Reviewer's report

Title: EFFECT OF TWO ALTERNATIVE METHODS OF POOLING SPUTUM PRIOR TO TESTING WITH XPERT MTB/RIF

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Reviewer: Jacob Creswell

Reviewer's report:

This manuscript presents data around alternate methods to test sputum using Xpert around pooling which is a poorly understood but potentially important development in TB as there is little data currently, but high test costs and limited budgets in many places so that such methods may be of great interest. I read the article with great interest and expectations. Unfortunately I was left disappointed. I think the group presented the methods well and the study was carried out well - it could be published - but only if there is much more thought and explanation in the results and discussion.

I tried to lay out my comments below as I read through the submission a few times.

The abstract says 'confirmed that pooling sputum specimens is a valid method of reducing the cost of testing sputum using Xpert for detecting pulmonary tuberculosis' which I would love to see, but nowhere is costs mentioned, and based on this data I am not sure I would agree that costs could be reduced - there were only two samples in the pool and there was no analysis to see how many cartridges could be saved - although with such high positivity in the overall sample - I would suspect it would not be cost saving to pool.

Line 43 - should be 'methods'

Line 44 should include very briefly how they were contacted to be assessed (ACF? PCF - hospital etc)

Line 55 is a very strong statement to be made from a small study in a specific population and lacks a larger thinking about the possible implications. It only says the method of reducing the buffer amount could produce similar results.

This brings me to the overall question - what is the objective and what are we ultimately trying to do. My feeling is that it would be good to have more data on the results of pooling Xpert samples as a valid way to test people and save costs, especially in ACF situations. Now, this study has a
pool of 2 people, but in reality - is not measuring any pooling effect - but rather the effect of diluting the buffer. This could be done with or without pooling I think? I assume that reducing the buffer could improve sensitivity as there would be more sputum to test that goes into the cartridge - but you are not really measuring 'pooling' then correct? I had a hard time with this.

The lines 71-76 talking about the Zishiri study are confusing to a reader (at least to me) about what was done - if you have not read the paper. There is both too much detail (20 and 17 samples, 3% modeling yield) and not enough what is acceptable sensitivity? How were people recruited?

Then, the Abdurrahman study used both ACF and PCF to recruit prospectively and test samples at the same time pooled and separately. The findings should be presented in terms of people missed and potential cartridges saved (as the Zirishi study did but is not discussed).

He next paragraph implies that the neither of the two previous studies did community based case finding but in fact the Abdurrahman study did and presents the results separately. What neither study did was try to directly control the amount/ratio of buffer that was used which could influence the results of any testing.

I am a little confused as well on the objectives of this manuscript as well as the stated methods in the two papers that are cited above. In the S Africa study the stated mixing is actually 3:1 while in Nigeria the methods were 1:1 - but say as per the manufacturer's instructions. This manuscript purports to test a 2:1 and 1:1 reagent mix which theoretically would give better sensitivity than the 2:1 o 3:1 ratios but again - the methods from previous work are not discussed properly. In the intro you are also not explaining why you would do this from a practical standpoint - and while it may be clear to a specialized reader, the introduction would benefit from that you would accomplish by simply reducing the ratio of the buffer.

In the methods section you begin by describing Xpert positive sample - but it is unclear how they were originally recruited? Is this part of ACF in Ca Mau or routine case finding at CSDP? Were the people tested first on Xpert and then requested to submit another sample for this study? After or before treatment initiation? A little more clarity would be helpful.

I am not sure the Cepheid… has to be repeated in line 102.

Starting with Line 104 you are describing a retesting process with the 'standard' instructions. But what is confusing to me is that these are people who have been tested positive on Xpert (And I assume they used a standard process as well?). Are you just testing again to ensure the new sample was Xpert+?
In line 111 - you introduce an Xpert negative stock solution which has not been described previously. I would say you did this for both + and neg up front and then move forward.

The methods discuss the cycle threshold categorization but do not discuss this measurement for the initial samples. It seems it might be important especially depending on how the patients with TB were initially recruited.

Results - line 134 - I had a hard time following this first part as the standard method is saying it was undiluted sputum but the standard method is a 2:1 dilution. This might be a wording choice or I am failing to understand the methods.

It would be relevant to elaborate upon "further four specimens were not tested for technical reasons". line 141.

I'm also not sure if the statistical analysis was appropriate. The study was only powered to detect a relatively high proportion of discordant results. Is 8 versus 2 errors is due to chance, especially when one would expect more errors in the reduced buffer arm?

38.6% positivity is extremely high and suggests that they were highly screened - again - this should be in the methods section up front and does have implications for the cycles I would guess. It should at least be discussed later.

Discussion

This is currently very weak and insufficient.

I am not so sure I agree with your conclusions. It is a very small sample, and you have 4 times the number of failed tests in the reduced buffer sample.

It found it surprising that there is no discussion of the increased errors, the also the possible bio safety issues. The issue in general is not well described. In my reading: there is a potential for increased sensitivity by reducing the ratio of the reagent since it dilutes the sample and with ACF you may get lower amounts of bacilli in the sputum. But the manufacturer has instructions on the preparation presumably tested, and it is in part to liquefy the sample and kill bacteria. With less sample you risk 'possible' bio safety issues which should at least be mentioned, and maybe more importantly, the error rates can be higher because the sample is not properly liquefied. These are major issues and I am not so sure that I would say that an effective 84% sensitivity is an
acceptable result if we are talking about a dilution of two samples which is also not a useful pool size, especially given the almost 40% positivity in the tested population. Pooling specimens in such a sample of patients might be even more expensive since almost all tests would have a positive result! The manuscript begins with a note of ACF but then the rest of the submission is not very relevant. I was left wanting more thought and discussion and see no reason why more space should not be dedicated to this.

**Are the methods appropriate and well described?**  
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**  
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