Author's response to reviews

Title: Evaluation of Simple Rapid HIV Assays and Development of National Rapid HIV Test Algorithms in Dar es Salaam, Tanzania

Authors:

Eligius F Lyamuya (elyamuya@muhas.ac.tz)
Said Aboud (aboudsaid@yahoo.com)
Willy K Urassa (wurassa@who.int)
Jaffer Sufi (mgumilasufi@yahoo.com)
Judica Mbwana (jmbwana@yahoo.co.uk)
Faustin Ndugulile (fndugulile@yahoo.com)
Charles Massambu (cmassambu@hotmail.com)

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Author's response to reviews: see over
Editor in Chief
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Liverpool, UK

Re: Responses to Reviewers Comments and Resubmission of the Manuscript (MS 5646694382211869) Titled “Evaluation of Simple Rapid HIV Assays and Development of National Rapid HIV Test Algorithms in Dar es Salaam, Tanzania”, for publication in the BMC Infectious Disease Journal

We thank the 1st and 2nd reviewers for their very good comments. Please find below point-to-point responses to individual comments (bolded) raised by the reviewers.

**Reviewer 1 Comments**

Major Compulsory Revisions

1- It does not appear clearly what gold standard was used to determine the performances of each of the rapid test. The authors confirmed all positive samples by Inno-Lia, however there is not mentioned of confirming the negative samples. Because the panel of samples used for this evaluation is not a panel of well characterized samples, not confirming all the specimens but only 10% non-reactive samples randomly and all positive (by any or all rapid test) may not be sufficient to determine the performance (sensitivities and specificities) of tests evaluated. The previous testing algorithm could have been used as reference to compare the performances of the rapid tests evaluated against.

The gold standard test used in this evaluation was Inno-Lia western blot assay. It is true that all positive samples were confirmed on Inno-Lia test while only 10% of non-reactive samples were confirmed on Inno-Lia test. This is due to the fact that it would have been very expensive to confirm 1043 non-reactive samples and the study budget was limited. However, the chances of having false negative results in all 5 rapid HIV assays is also limited and thus eliminates doubts on the accuracy of these results. It was also not possible to use the previous testing algorithm as Determine was among the 5 assays evaluated. A sentence about gold standard method which starts “Inno-Lia HIV I/II....” has been added
in paragraph 1 (under rapid HIV testing), line 14, on page 8 and the next sentence has been rephrased.

2- The author did not clearly explain the testing strategy (serial or parallel) used for both the previous and proposed testing combinations.

The previous and proposed testing strategies are serial. “Serial testing” has been added in the sentence which starts with “This……” in paragraph 2, line 2, page 5. The word “serial” has also been added in paragraph 2, line 5, on page 13.

3- What’s the rationale for using whole blood collected onto tube for this evaluation instead of serum, plasma or DBS. If the main purpose of this study is to evaluate rapid tests that can be used in hard to reach communities and been able to provide same day results, maybe a phase II/III evaluation will be more appropriate using whole blood from finger prick.

In Tanzania, rapid HIV assays are widely available in many peripheral settings including voluntary and counseling testing, PMCTC, HIV Care and Treatment Centre and whole blood specimens are usually tested in the presence of the client and results are provided within 15-20 minutes instead of serum, plasma or DBS. Certainly a phase II/III evaluation would be preferred in the future to assess the performance of the HIV testing algorithm in use in different settings.

Minor essential revisions
1) It does not appear, from the description of the method, if the HIV sero-status of specimens used for this evaluation is known prior the evaluation

The HIV serostatus of the specimens used in this evaluation was not known prior to evaluation. A sentence which starts with “The HIV serostatus of samples used was unknown prior the evaluation” has been added for clarity in paragraph 1, line 3, on page 8.

2) Pg 7: suggest replacin the word “indeterminate results” by either “undetermined discrepant, discordant or inconclusive results” and elsewhere in text. Indeterminate usually refers to western blot results. Thus, using it for inconclusive RT results may be confusing.

Reviewer’s comments have been incorporated, “indeterminate” has been deleted and word “inconclusive” has been added in paragraph 2 (under rapid HIV testing), line 7 on page 8.

3) Pg 8, 2nd sentence begining by “every testing laboratory .....to perform repeat testing” needs some editing for clarity. Maybe should be rephrased.

Reviewer’s comments have been incorporated and the sentence in paragraph 2, line 10 on page 8 has been rephrased for clarity.
4) Pg 8, sentence 5 “Data of all assay...... were also obtained” needs some editing for clarity.

**Reviewer’s comments have been incorporated and the sentence in paragraph 1, line 2 on page 9 has been edited for clarity.**

5) Pg 8, sentence 4 “All samples that were.... (Immunogenetics). Suggest rephrasing as follows “10% of non-reactive samples randomly selected were tested....”

**Reviewer’s comments have been incorporated and sentence 4, line 15 (under rapid HIV testing) on page 8 has been rephrased**

6) Pg 8: Test performances (sensitivity and specificity) should not be the only inclusion criteria for a national algorithms. Should also consider the PPV for a specific HIV prevalence rate. If whole blood will be obtained from clients/patients using finger prick, we recommend including the availability of sample collection devices as another criteria to be considered.

**Reviewer’s comments are useful and will be taken into consideration in the future evaluation since these inclusion criteria were agreed on by evaluation protocol committee and had already been used to formulate the national algorithm. However, another set of criteria (paragraph 2, under Selection of Rapid HIV Assays for Evaluation on page 6) including the availability of sample collection devices was used to select the 5 rapid HIV assays that entered into the final evaluation.**

Results
7) Pg 9: Not clear why the samples collected on the 1st two days were decided as pilot samples. Is the decision based on a certain proportion compared to overall sample size. Need to provide more information.

