Author’s response to reviews

Title: Development of a TaqMan Array Card to Target 21 Purulent Meningitis-related pathogens

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Author’s response to reviews:

Dear editors and reviewers,

Thank you very much for the comments about our manuscript. The manuscript entitled “Development of a TaqMan Array Card to Target 21 Purulent Meningitis-related pathogens” (Manuscript Number: INFD-D-18-01958) has been revised in details according to the comments. We appreciate your valuable comments very much, which are helpful to improve the quality of our present study. All the comments have been incorporated into the revised manuscript by highlighting. The point-by-point responses to the comments are stated below.

Reviewer #1:

1. This manuscript reports a TAC card array for bacterial meningitis (PM-TAC) in this study which can detect 21 PM-associated pathogens in 3 hours and shows a higher sensitivity than the
CSF culture particularly for CSF samples collected after empiric antibiotic therapies. This is not novel, as there are now many commercial molecular assays for meningitis such as Biofire FilmArray (14 targets), FastTrack Diagnostics (4 bacterial, 5 viral).

Response: Thank you for your question. Yes, there are now many commercial molecular assays for meningitis such as Biofire FilmArray (14 targets), FastTrack Diagnostics (4 bacterial, 5 viral). But PM-TAC in this study has its advantages such as the ability to detect more pathogens and low cost compared Biofire FilmArray (14 targets), and its advantages such as simple operation, sampling by centrifugation and simultaneous detection of 21 pathogens using only one kind of probe. Moreover, PM-TAC in this study can show the pathogen load correlation with patient's severity.

2. Line 45/46 The disease is characterized by acute onset, high fever, severe headache, vomiting, stiff neck, and high disability and mortality rates. In infants and young children, these "classical" signs are often absent. They may be poor feeding, lethargy, disorientation or reduced conscious level.

Response: This is an excellent comment. We have revised as suggested. For details please refer to lines 46-48.

3. Line 70: In the introduction, there is no mention of the Biofire FilmArray or FastTrack diagnostics panel as PCR platforms.

Response: Yes, we have added the mention of the Biofire FilmArray as PCR platforms. For details please refer to lines 71-77.

4. Line 81-84. Although TAC systems have been used successfully to identify pathogens, a comparison of the sensitivity of a TAC versus the CSF culture method to identify pathogens in CSF samples from patients receiving empiric antibiotic treatments is still lacking.

Response: Yes, the reason for a comparison of the sensitivity of a TAC versus the CSF culture method to identify pathogens in CSF samples from patients receiving empiric antibiotic treatments is still lacking is that it is really difficult to collect cerebrospinal fluid samples clinically.

5. Line 147-153: total of 32 children with PM were selected with a representative cross-section of the patient population in the Department of Infectious Disease of Beijing Children's Hospital. Of the 32 patients, 13 showing positive CSF culture before receiving antibiotics but negative after receiving antibiotics, 2 showing positive CSF culture before antibiotic therapies and remaining positive after the antibiotic therapies, 17 showing negative CSF culture before and after antibiotic therapies. Please can the authors explain how they got samples before and after
antibiotics samples. Was the CSF repeated after receiving antibiotics? Is that ethical? Was there ethics approval to do 2 lumbar punctures?

Response: Clinically, in order to better monitor the progress of the PM, it is necessary to perform lumbar puncture more than once. And that was approved by the Institutional Review Boards of the Institute of Microbiology and Epidemiology and Capital Medical University of China (IEC-C-008-A08-V.05.1). Written informed consent was obtained from a parent or guardian prior to collect CSF from the children.

6. This study is let down by the small sample size. Only 7 samples subsequently had a Ct value which makes any meaningful interpretation difficult.

Response: Yes, there was a small sample size indeed in this study, but it is particularly difficult to collect enough cerebrospinal fluid in children clinically. Many clinical examinations were performed so almost all cerebrospinal fluids were used up which reduced greatly the sample size. Although the sample size is small, it can reflect a very meaningful phenomenon. With the accumulation of sample size of cerebrospinal fluid, we plan to do more in-depth related research in the future.

7. The cost of $126/sample is still prohibitively expensive for resource poor settings and is more than the Biofire FilmArray system, which takes 1 hour.

Response: Yes, the cost of $126/sample equal to about $6/pathogen of PM-TAC is still prohibitively expensive for resource poor settings, but the cost of $400/sample equal to about $30/pathogen of Biofire FilmArray system is more expensive for resource poor settings. Additionally, with the amount of PM-TAC purchased increase, the cost of PM-TAC will decrease. Moreover PM-TAC can detect 21 or more targets in 3 hours but Biofire FilmArray system 14 targets in 1 hour.

8. The finding of pathogen load correlation with severity is not new, and has been shown with pneumococcal meningitis. (Carrol ED, Pediatr Infect Dis J.2007)

Response: Yes, there is report about the finding of pathogen load correlation with severity, but there is no report about the simultaneous detection of 21 pathogens in 3 hours and the finding of pathogen load correlation with severity.

Reviewer #2:

1. This a PCR technique to detect a panel of pathogens found in a paediatric population. The control samples were those who had similar symptoms but not meningitis. A artifcial CSF was also an additional control.
The study is well done and has taken care to safeguards against pitfalls in the study.

2. Apart from a few englsih errors the article bears merit and a larger study to test its effcetiveness especially in partially treated acuet bacterial meningitides would be valuable with clincal controls being other non acute bacterial meningitides, such as TBM, patients with non-specific CSF changes, but not normal CSF.

Response: The manuscript has been edited by Liwen Bianji, Edanz Group China.

Thanks to reviewers for their thoughtful and thorough reviews. Hope these will make it more acceptable for publication.

Sincerely yours,