Author's response to reviews

Title: Use of the GenoType(R) MTBDRplus assay to assess drug resistance mong Mycobacterium tuberculosis isolates from patients in rural Uganda

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Author's response to reviews: see over
RESPONSE TO EDITOR’S COMMENTS

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns. Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript.

Response. All changes made are in red text in the revised manuscript.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

Response: The files are correctly formatted. We have converted the word document into a rich text format.

We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional copyediting service. Examples are those provided by the Manuscript Presentation Service (www.biomedes.co.uk), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/). BioMed Central has no first-hand experience of these companies and can take no responsibility for the quality of their service.

Response: The manuscript has been greatly improved through careful review by the senior authors.

Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.

Response: A statement on competing interests has been added just after the conclusion.
RESPONSE TO REVIEWERS’ COMMENTS
Reviewer 1: Hendrik Simon Schaaf

The title is very long and mentions tuberculosis three times, which seems unnecessary. The reviewer suggests something like the following: Use of the Genotype MTBDRplus assay to assess drug resistance among Mycobacterium tuberculosis isolates from patients in rural Uganda.

Response

The title has been changed to “Use of the Genotype MTBDRplus assay to assess drug resistance among Mycobacterium tuberculosis isolates from patients in rural Uganda”.

Major Comments:

Q1. The main problem is the numbers of drug resistant cases. The abstract indicates MDR-TB in 4 of 125 (3.2%) cases, INH monoresistance in 5/125 (4%) cases and RIF monoresistance in 7/125 (5.6%) cases. However, what is indicated as “monoresistance” actually includes the 4 MDR-TB cases! In the manuscript (MS) in the results section under “Drug susceptibility” the authors indicate that there are only 6/125 RIF-resistant and 4 INH-resistant cases, of which all the latter 4 have MDR-TB, which adds up to 6 resistant cases in total. However, on the following page (Mutations associated…) suddenly there are 8 of 117 cases with drug resistance mutations (where did these extra ones come from and why only 117 isolates?). When one reads further, there most likely are 7 RIF-resistant isolates (if the #1 mutation is included), but no mention of the 5th INH resistant case? Finally, looking at the tables, hoping to get clarity, Table 1 indicates 5 INH-resistant, 7 RIF-resistant and 4 of these with combined resistance (MDR) which gives a total of 8 out of 125 drug-resistant cases. Table 2 agrees that there are 8 resistant isolates, but according to the reviewer’s understanding of this table, there are 6 RIF-resistant cases (no explanation given of the “-“ in cases 102 and 246), 4 INH-resistant cases, but now only two MDR-TB cases (cases 08 and 291). The reviewer is completely confused, and even if the interpretation may be slightly incorrect, the authors need to get their numbers correct and say exactly what they mean or how to interpret the data. As part of the above confusion, it seems from the methods section that only the MTBDRplus assay was used and no other intervention was done to confirm drug resistance – is this assumption correct?

Response
We regret the confusion. The numbers have been corrected as shown in the manuscript. The total number of Isolates analyzed is 125. Total number of resistant isolates is eight (six resistant to rifampicin, four to isoniazid, with two resistance to both drugs hence MDR) as shown on page 9. The indirect proportion method on Lowenstein-Jensen media was performed by the National TB and Leprosy Program (NTLP) for patient management, and results were kindly availed to us for comparisons. These were in agreement with the GenotypeMDR plus results except for one result of rifampicin as shown in Table 2 and in the discussion on page 9.

Q2. The conclusion in both the abstract and the MS is not correct – there is definitely not a “substantial proportion of INH monoresistance” and INH monoresistance is not “the highest” according to the numbers provided – there are actually nil or maybe one case of INH-monoresistance according to most of the data. Monoresistance means NO resistance to other first-line drugs.

Response

We agree with the reviewer. The conclusion has been changed. “Substantial proportion of INH monoresistance” has been deleted from conclusion.

Q3. Results, Study population…: Why were the 15 cultures not available for the study? Why were cultures only done on smear-positive cases and not on sputum smear-negative cases with suspected TB?

Response

The 15 cultures were not available for molecular analysis because there was no socio-demographic data of the patients. This has been pointed out in paragraph 1 of page 8 of the revised manuscript. Cultures were done only on smear positives because this was part of the study inclusion criteria at the beginning (this study was part of JB’s doctoral research and we had to adhere to protocols approved by the institutional ethics review board).