**The decision was not based on a proportion compared to overall sample size. The 1st two days were decided as pilot samples as agreed by committee of evaluation protocol.**

8) Pg 9: suggest moving up, in 1st paragraph, the following sentences “ A total of 1649 samples.... excluded from the analysis”. to the end of the paragraph begining by “ A total of 1649 whole blood samples were collected between June and September 2006....” on pg 7 and restruture accordingly for more clarity. Result paragrah will then begin as follows “ Of a total of 1433 samples that were tested....”

**Reviewer’s comments have been incorporated. The sentences have been moved up and restructed accordingly for clarity**
9) Pg 9, 2nd paragraph & sentence 2: Suggest rephrasing as follows “Of the five assays evaluated, HIV-1/2 Stat Pack Dipstick had comparatively lowest sensitivity and specificity on the initial screening.”

**Reviewer’s comments have been incorporated and sentence 2 in the 2nd paragraph on page 9 has been rephrased.**

10) Pg 9, 2nd paragraph & sentence 6: the authors refer to “.. using different types of samples from....”. Not clear if other types of specimens than whole blood were collected for the purpose of this evaluation. Need for clarification.

**Reviewer’s comments have been incorporated and sentence 6 on page 10 has been rephrased for clarification.**

11) Pg 9, 2nd paragraph & sentence 7: description of each study site should be moved up to the method section on page 6 under collection of blood sample paragraph.

**Reviewer’s comments have been incorporated. Sentence 7 in the 2nd paragraph has been moved up to the method section on page 7.**

12) Pg 10: The cost of the combinations should be based on more recent test prices.

The cost of the combinations is based on the test prices that was available during evaluation and were used to make decision on the national algorithm to be adopted country wide. One comments added is the fact that the cost of combination is subject to changes in the prices. A sentence which starts with “It should” has been added in the discussion in paragraph 1, line 16, on page 14.

13) Pg 11: last sentence of results paragraph needs some editing for more clarity.

**Reviewer’s comments have been incorporated and last sentence on page 11 has been edited for clarity.**

14) It will be useful to see a table summarizing samples for which a tie breaker was used.

**Proposed tie breaker test has been discussed in paragraph 1 on page 14.**

Discussion
15) Pg 11: In the discussion, 2nd paragraph can be moved to the introduction. However, would suggest deleting it.

**Reviewer’s comments have been incorporated and the second paragraph has been deleted**
16) Pg 13 1st paragraph: the last sentence is incomplete. Suggest rephrasing along the line as follows: “However, in the present study, .... of antiretroviral drugs, making it difficult to explain the lowest performance of some of the RTs evaluated.

**Reviewer’s comments have been incorporated and the last sentence in the 1st paragraph on page 12 has been rephrased.**

17) Discussion: It will be useful if the present study findings are discussed and compared against published data if any. (Exple: in pg 15, the last sentence of the discussion paragraph needs some reference).

**We have compared the study findings against the published data available.**

**Reviewer 2 Comments**

**Major Compulsory Revisions**

1. Sample collection is well described but it is not mentioned that the samples collected for the study were consecutive or a convenient sampling.

**Samples collected for the study were consecutive sampling and word “consecutively” has been added in the 1st sentence on page 7.**

2. The ability of the kit to detect different classes of immunoglobulin (s) should have been mentioned. It is ideal to have a table with important characteristics of the five kits used like storage temperature, ability to detect IgG/IgM antibodies, whether the kit can differentiate between HIV-1 and HIV-2 infection etc.

**The storage temperature (paragraph 2, line 7 on page 6 under Selection of Rapid HIV Assays for Evaluation) for each of these 5 rapid HIV assays evaluated was 2-30 °C and this was used as a criterion to prequalify the rapid HIV assay to be included in the final evaluation. A statement on the ability to detect IgG/IgM antibodies, and to differentiate between HIV-1 and HIV-2 infection has been added under Selection of Rapid HIV Assays for Evaluation, line 15 (last sentence) on page 6.**

3. The ability to detect and differentiate HIV-2 by each kit should be addressed as this can have implication on the treatment.

**Reviewer’s comments have been incorporated and a statement to address this issue has been added under Selection of Rapid HIV Assays for Evaluation, line 15 (last sentence) on page 6.**

4. In results section the text on table 2 can be shortened as most of the text is repetition of the table.

**Reviewer’s comments have been incorporated and text about table 2 has been shortened**
5. Several paragraphs in the Background and the Discussion are repetitive. Remove these and make the discussion more focused.

**Reviewer’s comments have been incorporated and repetitive paragraphs in the background and discussion have been removed**

Minor Essential Revisions
6. Will there be any change in the cost for this algorithm proposed compared to the existing one? If so that also should mentioned in the discussion.

**Yes the cost for the algorithm proposed is subject to changes in prices and a sentence which starts with “It is important to note……” has been added in the discussion in paragraph 1, line 16 (last sentence), on page 14.**

7. The Determine HIV1/2 at present is manufactured by Inverness Medical incorporation. This change should mention in the paper

**Reviewer’s comments have been incorporated**

8. It would have been more useful for the readers to provide the city/state and country name along with all the company names of the kit used.

**Reviewer’s comments have been incorporated. City/state, country and company names of the kit used have been added in the individual assay.**

9. What is the statistical method/software used for calculating the accuracy indices?

**Epi Info™ program version 6.7 was used**

In addition a paragraph on ethical consideration has been added in methods section on page 9.

All the changes (bolded) have been incorporated in the revised manuscript and have been also bolded.

Yours sincerely

Dr Said Aboud
Corresponding Author