Author's response to reviews

Title: Rapid screening of MDR-TB using molecular Line Probe Assay is feasible in Uganda

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Author's response to reviews:

Dear Editor,

Please find below our responses to the reviewers’ comments:

Reviewer 1:

In this well-written manuscript, the authors describe the results a validation study that compares the performance of a commercially available line-probe assay (LPA) with that of liquid culture-based drug susceptibility testing in a University laboratory and a well-equipped and staffed reference laboratory. The study design is scientifically sound. There are many publications that describe the performance of LPAs. The new information in this report is the performance in this particular population, which is important information for understanding the local utility of the test as well as the general performance of test in different regions of the world.

The report could be improved by including a description of other possibly novel informative aspects of the study such as describing training requirements, if performance improved with experience over time, if there is a need for supervision by an experienced molecular biologist, costs, infrastructure needs, etc.

A more detailed discussion of these points has been added to the discussion.
section. See page 13 and 15.

Minor essential revisions
Page 6 lines 13-15 and Page 7 lines 10-11. Please clarify the difference between these two sentences. Was the ZN microscopy (direct or concentrated?) done in a different laboratory or at a different time than the fluorescence microscopy?

Initial screening with direct ZN was done at the hospital laboratory. Concentrated microscopy was later done on the pooled sediments at the reference lab.

Page 7, line 9. Please clarify if sputum specimens or processed sediments were pooled.
Processed sediments were pooled. Have indicated in text.

Page 7, line 11. It should be ‘fluorescence’ microscope; not fluorescent microscope.

Changed
Page 7, line 14. DST (drug susceptibility testing) testing is redundant.

“Testing” removed.

Page 8, lines 6-7 and lines 13-14. These lines are redundant.

Removed lines 13-14.

Page 8, lines 13-14. Please clarify which sample was provided to the University lab, e.g., raw sputum, processed sediment, or DNA lysate?
Processed sediment was tested by University lab. Added to page 8, line 7.

Page 9, line 7. Please clarify if these were 118 individual sputum specimens from 118 patients or 118 pooled sputum specimens from 118 patients.

It was 118 pooled specimens from 118 patients. This has been clarified in line 1 of results section.

Reviewer 2:
The use of genotypic line probe assays for rapid drug resistance testing in sputum+ TB patients has been validated and is currently endorsed by WHO. However, additional information on its clinical implementation in TB endemic areas and performance under field conditions will help to guide more wide-scale implementation.

I offer a few comments for consideration - all are minor essential or discretionary revisions to consider. Comments follow the outline of the text/manuscript.
Title
The title accurately reflects the content of the paper.

Abstract
Accurate/good summary
1) Important to provide actual numbers and not only percentages.

Actual numbers added to the background, rather than abstract, by way of conciseness. Errors in percentage calculations in original abstract were also found and corrected.

Introduction
This provides sufficient background information and a clear study rationale.
2) The fact that only about 40% of new TB cases are sputum smear-positive is an important limitation of the new test in this setting, which is not adequately emphasized in the discussion.

This was added to discussion (p14) with reference to Uganda data (41% cases are ss+)
3) Use consistent references eg. no need to refer to WHO document in brackets; mix of Roman and non-Roman numbers used.

Removed from page 3. All in non-Roman numerals.
4) Discretionary - Consider using the term “amplification of” instead of or in brackets together with “development of further” drug resistance.

Changed as suggested. Page 4.

Methods
This is well documented, but clarity may be improved.
5) The paragraph at the bottom of page 6 is duplicated.

Duplication has been removed

6) It would be very helpful to include a schematic drawing specifying the exact specimen flow eg. which specimens were pooled and which aliquots went where or were first frozen etc.

Such a diagram would just duplicate what is described in the text. After making recommended changes/clarifications by reviewer 1 in the methods in terms of pooling specimens and order of testing, I think this is no longer necessary.

7) Measures taken to prevent cross contamination should be very clearly described, since this is crucial from an operational perspective.
This has been added to methods section, page 8, line 2, and discussed in discussion section (page 13).

8) Specify which MIC cut-offs were used in the conventional DST.

According to MGIT manufacturers’ instructions in package insert (rifampicin 1 microgram/ml and isoniazid 0.1 microgram/ml). Added to page 7.

Results
In general the results are well presented, but clarity may be improved.
9) Reflect actual fractions not just percentages in the text.

Added to last paragraph page 9. Otherwise the numerator and denominator is given either in tables and/or text. I have tried to avoid repetition.

10) Is there any indication that the mutations missed by DST are more likely to be associated with a fitness cost; therefore minor growth may not have triggered the detection signal during phenotypic testing?

We do not have any information on this available in our setting.

11) The fact that genotypic testing missed 1/7 INH resistant specimens on DST warrants discussion, since it is not widely appreciated that INH resistance may be particularly “underdetected” given the multiple possible mutations.

Mentioned in discussion on page 12.

12) Not only may genotypic DST provide false susceptibility results (most likely wit INH as above), but rpoB wild type mutations detected by the genotypic test may be functionally silent. Was this investigated at in the case where RMP resistance pattern was discrepant?

This was not further investigated.

Discussion
The discussion is well-written, although it may be slightly better focused.
13) No need to repeat sensitivity and specificity figures from results section.

Deleted.

14) Consider adding discussion on some of the points raised under results.

More discussion added, especially on implementation aspects.

15) It seems very important to clearly identify the main bottlenecks, risks and operational challenges identified during this study. Seeing that this is probably one of the most important study contributions, it may be worthwhile to summarize
this in a table.

I have added more detail in the discussion rather than a table, and referenced WHO recommendations which explain in some detail.

yours sincerely,

Heidi Albert