Q4. Discussion: the authors statistically compare three studies from Uganda, but the reviewer thinks that this cannot be done for the following reasons: a. no numbers (only percentages) are provided, and b. these are not comparable studies as they are from completely different settings. The reviewer suggests that the authors get a statisticians opinion or delete the statistical comparison. They may, however, describe what was found in other studies in Uganda and say why it cannot be directly compared.
Response

We agree with the reviewer, and the statistical comparison has been dropped. Only a description of the reasons that may be responsible for the differences has been given in the discussion section on page 10 of the revised manuscript.

Q5. The reviewer is concerned about the cases with RIF monoresistance, especially as these cases seem not to be HIV-infected. Is this a limitation of the MTBDRplus test or a true reflection of resistance in the community?

Response

We have compared the kit results with the indirect proportion method results proved by the NTLP and found that the two match except for one isolate where phenotypic resistance was not shown genotypically, and a possible explanation has been outlined on page 9. We think that this could be a reflection of the resistance pattern in the community.

Q6. The authors discuss and give the results of heteroresistance amongst their study isolates in the discussion. The results should be in the “results” section, otherwise it cannot be discussed.

Response

A section on heteroresistance has been added in the results section “Only one isolate resistant to rifampicin showed a double pattern, while all four isoniazid resistant strains showed double patterns (three in the KatG probes and the other in the inhA probes). This is shown in the last three sentences on page 9.

Minor:

Q1. Abstract: Not all patients were tested for HIV, therefore 67.9% may be misleading suggest give numbers with percentage.

Response
Q2. Abstract and MS: inhA is usually referred to as inhA promoter (region) mutation and not gene mutation.

Response

Changed inhA gene mutation to inhA promoter mutation

Q3. Introduction, 1st sentence: The global rate of TB is actually declining from 2004 (see most recent WHO reports) but the total number of TB cases are still increasing – this has to do with growing population and not increasing TB rates.

Response

We agree with the reviewer and have deleted the phrase “and the global rates continue to rise as do the rates of drug-resistance”.

Q4. The authors should indicate abbreviations with full description before using abbreviations in the MS (e.g. MDR, DST, PCR, AFB). Also, once abbreviated, do not repeat (e.g. NTLP in methods – already abbreviated in introduction, and tuberculosis – stick to TB).

Response

Noted, and this has been effected.

Q5. The authors use “susceptibility” mostly (which is preferred) but then uses “sensitive” on a couple of occasions – need to use same terminology throughout the MS.

Response

This has been corrected.
Q6. Was pre- and post-test counseling done and consent obtained for HIV testing?

**Response**

Yes, pre and post test counseling was done after consent was given by the TB suspects, and this has been indicated in the text on page 6.

Q7. Discussion: “Almost half of the study population…” This is not correct – of those tested two-thirds were infected, but a substantial number of patients were not tested for HIV and could also be dually infected.

**Response**

We agree with the reviewer, and the statement has been rephrased on page 11 of the revised manuscript.

Q8. The authors also try to do statistical comparisons (HIV-infected vs HIV-uninfected) but the numbers are really too small to make any reliable deduction.

**Response:**

We agree with the reviewer and this section has been deleted from the manuscript.

Q9. Middle of 2nd page of discussion: A partial sentence hanging in mid air “MDR rate in Uganda…National survey.” Should be deleted.

**Response**

We regret the error; the hanging statement has been deleted.
Q10. The references need tidying up! Many “initials” with no authors, any number of authors from one to nine before “et al.”, style not consistent, etc. This does not look good.

Response

We thank you for the observation. Tidying up has been done on the references, and a reference manager system (EndNote X3) has been used for consistency.
RESPONSE TO REVIEWERS’ COMMENTS

Reviewer 2: Zeaur Rahim

Q1. Specific comments: Rapid tests are usually run parallel with conventional test for validation. One of the major drawbacks of this study is that DST was performed by the MTBDRplus assay, but without proportion susceptibility testing (PST) (a conventional method of DST). PST must be run parallel with rapid assay for validation of rapid test.

Response: All patient samples processed and cultured at the National TB Laboratory undergo drug susceptibility testing for patient management. The laboratory has kindly provided the results for comparison with our study. A section for conventional DST has been included in the methods section, findings included in the results section under DST and the results discussed. All these changes are in red text of the revised manuscript.

Q2. In the discussion section, authors mentioned discrepancies between the DST of the present study and previous National DST surveillance data. The difference might be partly due to time difference of experiment and also due experimental problem of the new tests. However, it creates doubt about the validity of the data generated in the present study using MTBDRplus rapid kit. Data generated by the rapid tests must be validated by parallel testing of at least half of the strains.

Response: As explained in 1 above, data was validated as shown in Table 2 and discrepancies in the results obtained by the indirect proportional method on LJ have been discussed. Furthermore, Table 3 showing comparisons with previous studies has been deleted.