Author's response to reviews

Title: Use of Real time Polymerase Chain Reaction for detection of M. tuberculosis, M. avium and M. kansasii from clinical specimen

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Author's response to reviews: see over
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Title: Use of Real Time Polymerase Chain Reaction for Detection of M. tuberculosis, M. avium and M. kansasii From Clinical Specimens

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Authors response: Appear in red below
Editors’ comments:

1. Reviewer 1 question 5. Please include answer in the paper. (Discussion states 'could probably might have been'; this needs some correct editing).

Included statement ‘This is further supported by the theory of *M. avium* cells being non-viable in liquid culture yet their DNA was identified by Real time PCR assay’ in the discussion on page 7.

2. Reviewer 1 question 7 was not answered well: the reviewer suggests to stratify the results by smear positive and smear negative. I agree this would be a useful addition to table 2 (may label as table 2b and 2c).

To stick with the study objectives, the authors agreed not to include smear results because they cannot be used to differentiate the three mycobacteria species.

3. Reviewer 1 question 11: please include the answer in the paper.

Included statement “Since this assay has amplification and detection done by the Roche 480 II instrument, there are limited chances of assay contamination than other assays like DNA line probe assays that involve further manipulations after amplification” in the discussion on page 8.

4. Reviewer 2 question 6. I also redid some calculations and also do not get the same numbers. Eg. The specificity of the assay for avium is 132/145= 91%. How did the authors get to 95.4%?

Re-examined our calculations and we get the same results:

Specificity for *M. avium* = true negative (2) / (false positive (0) + true negative (95 mtb+1 m.kansasi+132 negative)) *100. Specificity 95.4% at 95% CI (91-97). Note: there were three species identified by one assay and no mixed infections identified, so if it was positive for one species (eg MTB), then the positives for *M. avium* and *M. kansasi* are negatives for MTB.

5. Since only 1 Kansasii was found; this cannot be a major item in the abstract, results and discussion. It can only be a minor result and needs discussion that larger sample size is needed. One sample cannot support the statement that the new test has the ability to detect 3 mycobacterial species.

We feel the identification of *M. kansasi* in this country is good indicator for presence of rare mycobacteria species in the Ugandan population “With the demonstrated advantages of using this technique such as capacity to identify the *M. tuberculosis* and *M. avium*“ but reviewed the
6. The tables, results and abstract give 1 or 2 decimals. This gives a false idea of prevision for low numbers. Please use zero decimals throughout for %, sensitivity, specificity, PPV, NPV

Changed all numbers to zero decimals