Reviewer's report

Title: A novel detection device for Mycobacterium tuberculosis antigen in breath

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Reviewer: Carlton Evans

Reviewer's report:

1. This clear, well-written manuscript describes thorough research addressing an important research area. It will make a valuable contribution to the literature and usefully advances knowledge. The study assesses a new point of care device that may be useful for diagnosing TB or for diagnosing infectious TB. The paper would be more clear and robust if it conformed more closely and explicitly to the STARD guidelines for reporting studies evaluating diagnostic tests. Most of the key STARD requirements can be inferred from the text but some need clarifying. For example, specifying the inclusion criteria, any exclusion criteria, whether there were any drop outs from the study, any missing data and importantly what the pre-study analysis plan was - what was to be considered the 'reference standard' and how was the study powered? If this was a proof of concept study then that should be clarified and the statistics removed. However, I assume that the intention was to assess the sensitivity, specificity and predictive values of the new test against the reference standard of diagnosis (from smear, x-ray, blood tests, history, examination etc) and if these quantitative evaluation statistics are to be stated then this issue should be clarified and all these quantitative measures should be clearly stated in the abstract and manuscript, with their 95% confidence intervals (that are currently missing). At the moment, the manuscript has some features of a proof of concept study (with interesting preliminary results that warrent future formal evaluation) and an evaluation study (with quantitative assessment). The latter approach seems most appropriate to me, but will require somewhat more rigorous description.

2. A fundamental challenge for the study was the logistical difficulty that prevented culture results from being available. Samples were sent for culture but no results were received. A reality of developing country research: research in the type of setting where this type of new test may be have the most to offer. Because of the low sensitivity of sputum microscopy and the poor accuracy of clinical diagnosis (even with X-rays), the evaluation of the novel diagnostic test was impaired. Despite this unfortunate problem, the study still has great value and should still be published. Indeed, the missing LJ cultures would not have been available for many weeks (or months) anyway, so would have had limited clinical relevance - a point that the authors might make in discussing this limitation of their study. The authors should be more clear in the discussion and especially in the abstract that this issue somewhat limits their conclusions, and necessitates more research.

3. It seems very important that the breathalyser was positive in 6/29 (21%)
people who were thought not to have TB. Without culture results, this is difficult to interpret. However, it seems likely that the new test may have some false positive results in people who don't have TB. This should be worded more clearly in the abstract and might be discussed more explicitly in the discussion section.

4. It doesn't yet seem to be clear whether the importance of this new device may be as a new diagnostic test or as a new test of infectiousness. For the latter, it will be important for the revised manuscript to mention the issue of cough-aerosolized particle size. For diagnosis, this seems to be irrelevant. However, for predicting infectiousness this may be a key issue. There is good evidence that only particles within a certain size range are infectious to humans. Some previous work (cited in the introduction) has focused on particles in the infectious range whereas the new device presumably samples a much wider range of particle sizes - anything that the patient coughs onto a prism. Indeed, this capture system might favour larger particles, whilst smaller infectious particles may pass around the prism in the cough-generated air flow. Thus, cough aerosolization of infectious particles versus particles detected by the new device may, or may not correlate. This should be the subject of future research and this limitation should be discussed.

5. The new test actually provided a quantitative result around a (presumably somewhat arbitrary) cutoff. In untreated patients (as were studied here), sputum smear microscopy also provides a semi-quantitative result, usually reported according to National, IUATLD, CDC or WHO criteria as negative, borderline, +, ++ or +++ (i.e. a five-point semi-quantitative ordinal scale). The authors might either mention why sputum smear gradings were not available (maybe the Ethiopian lab only reported positive or negative, not the five-point scale) or preferably instead present the quantitative Breathalyzer results analyzed against the semi-quantitative sputum smear microscopy results for each sample. Did they correlate? Would these results usefully be presented graphically? Whether or not there was an association, these data will be of considerable interest. Would a different Breathalyzer cut-off have performed better? An ROC curve might be a good way to clarify this.

MINOR REVISIONS
6. Abstract: nebulisation with saline might be better described than 'lubricating the trachea'.

7. Abstract conclusions: typo... investigations as A tool...

8. The background (introduction is clear, well written and interesting but seems disproportionately long compared with the rest of the manuscript. Suggest shortening a bit.

9. Background sentence 'In the absence of an effective vaccine...' should be re-worded.

10. Probable typo: [7] bit it is not known how early in their infection THAT patients...
11. Please clarify whether the study was formally double blind. The study should be published whatever the answer, but where the people making the diagnosis actually 100% unaware of the results from the Breathalyzer? It is clear that the Breathalyzer results were not explicitly considered in the diagnostic algorithm, but the current wording is unclear concerning this important details. If the Breathalyzer results were known (but notionally ignored) by the diagnosing health care providers, then the 'reference standard' diagnosis may have been influenced somewhat by the experimental test that was under evaluation. Either way, this should be made clear in the manuscript.

12. Please clarify if patients coughed once into the new diagnostic Breathalyzer machine, or severla times. For example, the guineapigowrk involved weeks of coughing and the 'cough box' work cited in the introduction samples multiple coughs. If the research reported here sampled a single cough then that is a distinction worth clarifying and discussing.

13. Did the test take 5 (results) or 10 minutes (introduction)? A minor detial, but worth being consistent about.

14. A minor detail, but with a sample size of 60 (and much smaller diagnostic groups of 11-29) the percentages (and their pending 95% confidence intervals) should think be stated to a level of precision of 2 significant figures. The current use of threesignificant figures seems inappropriate to the level of precision obtainable with this samples size.

15. Typo in competing interests: 'and no conflicting intere'

16. The patients weren't nebulised, they used a nebuliser. Suggest re-wording this sentence in the methods section.

17. Reconsider title wording: Up to the authors, but it seems to me that this device tests for TB in cough-generated aerosols, not breath. Other devices do test breath for volatile organic compounds etc., but this device tests nebuliser-facilitated cough aerosols.

Although these comments are numerous, they should all be easy to address and with these straightforward improvements, this manuscript should make an important contribution to the literature. This novel device may well make a significant contribution to patient care.

Carlton Evans

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

I have met one of the authors about 5 times.
I live and work in the tropics but have an honorary appointment at the same
institution as at least one of the authors.
I consider that I have no actual conflict of interest in reviewing this manuscript.