Reviewer’s report

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

Version: 0 Date: 18 Sep 2018

Reviewer: Pamela Nabeta

Reviewer's report:

This is a report on alternative methods for processing pooled sputum samples for the diagnosis of tuberculosis in settings with limited resources. The authors, building on prior research (Ho et al. 2017), try to assess whether a reduction of the proportion of Xpert MTB/RIF sample reagent (SR) could increase the sensitivity of MTB detection when using pooled sputum samples.

Major Compulsory Revisions

Reducing the manufacturer recommended ratio of SR to sample could have an effect on the number of invalid and/or error results in addition to incomplete inactivation of MTB in the samples/pool.

Inadequate liquefaction of very viscous samples could cause blocking of the Xpert MTB/RIF filter, which might result in errors due to increased pressure. In addition, inhibitors that may be present in the samples would be “more concentrated” if the proportion of SR is reduced resulting in PCR inhibition with invalid results. Furthermore, the incomplete inactivation could have biosafety implications if samples are not properly handled.

The authors would need to state whether there was a consideration for these potential issues and how did they address/mitigate them during the study.
Minor Essential Revisions

Methods

- Ct value categories are not accurate and should follow published/validated citations. Authors could refer to Blakemore et al. 2011.

Results

- A higher number of non-determinate results is reported among the "reduced buffer pooling" group. Authors should assess whether this difference is statistically significant and discuss the type of non-determinate results (i.e. error, invalid, no result) and whether this may be due to a lower concentration of SR (e.g. invalid results may be due to a sample which was not properly processed per description on the Xpert MTB/RIF package insert, per major comment above).
- The authors should consider adding the different Ct value categories among the MTB detected cases on Table 1.

Discussion

- The sentence in line number 154-156 refers to two false negative tests. Unless I'm missing anything, these should say "four" instead of "two".
- Per major comment above, the authors should address the implications of a "reduced buffer pooling" strategy. Although this strategy would allow to maintain the original concentration of MTB in the sputum, this comes at the expense of: i) reduced MTB inactivation to comply with international decontamination standards (Banada et al. 2010) potentially introducing biosafety concerns; ii) increased number of non-determinate results i.e. error, invalid due to incomplete liquefaction and higher concentration of inhibitors.
- The authors should acknowledge the study limitations.
- Importantly, the use of sputum pooling may not be generalizable given the requirements of adequate biosafety control and availability of ancillary equipment like a vortex to ensure sputum homogenisation.
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?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
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