40

Utility of a Reverse-Hybridization Strip Assay for *Streptococcus pneumoniae* serotyping

12th EUROPEAN MEETING ON THE MOLECULAR BIOLOGY
OF THE PNEUMOCOCCUS
(EUROPNEUMO 2015)
7th-10th July 2015 St Catherine's College, Oxford, UK

Authors : Ercibengoa M[3]*, Gamen S[4], Manrique A[4], Marimón JM[1,2], Pérez-Trallero E[1,2,3]
Affiliations : University Hospital Donostia[1], CiBERES[2], Basque Country UPV/EHU[3], San Sebastián, Spain and OPERON S.A.[4] Zaragoza, Spain

INTRODUCTION

Streptococcus pneumoniae is one of the leading causes of sepsis, meningitis and respiratory disease worldwide specially in children <5 years of age and adults >65 years. Therefore, specific typing tools are needed for accurate epidemiological surveys.
The Microbiology Department of Donostia University Hospital (San Sebastián, Spain) and the Immuno and Molecular diagnostic company Operon SA (Zaragoza, Spain) are developing a reverse-hybridization strip assay to make pneumococcal serotyping a rapid and simple process with high sensitivity and specificity.

OBJECTIVES AND METHODOLOGY

Objectives

- Design a multiplex-PCR and a reverse hibridation assay to help to identify the *S. pneumoniae* serotypes included in the PCV13 and another 4 related serotypes (6C, 6D and 18A, 18F).
- Extend the assay to all described serotypes, assessing the possibility of detecting some serotypes as a group.
- Patent the technique based on the results and subsequently publish the validation results.

Methodology

✓ Specific primers (GenBank, Sanger Inst. Cambridge, accession CR931632 to CR931722) were used for PCR, the resulting amplicons were placed on the nylon strips containing the 17 probes and analyzed in a multiple sample device by reverse hibridation (Auto-LiPA (INNOGENETICS) .

✓ The PCR and the hybridization reaction with serotype-specific probes was validated using reference strains for each serotype.

✓ The initial performance of this serotyping technique was assessed with 66 *S. pneumoniae* clinical isolates collected at the Donostia University Hospital Microbiology Department that had been previously serotyped with the Quelling reaction (gold-standard technique) .

Fig. 1. The two work centers involved in the project and the prototype being developed

RESULTS AND CONCLUSIONS

- This first prototype (serotypes included in the PCV13) was initially validated against reference isolates of 94 pneumococcal serotypes showing 100% specificity

- The concordance of this technique in the serotyping of the 66 previously serotyped clinical isolates with the Quellung reaction was 100% : the 59 strains belonging to the 17 serotypes included in the strip were correctly identified and another 7 strains of serotypes not included gave no reaction. A prospective study is now being carried out.
- Because of similarity of capsular sequences, serotypes 7A/7F, 9A/9V and 18B/18C could not be differentiated between them.

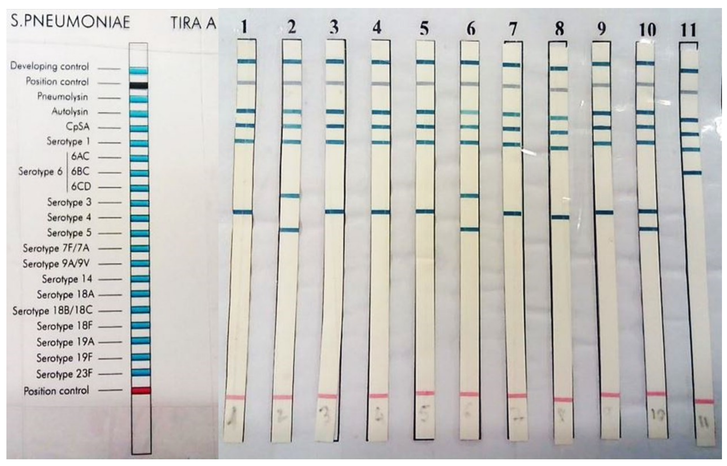

Fig. 2. Template with serotypes included in the first prototype and one experiment conducted

Conclusions

- The reverse-hybridization strip assay proved to be a good tool for pneumococcal serotyping. This technology is simple and applicable to most laboratories that carry out PCR techniques.
- Its advantage compared to Quellung reaction is (1) the possibility of working in batches of samples (2) no experienced staff is required to obtain and interpret results and (3) results records (hybridized strips) can be stored.
- With this technique, some closely related serotypes could not be discriminated because of sequences similarity.