Reviewer’s report

Title: Evaluation of Mycobacterium tuberculosis viability in OMNIgene-SPUTUM reagent upon multi-day transport at ambient temperature

Version: 0 Date: 27 Jun 2017

Reviewer: Norbert Heinrich

Reviewer's report:

Review INFD-D-17-00730

General: this paper describes a validation study for OMNIGENE sputum, a stabilizing reagent for preserving samples for later culture. The main comparison seems to be between culture and further testing at the site of collection in Albania, and at the site of receipt of OMNIGENE - preserved samples in Milan, Italy, with some delay of testing in Milan. The paper is well written and merits publication after some changes.

The fact that results for the two methods are generated in two differing laboratories is the main weakness of this study, since TB decontamination and culture are procedures that are not very well standardisable and results differ between labs. This is appropriately mentioned in the limitations section. Another weakness is that the number of positive cultures is not extremely high.

Nevertheless the presented data are of great interest; since there is an urgent need for published information on this reagent - which could solve pressing problems in TB sample shipment and remote analysis. The problem of contamination when sending samples for remote analysis in many high burden settings makes quality diagnostics unavailable for a large part of persons in need of testing.

The main statement of this paper is correct in emphasizing that an approach of splitting samples and sending them at room temperature to a distant (reference) lab in OM-S is a viable option, based on the data presented. What this data cannot support are claims of sample processing method’s equivalence due to the mentioned limitation.

In general, analysis at the remote lab would probably benefit of a combination of culture and molecular analysis, since there is some loss of viability observed at remote analysis.
In detail:

Abstract: it is appropriate to add that such transport media will make available quality diagnostics to a large population in need.

Introduction

Line 44: sentence not complete

Methods:

Line 77: how was the sample size calculated - or was this a convenience study?

Line 79: how was the sample divided - was there any homogenization done prior to this step? Was sample split into portions of equal volume?

Line 102: student' s t test requires a normal distribution of the sample, which in my experience in MGIT TTP is not the case.

Results:

Line 109: this reads like the entire sample was either decontaminated or treated with OM-S; but in methods there is mention of a splitting of sample. Please clarify.

Line 113: if sample are mixed with equal portions of OM-S, and sample volumes differ, how can 1/3 rd of the sediment always be 0.5 ml? Did you resuspend it after spinning with 1,5ml? When comparing TTP, it is important to state fractions of total sample inoculated.

Line 117/118: please clarify which samples were analysed where.

Line 119: since samples with OM-S and NALC treatment are not processed in the same lab, one cannot say that OM-S "reduced" contamination - baseline contamination rates in the two labs might be fundamentally different.
Line 137: what does "rescued" mean - positive or negative result? Negative here might be false negative. Propose to avoid this word; since this sounds overly supportive for OM-S.

Line 183: please explain the disadvantages of CPC.

Discussion: Line 190: how are the 17.7% loss of viability calculated? Overall there were 45 culture pos samples in Nalc/NaOH, of which 9 were negative in OM-S, making 20%?

Figure 1: change title to "comparison of time to culture positivity between OM-S preservation and Nalc-OH decontamination, dependent on time in OM-S.

Further, there are four symbols in OM-S groups with a TTP above 41 days; while the methods section states that culture in MGIT was performed for up to 41 days - and I assume all samples without a positive signal up to that time were declared negative. Either update methods or clarify on these.

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?
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.

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I have received funds for being a trainer in a TB course offered by BioMerieux. None other to declare.

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