Author’s response to reviews

Title: Clinical Diagnostic Value of Simultaneous Amplification and Testing for the Diagnosis of Sputum-scarce Pulmonary Tuberculosis

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Author’s response to reviews:

Editor Comments:

1. In your declarations section:

   a. Please include details of the consent for inclusion in the study in the 'Ethics approval and consent to participate' section

   We have changed according to your requirement.

   b. In your 'Consent to publish' section please write 'Not applicable' as you are not publishing identifiable patient information.

   We have changed according to your requirement.

Reviewer reports:

Lucilaine Ferrazoli, Ph.D. (Reviewer 1): The data of the manuscript contribute with important information to the field, but need to be reviewed.
Abstract:

Replace the subtitle Objective to Background.

We have replaced.

The smear is used to confirm PTB, but not in the text. To review.

Culture is used to confirm PTB, not smear. We have changed.

The conclusion is different from the text. The accuracy of 63.9% is not high. To review.

63.9% is for sputum scare patient, not all TB patients.

Introduction

Line 84 - Abbreviate Mycobacterium tuberculosis complex - MTBC

We have corrected.

Line 85 - Correct non-tuberculosis to non-tuberculous

We have corrected.

Line 88 - Replace Mycobacterium tuberculosis complex to MTBC

We have replaced

Patient and methods

Line 124 - Write in full AFB

We have changed.

Line 129 - Replace Who were suspect of having active TB to patient to be evaluated for TB

We have replaced

Line 131 - Give more details about the pathological examination.

We have added find the acid fast bacilli

Line 143 - The third group was the patients excluded. Give more explanation.

We have added with obscure diagnosis or the patients lost to follow-up
Line 154 - Replace acid-fast bacilli to AFB
We have replaced

Line 157 - The reference 22 is not appropriate.
We have added by following the standard procedure of the manufacturer

Line 162 - Correct MT to MTBC
We have corrected.

Results

The Table 1 is confusing. Clarify.

On Microbiology - would be clear if culture positive was Only culture positive = 96 and Positive smear was Positive smear and = 20

We have changed.

Clarify in the text why 68 patients are in Microbiology if all patients showed a negative results?.

We have changed

Clarify why the four positive cultures were on the clinical diagnosis, since the criteria for classify the patients clinically were to have negative culture?

four positive smear not culture

Line 257 - use NTM abbreviated
We have changed.

Line 275 - Correct non-tuberculosis to non-tuberculous
We have corrected.

Table 2. The numbers used to calculated the sensitivity, specificity, PPV and NPV should be clearly informed into text or Tables for the reader to easily identify them.

We have changed.
Discussion

The sensitivity of 50.75% for SAT-TB x 21.64% for culture is not well explained and justified.
We have changed.

The presence of inhibitors of enzymatic amplification would give a lower sensitivity not higher.

The presence of inhibitors of enzymatic amplification was the reason why the sensitivity of SAT-TB is only 50.75%.

List of abbreviations

Correct non tuberculosis
We have corrected.

Correct MTB to MTBC
We have corrected.

Max Salfinger (Reviewer 2): This manuscript describes the comparison between a NAAT and AFB smear and culture from BAL collected from 764 patients suspected with pulmonary TB.

L85- 'nontuberculous' mycobacteria (NTM)
We have corrected.

L90- Is SAT-TB really a point-of-care molecular test?

Yes, it is.

111- sputum-scarce PTB individuals; did the authors try sputum induction in patients not able to expectorate a sputum?

Yes, we did.

L157- What was the final concentration of NaOH used for decontamination of the BAL? How long were the samples exposed to NaOH-15, 20, 25 or 30? Did the authors used NALC as well?
The final concentration of NaOH is 4%. The samples exposed to NaOH for 15-20 minutes. We did use NALC.

L166-Please explain in more detail how the samples were processed for conventional smear and culture and for SAT-TB. Was SAT-TB performed on a processed sediment obtained by the conventional work-up? Please provide more details about SAT-TB. Is there an amplification control for detecting amplification inhibitors built in? What was the concentration used and exposure time for the SAT-TB work-up?

We have added in the text.

190-764 patients were analyzed and 70% were diagnosed with TB; however, only 116 out of 536 (22%) patients with TB were confirmed by microbiology. This is a low figure. Can you explain further in the DISCUSSION section?

We choose the sputum scare patients, not all TB patients.

L197-120 PTB patients (96 were only culture positive and 20 smear positive) - what happened to the missing 4?

4 were only smear positive.

L235-A GeneXpert cartridge costs $100 - Is China not eligible for a discounted price of $10?

In China, the cost of a GeneXpert is $100 and not covered by public medical insurance.

L239-Was SAT-TB assay performed 7 days a week?

Yes.

L245-How does the SAT-TB control for amplification inhibitors?

We have added in the text.

L259-Only 2 patients were diagnosed with NTM - any explanations? Smears can be AFB positive in NTM disease; therefore, one should not call them false-positive.

Yes.
References- This author suggests deleting reference 8 and 9. They are not relevant for this study. The Roche TB assay is no longer on the market.

We have deleted reference 8 and 9.

Table 1

Were 764 SAT-TB assays performed? How do you explain in the Non-TB patients 12 positive SAT-TB (false positives?), 4 positive culture (false positives?), and 4 positive smear (false positives or NTM?) false positives

Table 2

Please provide the actual figures and not just the % value.

We add the actual figures in the text.

Laura Forsberg White (Reviewer 3): Clinical diagnostic value of simultaneous amplification and testing for the diagnosis of sputum-scarce pulmonary Tuberculosis

This is an interesting paper with a generally well-designed study. I am struck by the large number of clinically diagnosed individuals, which makes me question the generalizability of these results. Additional stratified analysis might help in interpreting the test diagnostics in other settings. As I was asked to assess the statistics in this paper, I will comment on these issues.

1. The power calculation given does not really make any sense for the results presented for multiple reasons. First, what is a power test at the 95% level? What test is being done? This is not clear at all and it is impossible to determine if this calculation is appropriate for the results presented. Further it is not clear why the authors state that only 100 people were necessary, but then enroll over 700 people.

This part we have modified.
2. In reporting the sensitivity values on page 10, the authors should also include n/N for these values. It is also not necessary, or customary, to report the X^2 statistic value. Usually a p-value alone is reported. Reporting confidence intervals for these values is also warranted. We have added n/N and deleted X^2 statistic value.

3. In general, I would like to see an AUC analysis to compare the performance of the tests. This is generally better since it takes into account both sensitivity and specificity. It is fine to report the individual test characteristics (sensitivity, etc), but AUC is also important.

4. The kappa results reported are a bit confusing. There is n=382; 76.44% agreement. What are these numbers? This part we have modified.