MODS inoculation before aliquoting 900µl of this suspension into each of 4 wells while 100µl of processed sample was directly inoculated to LJ (lines 160-163).

Reviewer 3: Howard Takiff

A. Major compulsory revisions

There are a few minor substantive problems that will be addressed below, but it is not feasible to list all the instances where the text needs work. There many places where the article “the” is left off, there’s a lack of noun/verb agreement or simply awkward phrasing. A few examples will be noted below. Careful editing by a native English speaker or a copy editor could easily and rapidly resolve these problems, which detract from the value of the manuscript.

The errors have been corrected.

B. Minor essential revisions

1. Ln 28 Why is Drug capitalized?

   We have corrected “Drug” to “drug” (line 28).

2. Ln 39 of which (not Of which) “which ‘is used only after a comma. If there is no comma, it is correct to use “that”.

   We have revised the text as requested by the reviewer (line 39).

3. Ln 42 None of these isolates “were” identified.
We have corrected “was” to “were” as requested by the reviewer (line 42).

4. Ln 45 for “detecting” RIF resistant isolates.

We have revised the text as requested by the reviewer (line 45 and line 47).

5. Ln 58 “documented by the World…(WHO), with estimates of nearly …annually, and 150,000…

We have revised the text as requested by the reviewer (lines 60-62).

6. Ln 66 According to the WHO ……only 22 (settings means countries? Then why not say only 22 of these countries?)

We have revised the text as requested by the reviewer (line 680).

7. Ln 76 (and other places) M. tuberculosis has a space between the M. and tuberculosis.

We have corrected M. tuberculosis with a space between M. and tuberculosis throughout the manuscript.

8. Ln 88 Isn’t the color of the indicator proportional to the growth of bacteria in the medium? If the bacteria are quiescent but viable, the color may not change.

Yes, the color of the indicator is proportional to the growth of bacteria in the medium, and does not detect quiescent bacteria. We have modified the sentence to clarify this (line 90-92).

9. Ln 112 the form is not grammatically correct. It might be better to say something
like, “MDR-TB, which would be rapid, low-cost, easy to perform and highly sensitive and specific.”

We have corrected this sentence as requested by the reviewer (line 116).

10. Ln 163 “Daily, one…” What is meant here? Controls was mounted just once daily, or one per each plate?

MODS was performed once each day (at least one MODS plate/day). Controls were therefore innoculated into a single plate each day. We have clarified this in the manuscript.(lines 167-169).

11. Ln 164. H37Rv is not really a clinical isolate. Where were the INH and Rif resistant control isolates obtained?

We have corrected this sentence in the manuscript. The INH and RIF resistant control isolates are clinical isolates, from Pham Ngoc Thach (PNT) hospital patients, with well-characterised DST patterns determined by proportional DST method. These isolates are used routinely as DST control in the PNT microbiology routine practice (lines 167-170).

12. Ln 184 “PNT” was never defined

We have defined PNT as Pham Ngoc Thach hospital in the text as requested (line 169-170 and line 189).

13. Ln 215 A reference for the 5% and 20% of resistant strains without mutations would be appropriate.

We have added a reference for INH and RIF resistant strains without mutations as requested (line 220).

Please find the related information in the link below:
14. It would be good to have a table with all of the Sensitivities, Specificities, PPV, NPV etc. and their respective number of isolates in each case. This is included in the abst., but it would be good in a small table.

We have removed table 1 and replaced with a summary table as requested (lines 646-652).

15. Ln 326 – 328 The resolution of discrepant results is still not easily understood from the text or table 2, and in this table there are no INH results shown for isolates 2,3,4, although 2 and 4 were supposedly INH resistant. Could there be a summary of the number of false positives and false negatives for each antibiotic?

In table 2, the blanks represent concordance between DST-MODS and DST-LJ as in footnote. This format was chosen to highlight discrepancies for readers. We have added all DST-MODS and DST-LJ results in table 2 as requested (lines 658-659).

There are some isolates for which no conclusion could be reached regarding the ‘true’ result, due to the fact that the absence of a mutation does not rule out resistance.

16. Ln 356. If the samples had discrepant results, there must have been a culture and DST subculture on LJ, as well as on the original MODS, so how can there be no samples for repeating DST tests? From the responses to this question, the authors state that the strains didn’t grow on reculture. However, if they grew enough for DST testing using both methods (they were selected for discrepant results), the bacterial load in the original specimen should no longer be relevant.
The MODS technique has been developed for doing direct DST. In this study, we performed DST-MODS directly on processed sputum samples. The repetition of DST-MODS was also conducted using a stored aliquot of processed samples. Since direct DST-MODS was endorsed by WHO, repeated DST-MODS using cultured isolates was not attempted in this study. Please find the following reference for your information.


17. Ln 365 – 369 No DST testing was performed on these one well positive strains?

The DST-MODS results of strains positive in only one well were not recorded as these results are not eligible for reading DST result as per the standard MODS protocol.

18. Ln 377 The “average or median” time to positive with MODS” Please specify which was used.

The median time to positive is used through the manuscript. We have modified the manuscript, as requested (line 358).

19. Ln 447 – 463 Can the authors give a reference for problems with clumping affecting DST results?

Please find related information in this reference -

Todd P. Primm and Scott G. Franzblau. Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs. 2007. Current Bioactive Compounds 2007, 3, 000-000
C. Discretionary revisions:

20. Couldn’t clumping be overcome by vortexing, perhaps with glass beads?

We agree with the reviewer that vortexing processed samples with glass beads may help to address the clumping issue. However, this step is not included in the standard MODS protocol; and performing this step will require additional equipment and facilities with higher bio-safety containment, as are required for conducting traditional DST method. The purpose of the MODS test is to be applied as an interim solution for countries where traditional DST is not available. Furthermore, applying vortexing with glass beads on processed samples in direct DST may compromise the bacterial load after vortexing due to the adhesion of bacteria onto the beads. The addition of vortexing may merit evaluation.

21. Ln 447 Would the authors advocate in addition to MODS, the inoculation of LJ tubes with INH and RIF?

As mentioned in the response to the comment 20, the development of MODS is aimed for use in laboratories where DST-LJ is not available due to financial and infrastructure constrains.

22. Could the authors estimate the cost per specimen of MODS testing for MDR?

The estimated cost of MODS-DST is approximately 2.5USD (consumable costs only, not including labour and infrastructure costs). We have not included this estimate in the manuscript as it is not a full economic costing and this was not the purpose of the study.