Reviewer's report

Title: Sequential multiplex PCR assay for determining capsular serotypes of colonizing S. pneumoniae

Version: 2 Date: 13 January 2011

Reviewer: Judith E Mueller

Reviewer's report:

This is a well written paper reporting an interesting experience of significance for research on pneumococci.

The research question has been well formulated, methods appear appropriate and well described, with two exceptions of minor significance:

1. The methods of the carriage study that yielded the Sp strains should be briefly described, to allow the reader judging on whether this was a representative sample of the age group, the number of colonies analysed per participant, and whether multiple carriage was detected.

2. Part of the result section (approximately lign 134 to 163) appear to be a mix of methods, results and discussion. Maybe the manuscript could gain on clarity by re-structuring this section.

A technical question is, why only 332 isolates were included in the study. I understand that this is because 30 non-typable isolates were excluded, but I guess that it may have been interesting to see how specific PCR testing is for these non-typable carriage isolates. Maybe this can at least be addressed in one sentence.

All data appear well reported, the discussion and conclusion balanced. Limitations are listed.

The theoretic geographic limitation of one algorithm should be discussed a bit more. Although the main serotypes in carriage seem to be similar across the world, as the author write, the minor serotypes may vary between countries and continents, as disease serotypes vary a lot. This is important for recommending that laboratories in Africa could simply adapt the presented algorithm or whether preliminary studies are required.

In the discussion, I would suggest addressing the need for ongoing control with Quellung technique of PCR non-typable isolates, to assure that no emerging serotype is missed in carriage studies.

Minor corrections

The year of publication is missing for some of the references.

Figure 2: the cumulative percentage is missing after PCR 4.

Table 1: column “aim of the study”, section “no plating” should be “PCR-based serotyping directly on clinical specimens”, not “directly on clinical isolates”.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I have received honoraria for consultation from GSK, a producer of a pneumococcal conjugate vaccine. I do not have any other competing interests.