Author's response to reviews

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

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Spot sputum samples are at least as good as early morning samples for isolating Mycobacterium tuberculosis
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BMC Medicine

Dear Dr Szyszka,

Thank you for reviewing the above manuscript. We are very grateful for the positive comments provided by the reviewers and the editorial team.

I have responded to the points raised in sequence and provided responses and the line numbers (for the version without track changes). I have also highlighted the changes in blue below alongside the relevant comment.

I trust that this revised manuscript meets with your approval.

Best regards,

Dr Michael E Murphy
MBChB MD(Res) MRCP FRCPath

Reviewer reports:

Reviewer #1:

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.

The authors are grateful for these positive comments and have taken steps to amend the manuscript where indicated.

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?
The same criteria apply to both EMS and spot samples; I have clarified (Lines 164-166)

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.

No difference in EMS and spot sample inclusion; all used same criteria for quality so no stratified analyses required

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

I have clarified as requested (Lines 335-355)

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.

Agreed. I have revised the discussion section in order to more strongly advocate spot samples. (Lines 313-321). I have added the additional references to support this (Lines 344-352).

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

We are grateful for this comment and we have added a comment (Lines 337-339)
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_microscopymethods_nov09.pdf?ua=1)

Thank you for this. We have added a comment and inserted the relevant references. (Lines 353-357)

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.

As Xpert is replacing smear is some programmes, we think it is useful to highlight that while the MGIT TTP does not have bearing on the conclusions we reach with regard to smear, these findings may not equally apply to Xpert.

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.

The authors agree with this comment and the text has been amended to take account of it. (Lines 346-349)

Reviewer #2: Summary

Murphy et al present the findings of a secondary analysis of the ReMoxTB trial designed to answer the question, "Are early morning sputum samples superior to spot samples for TB diagnosis and monitoring for adverse outcomes of treatment?" (ReMoxTB was a large, multi-country study which evaluated the efficacy of moxifloxacin-based regimens for shortening of treatment duration for active tuberculosis). Comparing the results of smear microscopy, solid culture, and liquid culture for 1115 paired baseline and 2995 paired follow-up sputa from 1931 patients, the authors show that either timing of sputum collection - spot or early morning - identifies similar proportions of TB patients. For some key outcomes, such as time-to-positivity in solid culture as well as in liquid culture, spot specimens are superior for TB diagnosis. For treatment monitoring and prediction of adverse treatment outcomes, the two specimen types performed similarly.

These findings provide important information for global TB control policy because a large amount evidence shows that requiring an additional clinic visit for submission of an early morning sputum specimen is associated with high rates of loss to follow-up from the diagnostic process, especially in routine clinical settings. For patients who do return to clinic to provide early morning sputum, the extra visits are associated with substantial financial harm for poor
patients, who make up the majority of those undergoing TB evaluation in high-burden countries. These findings challenge nearly 60 years of dogma that early morning specimens are superior to spot specimens. As the authors note, these data also provide support for a widely overlooked 2011 WHO policy recommending that same-day collection of sputum specimens be allowed as an alternative to two-day sputum collection strategies. Because WHO's 2015 End TB Strategy calls for patient-centered strategies for control and elimination of TB as one of its three main pillars - as well as an end to catastrophic costs of TB evaluation and treatment - this manuscript provides important information to advance that goal.

Main criticisms and concerns

Methods

Reporting should follow the STARD guidelines and checklist (Ann Intern Med. 2003;138:40-44.)

This additional information has been added as requested. (Figure 1 – page 30)

Please provide a definition for MGIT false positive results. Is this when MGIT machines report liquid cultures positive, but the speciation algorithm excludes Mtb?

MGIT pos – but AFB negative and no contamination. We have added the definition as requested. (Lines 180-181)

Please state the definition of unfavourable TB treatment outcomes.

This definition has been added (Lines 205-207)

Results

Please present information in the text or a flow diagram to show how patients / samples were selected / excluded for the sub-study.

We have now included the requested Flow chart as figure 1 (Page 30)

Are you able to present characteristics of sputum, e.g. distribution of sputum quality and volume? Did these characteristics differ between spot and early morning specimens?

All samples were considered ‘quality’ samples as laboratory staff requested repeat spot samples if the sample provided, either EMS or spot, was considered of insufficient volume (<2ml) or of poor quality (e.g. salivary sample). This issue has been clarified in the text. (Lines 164-166)

For follow-up specimens, was there any difference between spot and early morning specimens in the proportion successfully collected?
We included only paired EMS and spot samples in this study, so these data are not available.

While I understand that all patients received instruction on how to produce a good quality sputum, I would suppose that collection of most if not all spot specimens is supervised, while most if not all collection of early morning specimens is unsupervised. Do these results suggest to you a possible beneficial effect of supervision on the quality of sputum collection (for baseline and/or follow-up specimens). Could this explain some of the difference between your findings and those of previous studies, which I believe came primarily from operational settings, where supervision of specimens is less common?

A comment addressing this point has been added to the discussion. (Lines 345-348)

Please report the number of patients in this sample with an adverse outcome.

We have added the number of unfavourable outcomes to the ‘Predicting outcomes’ section of the results. (Lines 288-289)

------------------- Editorial Requests -------------------

1. Reporting guidelines

Please revise your manuscript in line with STARD 2015 reporting guidelines for Diagnostic Accuracy Studies and provide completed STARD checklist (attached). More information available here: http://www.stard-statement.org/

The paper has been amended as requested. Figure 1 (page 30) depicts a flow chart of the samples collected as part of the REMoxTB study which were included in these analyses.

2. Ethics approval and consent to participate

Please note that the names of all ethics committees that approved the study need to be listed in the Declarations section at the end of your manuscript.

I have added this statement to the declarations at the end of the manuscript (Lines 487-490)

3. Availability of data and materials

Please provide information about where the data supporting your findings can be found. We encourage authors to deposit their datasets in publicly available repositories (where available and appropriate), or to be presented within the manuscript and/or additional supporting files. Please note that identifying/confidential patient data should not be shared. Authors who do not wish to share their data must confirm this under this sub-heading and also provide their reasons.
More information available here:

http://www.biomedcentral.com/submissions/editorial-policies#availability+of+data+and+materials

All data related to REMoxTB is being made available through CPTR. We have added this information as a heading at the end of the study and as reference 15. (Lines 511-514)