Reviewer's report

Title: Comparison of the specificity of different Streptococcus pneumoniae specific PCR assays

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Reviewer: Jim Dunn

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El Aila at al. have developed a novel classic PCR assay that demonstrates excellent specificity for S. pneumoniae. The assay was negative when tested against a number of other non-pneumococcal viridans streptococci including S. pseudopneumoniae. This latter species has been known to cross-react and generate a positive result with other pneumococcal PCR assays, the S. pneumoniae AccuProbe test, and a commercially available S. pneumoniae antigen detection assay.

S. pseudopneumoniae was first described in 2004 and since that time has been identified in a number of clinical samples. The pathogenicity of this particular species is still under investigation but it appears to be less virulent than S. pneumoniae. As the authors of this study point out, it can be useful to rapidly and accurately identify S. pneumoniae, particularly from blood and CSF specimens. The data presented in this paper suggest that the species-specific identification of S. pneumoniae can be accomplished without cross-amplification of S. pseudopneumoniae, other viridans streptococci, or other organisms commonly found in the respiratory tract.

The majority of the data presented in this paper has been derived from previous studies. Most of the novel data appears to be that derived from testing of the listed streptococcal strains by the newly described PCR assay (Spne-PCR) and the previously described Snpn9802-PCR. In addition, three more S. pseudopneumoniae isolates have been assessed by using the five different PCR assays.

Minor Essential Revisions

There are several items in this report that require correction and/or clarification before publication.

Table 1 requires a number of modifications and clarifications including:
1. The first two header rows are duplicates.
2. There is no reference to the meaning of the first two columns of the table labeled "Rek" and "LBV". These need to be clarified and the relevance of the values in those columns stated or the columns should be deleted.
3. There is no legend for the abbreviations used after the Group I, Group II, and Group III notations. It appears that o=optochin, C=capsule, and A=AccuProbe but
this is not made clear. If these are the correct abbreviations, the optochin and capsule results would then be duplicated in the subsequent columns. If AccuPorbe results are to be included, they too should be stated clearly in a separate column.

Title page: A more appropriate title might read better as "Comparison of the specificity of five different PCR assays for Streptococcus pneumoniae"

p.2, line 5: The PCR product appears to be 217 bp instead of 216 bp

p.2, line 4-6: It's unnecessary to include the primer names in the abstract. The sentence would read better as "This report describes a newly-developed PCR assay (Spne-PCR), amplifying a 217 bp product of the 16S rRNA gene of S. pneumoniae, and its performance compared to other genotypic and phenotypic tests".

p.2, line 9: The four previously described assays should fall in parentheses as "PCR assays (psaA, LytA, ply, spn9802-PCR) for the specific amplification of S. pneumoniae".

p.2, line 10: Make this a new sentence.

p.3, line 7: "cut-offs" should be "antibiotic susceptibility breakpoints"

p.3, line 8: "resistance" should be "susceptibility"

p.3, line 11: omit "and" before the period

p.4, line 24: should read "Spn9802-304R"

p.5, line 5: should read "Spne-PCR assay". From the heading it appears that there is more than one newly developed assay.

p. 5, line 13: Was the concentration of input bacterial DNA equivalent for each assay? Please clarify.

p. 5, line 17: "respective annealing temperatures" suggests that multiple new assays are being assessed. Should this be 57 degrees C?

p.6, line 10-11: No S. parasanguinis isolates are listed in the Methods nor in Table 1.

p.6, line 12: Reference [15] should be reference [9].

p.6, line 16: should read "PCR assays were negative for the 12 organisms commonly found in the respiratory tract, 8 S. mitis group isolates, 7 S. oralis isolates"

p.6, line 17: The 3 S. sanguis and 2 S. parasanguinis isolates mentioned here are not listed in the methods or in Table 1.

p. 7, line 4: The AccuProbe results are not clearly listed in Table 1.

p. 9, reference 9: Should use correct citation as "J Clin Microbiol"

p. 11, line 3: Should read "amplification of S. pneumoniae and the homologous sequence for S. pseudopneumoniae"

p. 14: primers should read Spn9802-143F and Spn9802-304R and should be from reference [9], the original citation.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.