Author’s response to reviews

Title: A systematic review of the diagnostic accuracy of Loop-mediated-isothermal AMPlification (LAMP) in diagnosis of invasive meningococcal disease in children.

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Version: 1 Date: 19 Nov 2018

Author’s response to reviews:

We thank the editor and all three reviewers for their time and their detailed peer review. We have worked hard to address all of your questions and suggestions and we have essentially re-written the entire review. We also took additional advice from members of the Cochrane Collaboration regarding the meta-analysis in direct response to reviewer 1. We agree with reviewer 1 that the number of studies and the differences between them make a meta-analysis unsafe statistically and we now offer simple descriptive statistics summarising the results of individual papers.

Below are our responses to the editor and then the reviewers.

Editor

Technical Comments:

Title page: We have noted that the corresponding author identified on the title page is different to the author on file in the editorial manager system. Please ensure that the corresponding author provided on the title page matches in the manuscript file and in the editorial manager system.
This has been amended

Headings: Kindly rename the "Introduction" heading to "Background".

This has been amended

Editor Comments:

Thank you for submitting your article and apologies for the long time to respond. Please note the reviewer comments below, which all need to be addressed.

Please note Reviewer 1's comments and add this to the limitations in the discussion.

We agree in part with reviewer 1. There is insufficient data for the meta-analysis to be reliable and we have therefore changed the analysis to a purely descriptive analysis. We also acknowledge that Lee et al is the largest study and acknowledged the limitations this brings.

The Lee et al study however, only used CSF specimens and we feel there is value in including other studies that have determined the DTA of LAMP on Blood and Naso/oropharyngeal swabs.

Reviewer 1

This is a systematic review of the diagnostic accuracy of LAMP in diagnosis of invasive meningococcal disease in children. For this purpose, authors used just three reports. One of them included really large number of samples compared to other two reports. Author's results were dramatically influenced by this one report by Lee et al. I don't think there is any value on such complex calculations.

We thank you for your review. We agree that given the small number of studies a meta-analysis is inappropriate and in the revised manuscript we report a systematic review without meta-analysis.
The Lee paper whilst large only reported on the use of LAMP for CSF specimens whereas the other two publications included blood and naso/oropharyngeal swabs. As such we feel there is value in including their reports.

LAMP technology for detection of invasive meningococcal disease is relatively new and as such there are few diagnostic accuracy studies published. We do however, feel this is an important review and that the data provided is relevant to the meningitis community.

Reviewer 2

This article provided a systematic review and meta-analysis of Loop-mediated-isothermal AMPlification (LAMP), a PCR-based, rapid test for the detection of meningococcal diseases. Through the review and meta-analysis, the authors concluded that LAMP is a highly accurate test for invasive meningococcal infection that can be performed on a range of clinical specimens and offers potential to improve the rapid diagnosis of invasive meningococcal diseases. The paper is well organized and scientifically important for meningitis community. Given the severity of this disease, early detection is critical for case management. LAMP test may serve as the next generation rapid diagnosis test for meningococcal diseases.

We thank you for your thorough review and we thank you for your recognition of the value of this review. We agree this review is highly relevant to the meningitis community.

Below we respond to your points raised:

1. Pg 4 Lines 11-18. Were patient age and gene target (ctrA) the only criteria for inclusion/exclusion selection? If so, I wonder why the analysis was limited to less than 18 yrs. Young adults such as college students are also at high risk for meningococcal diseases. It would be interesting to know LAMP performance for that population. Among the published literatures you have screened and reviewed, were there any other gene targets used? ctrA is a good target for encapsulated meningococci but not for nongroupables.
We are a team specialising in the diagnosis of meningitis and severe infection in children and as such we felt our expertise was only to explore the DTA in a paediatric population. We acknowledge the suggestion and we would consider a future collaborative review to explore LAMP DTA in adolescents / adults. Having already published the protocol we are concerned that any change to the inclusion criteria now would undermine the credibility of the study.

We thank you for highlighting the point regarding the ctrA region. We apologise for causing confusion here. The inclusion criteria for the review were actually “LAMP for Neisseria meningitidis “ – please see the attached search strategy and the original open access protocol. The review found that all of the LAMP assays were directed against the conserved ctrA region specific to all pathogenic forms of Neisseria meningitidis. To make this clearer we have changed the inclusion criteria in the methods to reflect to protocol and search strategy to remove reference to the ctrA region and then made it clear in the results that all of the LAMP assays for Neisseria meningitidis have primers for the ctrA region.

Furthermore regarding the ctrA as a target, it is generally accepted that acapsular (ctrA-negative) meningococci are associated with carriage, but do not cause invasive disease. Acapsular strains are certainly of interest in terms of meningococcal carriage, epidemiology and immunity. However, detection of the ctrA gene, allowing selective detection of capsular strains only, has been the focus of molecular assay development for diagnosis of invasive disease.

2. Pg 6 Lines 3-5, of the 36 studies screened, most were excluded in the analysis. Were the findings from the excluded studies consistent with your conclusion? If not, suggest including some comments to rule out biased selection.

We have added additional detail to the results section. We have explained that 31 studies were excluded as they were clearly irrelevant from the title/abstract. The two studies excluded following review have been referenced and an explanation given. This is in keeping with PRISMA guidance.
3. Table 1. What was the age range in the original study and the proportion of cases excluded? Suggest including columns for these information. Did the reference qPCR use the same gene target ie ctrA?

We have added the age ranges to table 1 and we have included the total number to show the proportion excluded. We have also clarified in the results section that in all studies the LAMP and qPCR testing were directed at the ctrA region of Neisseria meningitidis, for the reasons outlined above.

