Reviewer’s report:

Manuscript: LAM-ELISA shows a low sensitivity for the diagnosis of pulmonary tuberculosis in urine

This manuscript provides novel & important “real-life” information on the performance of the much anticipated urinary LAM_ELISA test for rapid TB diagnosis. The topic is clearly highly relevant and should be of interest to the readership of the journal.

I offer a few comments for consideration; comments follow the text outline and are not arranged according to importance.

Abstract
Summarizes the content of the paper well

Introduction
This provides sufficient background information and in a concise format.
The main objective of the study is clearly stated.

Methods
The fact that the study was performed in a “real-life” setting evaluating patients with symptoms suspicious of TB, who represent the group in whom it would be utilized in actual practice, is highly commendable.
Laboratory techniques are clearly described and sufficiently rigorous.
All data was double entered and routine errors were corrected.

1) I am uncertain if the expanded discussion about diagnostic likelihood ratio’s is necessary, this could be shortened.

2) The exact symptom definitions used are not stipulated and could be provided on-line as an addendum. This is relevant given the importance of accurate symptom definition for comparative purposes. It has been documented that in children the use of “well-defined symptoms” provides greatly enhanced diagnostic value.

Results
In general results are comprehensive and well presented.
3) It would be of interest to state the proportion of HIV-infected patients who has sputum smear-positive TB.

4) Despite the fact that the gender distribution among HIV-infected pts was very similar, it would still be interesting to test for interference(confounding). Given that the LAM assay performed better in HIV-infected individuals, HIV and/or immune status may potentially explain the gender association observed.

5) Paragraph 3
Language error – should read “… patients with culture confirmed TB…” (not “culturally confirmed”).

6) The explanation provided for the interpretation of the positive DLR of fever and LAM-positivity is not very clear or helpful.

7) The discussion on optical density is not very clear to me. In methods it is stated that an OD difference of 0.1 defined positivity. Figure 1 creates the impression that apart from a few outliers none of the levels measures crossed the “defined diagnostic threshold” – seems important to ensure that this is absolutely clear to the reader.

8) May the fact that proteinuria had a significant impact on LAM-positivity indicate that LAM excretion is enhanced in this situation i.e. that LAM excretion in urine may be reduced (to greater degree than previously appreciated) in patients with normal kidney “function”.

Would be interesting to review the data regarding LAM urinary excretion and how this is influenced by glomerular integrity.

Discussion
Well written, but one or two language errors should be corrected.

9) Please clarify the statement that MTB Ag testing may provide an opportunity to “quantify the burden of disease” – this is usually a public health/epi term - seems as if “organism load” would be the more appropriate term to use in this context.

10) The most important/relevant observation is the poor sensitivity of the current LAM assay. It is not unexpected that the addition of symptoms will add little benefit, given the poor sensitivity of the assay in this study.

11) The detection of LAM in nearly 10% of pts with NTM disease is interesting and warrants some additional discussion.

12) I disagree with the comment that “molecular differentiation between MTB and NTM in this study could have contributed to the low sensitivity observed”, in fact, I would have expected that the use of more precise reference diagnostics (better phenotyping) should have improved both sensitivity and specificity.

13) A potential application seems to be in the diagnosis of people (especially HIV-infected) with extra-pulmonary TB, since it seems as if poor
“compartmentalization” of disease may improve urinary LAM sensitivity and this is also the subgroup that poses the greatest diagnostic dilemma at present. Would be interesting to identify such a sub-group, but unlikely that they would have been included in the study.

14) High specificity in those presenting with fever implies that it may have utility as a “rule in test” in this subgroup, but more validation required.

Table 1
Why was a temp of 37.4 used as cut-off value; 37.5 or 38 would be a more traditional cut of value for a single reading.

Heading for column 2 – should this read “Symptoms DURING the 3 months prior to enrolment”?

Chest pain seems exceedingly common – this is rarely reported. What was the definition of “chest pain” and is there a possibility that “chest pain” and “cough” are very similar word/concepts in the local language?

In summary
I think this manuscript is highly relevant and important to publish, despite the negative outcome reported. The manuscript is generally well-written, but may benefit if attention is given to some of the comments made.