Reviewer’s report

Title: Spot sputum samples are at least as good as early morning samples for identifying Mycobacterium tuberculosis

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Reviewer: Adithya Cattamanchi

Reviewer’s report:

Murphy and colleagues present the largest and most complete comparison of the yield of spot vs. early morning sputum samples for TB diagnosis. They find minimal differences between the two across a number of analyses, strongly supporting that spot samples are sufficient for TB diagnosis. I have never said this before in a review but my main concern is that the authors undersell the implications of this high quality and well written analysis. I believe the Discussion should more affirmatively support a switch to spot samples as described below along with a few additional minor points.

1. In lines 43-48, the authors note that there were criteria for rejecting spot sputum samples based on volume or quality. Were there any criteria for rejecting early morning samples?

2. Related to the point above, it would be useful to add a comparison of the volume and quality of spot vs EMS samples before getting into the comparison of smear positivity in the Results section. If there are significant differences between the two types in either volume or quality, stratified analyses might be useful to make sure we are comparing apples to apples when assessing smear positivity, smear grade, culture positivity and time to culture positivity.

3. 4th paragraph of the discussion - I might clarify that this paragraph is referring to value of EMS vs spot samples for culture.

4. I would consider revising the Discussion and conclusion to more strongly advocate for moving away from EMS. It is disappointing to see the first paragraph of the Discussion end with limitations and for the last paragraph to call for further research. Is there really going to be a larger or better quality study of this issue in programmatic settings - this would be very difficult to do. Clinical trials of anti-TB regimens, such as ReMOX, are not repeated in programmatic settings before their findings are recommended or implemented. In addition, there are a number of additional publications not cited that support the use of spot specimens, including a systematic review of same-day vs. standard microscopy (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3836432/) and a study comparing the yield of multiple smears on a single spot specimen to that of spot-morning strategy (https://www.ncbi.nlm.nih.gov/pubmed/20851925). Given the analysis presented here and these studies, the burden should be on those who still advocate for EMS to now show that they add value. A few points regarding some of the limitations mentioned in the Discussion:
a. All patients are smear positive at entry - this would only impact culture, which is not done for diagnosis in the programmatic context. I don't see this as a limitation to extrapolating results to the programmatic context

b. Direct smears vs decontaminated smears - is there reason to think there would be a difference? The WHO has recommended against concentrated microscopy based on a meta-analysis showing no difference in sensitivity between direct and concentrated microscopy (see: http://jcm.asm.org/content/48/7/2433.full and http://www.who.int/tb/laboratory/egmreport_microscopymbodsmethods_nov09.pdf?ua=1)

c. Contention that small differences in time to positivity (i.e., burden) may be important for Xpert - not sure why this is raised as a potential issue. Smear microscopy also has an operational limit for detection, and the smear positive proportion was higher in pre-treatment spot vs EMS samples.

d. A clear difference between the clinical trial setting and programmatic settings is that patients were coached when providing spot specimens. Rather than highlight that further research is needed in programmatic settings, I would consider emphasizing the importance of instructing patients on how to provide a sputum sample.

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.

Yes

Are the conclusions drawn adequately supported by the data shown?
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Yes

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