Reviewer's report

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

**Version:** 2 **Date:** 11 January 2010

**Reviewer:** Guido Bloemberg

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The manuscript by Abdullah El Aila et al. describes the construction of a 16S rRNA gene based identification PCR for the differentiation between P. pneumoniae and S. pseudopneumoniae. The authors compare this PCR to four other PCR’s which were published before. The paper does not really make clear why an additional 16S rRNA gene based assay is required since the psaA assay is as specific.

**Major Compulsory Revisions**

1. The title is not very adequate for the paper. I would suggest to change it in a more specific title such as: `The development of a 16S rRNA gene based PCR for the identification of Streptococcus pneumoniae`.

2. Page 2. Delete the second sentence of the conclusion, since this is already implicated by the first sentence.


4. Page 3, lines 14-15. ‘Because tests, …past decades’. This is an unclear sentence, probably part of this sentence is lacking.

5. Page 3, line 21. Please specify in detail the criteria that were used to consider a sequence as erroneous.

6. Page 5, line 14-15. It would be important to show a gel electrophoresis of the stringent annealing temperature analysis. For example it would be useful to show the results with annealing temperatures of 55, 56, 57, 58 and 59 degrees.

7. Page 6, line 5-6. Please specify the remaining problems for specific strains after the publication of Verhelst et al 2003, since the conclusion of this paper is that the psaA PCR, which was used in the Verhelst et al 2003 publication is as good as the 16S rRNA gene PCR presented in this manuscript.

8. Page 6, line 22-23. In my opinion the strains which were negative for the Spne-PCR but positive in other assays should be confirmed for possessing the specific T at position 818 (see figure 1).

9. Page 7, line 3. The term ‘unambiguously’ is in my opinion questionable, as a sequence homology of more than 99.7% of S. pneumoniae and S. pseudopneumoniae was demonstrated in earlier studies (Arbique et al., JCM, 2004, 42:4686-96).

10. Page 7, lines15-17. What is the advantage of sensitivity when cultures are
used as material. I can only imagine that sensitivity could be of relevance when direct detection is required. Please state that as such.

11. Page 11. Figure 1. Please add to this figure the sequences of all Streptococcus spp. of the S. mitis group in order to show the specificity of the primer set. Is the sequence between the two primers 100% identical for S. pneumoniae and S. pseudopneumoniae?

12. Page 14. Table 2 can be deleted since the new primer sequences are already shown in Figure 1. The annealing temperatures can be mentioned in the text.

Minor Essential Revisions

13. Page 4, lines 7-8. For better understanding, the 6 strains of S. mitis and 3 strains of S. oralis might be described as being ‘reference strains’ from the previous study (Reference number 11).

14. Page 5, line 5. Replace ‘Newly developed PCR assays with `16S rRNA gene PCR assay’.

15. Page 6, line 10. Mustn’t it be the correct reference number 11? Otherwise, those strains are not described in the methods.

16. Page 6, line 11. the strain S. parasanguinis wasn’t mentioned in the methods as being tested, as well as the strain S. sanguinis (line 17).

17. Page 6, line 15. Is the number 12 the correct reference? (in the methods are these strains described as being tested in reference number 11)

18. Page 11, line 3: the species (S. pneumoniae) name lacks the letter ‘e’.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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