4. Table 2. Most of meningococcal strains in oropharynx do not produce capsule and are negative for ctrA. It is surprising to see that LAMP targeting ctrA showed high sensitivity and specificity unless the reference test used the same target. The concern is that ctrA LAMP may have missed meningococcal strains that were negative for ctrA. Would suggest discussing this limitation in the paper.

Thank you for highlighting this, we have added a section in the discussion relating to this. We suspect that the high accuracy of naso/oropharyngeal swab testing in predicting invasive MD in children relates to (i) the low carriage rates of Neisseria meningitidis in young children and (ii) acapsular (ctrA negative) strains of meningococcus do not cause invasive MD.

We are unclear as to the value of a diagnostic test that detects acapsular non-pathogenic meningococci.

Molecular reference tests (Eg. qPCR) and the LAMP tests target the ctrA gene, for the reasons outlined above. The LAMP assays and reference assays are designed to selectively detect ctrA-positive capsular strains, not ctrA-negative strains.

5. Pg 11 Lines 8-10. If the purpose was to determine the accuracy of LAMP tests for invasive MD, can you explain why NP and OP swabs were included in the analysis? They are not good specimen types for invasive diseases.

We agree the rationale here was not well explained. We have re-worded the methods, results and discussion to reflect that we are looking at using testing of NP and OP swabs to predict those children with invasive MD. The rationale has been expanded to outline that in young children that carriage rates are low and as such a positive/negative NP or OP result in a young child might be used to direct treatment without the need for more invasive testing. It maybe that NP/OP rapid testing could be used as a non-invasive way to predict MD in children.
To better understand the context of the review and meta-analysis, it would be extremely helpful to include a table describing study characteristics for the 31 studies excluded, similar to table 1.

The 31 studies excluded were not relevant to the review and a table similar to table 1 could not be completed. We have however, expanded on this in the results section and provided greater detail on the 2 studies using spiked specimens that were excluded. This is in keeping with PRISMA guidance.

Reviewer 3

This systematic review and meta-analysis appears to be well conducted, however, suffers from a paucity of relevant studies that could be included. This is, of course, not the fault of the authors and not something that can be amended.

We thank you for your review and we agree there are only a small number of available studies. On reflection from your comments and those of reviewer one we have removed the meta-analysis and instead provide a systematic review of the available literature.

Some minor comments:

1. The statistical methods appear to be appropriate however there are some obvious errors that need amending.

   For instance in Table 2 the specificity is reported as 100% for the Lee study yet there are false positive results so this is impossible. Similarly for the Bourke study, 100% sensitivity is reported for Swabs even though there are 4 false negs. The McKenna results don't look right either.

   The results in table 2 don't appear to be the ones in the meta-analysis as figure 3 reports different sens and spec so I think these are just typos in table 2.

   Table 2 has been corrected. All statistics are reported to 2 decimal places so for example the specificity for the Lee study is 0.996 which is why it appears as 1.00 or 100% in the table.
2. The Stata package used for the meta-analysis should be cited in the methods section and the authors of the package appropriately referenced.

This was referenced in the methods on page 6 lines 7-8 as follows “All analysis was performed using STATA version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).”

3. It would be helpful to have the references for the three studies included in Tables 1 & 2 reported in the tables so that these paper can be easily found. There are two Lee 2015 papers so name and year alone is not sufficient to point the reader to the correct paper if they want to read it.

The references have been added to the table.

These references have been added

4. CSF results for the Bourke study have not been included. Can the authors provide an explanation/justification for this exclusion?

These have been included

5. McKenna study: the Blood EDTA and the serum results have been combined under one heading 'blood'. This may not be the best approach as these may be replicates of multiple samples on the same participants. In Bourke it is Blood-EDTA only so for consistency EDTA and serum should probably be reported separately as they are in the paper. In addition the tables should have footnotes to give details of all acronyms and definitions.

We have contacted the author (James McKenna) and clarified that testing was on either serum or EDTA but not both i.e. one blood test per patient.

We have added footnotes to all of the tables.
7. McKenna report 139 throat swabs in Table 3 of their paper yet there are 255 in the review - I can't see where the numbers are coming from

Apologies for the error there was a typo in the table. Having contacted the author (James McKenna) we can confirm there were 203 separate patients with blood samples (EDTA or Serum) without duplication. There were 21 patients with CSF samples and there were 155 swabs (a mixture of naso/oropharyngeal and respiratory swabs). We have expanded the results section to make this clearer and amended the table.

For the discussion some additional thoughts:

Can the authors make it more clear that this review compares the diagnostic accuracy of the LAMP test to conventional RT-PCR +/- blood culture tests. Not the accuracy of LAMP in diagnosing MD.

This has been made clearer throughout the manuscript

The gold standard for diagnosing MD is RT-PCR +/- BC however this gold standard is an imperfect gold standard and may not detect all true cases of MD. Those isolates classified as false positives by LAMP may in fact be true disease cases that were not detected by PCR and the authors could include some discussion of this possibility. What are the implications of having false positive LAMP samples and are they really 'false'?

This is a really important point and we have specifically added a section to the limitations highlighting this important point.

In addition, what are the implications of having false negative samples by LAMP? Presumably this is a much more worrying possibility and as the confidences intervals for the sensitivity of LAMP is as low as 71% (or 24% for blood and CSF only), this means potentially 29% (or up to 76%) of cases may be missed by LAMP compared with PCR.
Further comment has been made around this area in the discussion.