Author's response to reviews

Title: Accuracy of Real-Time PCR, Gram Stain and Culture for Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae Meningitis Diagnosis

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Author's response to reviews: see over
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Dear Editor:

We are resubmitting our manuscript, titled, “Accuracy of Real-Time PCR, Gram Stain and Culture for *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* Meningitis Diagnosis” for publication in *BMC Infectious Diseases* (MS: 1615755798799552). We have made revisions to the manuscript based on reviewer feedback. Our specific responses to each comment are listed below.

We greatly appreciate your consideration of our manuscript. Please do not hesitate to contact me if you have any questions or concerns.

Sincerely,

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Responses to reviewer comments:

**Reviewer: Paul Levett**

In the Methods, under heading Study population and routine clinical testing, first paragraph, it would be helpful if the authors would specify that cell counts and chemistry values were derived from CSF specimens.

We have revised the wording in the Methods section revised to make it clear that the criteria applies to CSF specimens (page 6, lines 12-16).

**Reviewer: Julio A Vazquez**

- Authors mention in page14 second paragraph that 4 specimens were defined as indeterminate by RT-PCR due to its cycle threshold value. However two of them were positive defining genogroup by RT-PCR and apparently they were still defined as indeterminate. Some type of discussion should be desirable in this
context. Has the author done some additional analysis of those specimens? ctrA false negative has been already described (just in a few number of invasive strains) using the most common protocols and in addition, protocols for genogroup are generally less sensitive than ctrA protocols etc

Upon further review of the data, the statement referenced here about the serogroup-specific RT-PCR was incorrect using current CT value definitions for positivity. Therefore, we have removed the sentences in the Discussion discussing the indeterminate specimens.

- The authors state in page 15 line 20 that "antimicrobial susceptibility testing requires that the pathogen be cultured" and the statement might be refined: there are data bases with public access for those genes potentially involved in resistance (particularly in meningococci) associating allele and susceptibility level, at least for penicillin, rifampicin and quinolones.

We have revised the sentence to state that “…conventional antimicrobial susceptibility testing requires the pathogen to be cultured…” (page 15, line 19).

Reviewer: Rodrigo Hasbun

1) I would comment on the fact that a CSF culture was only done when the CSF WBC count was > 1,000. The Dutch Meningitis Cohort study of 696 patients documented that a CSF <1,000 was present in 29% of their patients and was a prognostic factor for an adverse clinical outcome. It is unclear if the results would be different for patients with a CSF WBC <1,000. This needs to be clarified and added as a limitation of the study.

Dr. Hasbun raises a good point, and we have added language emphasizing that local testing recommendations might have selected a unique study population (page 16, lines 20-22). In addition, we already have language in the discussion that addresses the potential for different results in different study populations (page 17, lines 6-9).

2) In figure 1, in the last boxes, It is redundant to include both CSF culture negative and CSF culture positive cases. I would consider placing one box with CSF culture positive and the other box with CSF culture + CSF Gram stain positive

Since we are presenting the data in a flow diagram showing the test result of each case included in the study, some redundancy is unavoidable. To accommodate Dr. Hasbun’s suggestion for CSF Gram stain results in this chart, we have revised it to include another row of boxes that further stratifies the cases by CSF Gram stain result.

3) The utility of table 5 is questioned. There is a small sample size (n=20) in the RT PCR group that is the focus of this table and it is compared to the other two groups (All test negative and all test positive) with no statistical analyses. I would
consider deleting this table and if needed adding a small section in the
discussion stating that there were no significant differences between the three
groups regarding key characteristics with a P value <0.05.

Dr. Hasbun is correct that this table could potentially be summarized in the text.
However, we believe there is value in its inclusion to visually show differences and
similarities in each group. The differences are empirically clear enough that we do not
believe statistical comparisons would add much to the table; however, if the editors
prefer this we would be happy to do this.

4) There is no mention of the potential use of Binax NOW for Streptococcus
pneumoniae in the discussion given it’s high sensitivity and specificity and it’s
cost and rapid results (<15 minutes). It would be worthwhile testing the isolates
that were + for Streptococcus pneumoniae if the samples are still available.

Unfortunately, the original CSF specimens are no longer available for testing. However,
we agree that the rapid immunochromatographic test Dr. Hasbun is referring to has
promising test characteristics and might play an important role in diagnosis of
pneumococcal meningitis. We have added some language to mention this test and
reference it in our discussion of other tests that might be helpful in evaluating RT-PCR
(page 15, lines 6-10).

Minor revisions:
1) Change "was" to "were" in page 13, line 6.
2) Typo in page 25, Table 4, Sensitivity of Gram stain in antibiotic negative is
"1.0"?

We have corrected the errors as suggested.