

## Real-time PCR targets for *Streptococcus* species detection and antibiotic susceptibility in SPN\*

| Real Time PCR Target  | Primer/ Probe | Sequence (5' – 3')  | Gene         | Accession No. | Location | Conc. (nM) |
|---|---------------|---|--------------|---------------|----------|------------|
| <i>Streptococcus pyogenes</i> <sup>1</sup>                    | Forward       | GCA CTC GCT ACT ATT TCT TAC CTC AA                        | <i>spy</i>   | AE006565      | 295-320  | 300        |
|   | Reverse       | GTC ACA ATG TCT TGG AAA CCA GTA AT                        |              |               | 367-392  | 300        |
|   | Probe         | 5'-FAM-CCG CAA C"TT" C ATC AAG GAT TTC TGT TAC CA-3'-SpC6 |              |               | 325-353  | 100        |
| <i>Streptococcus pneumoniae</i> <sup>2</sup>                  | Forward       | ACG CAA TCT AGC AGA TGA AGC A                             | <i>lytA</i>  | EA005672      | 1841014  | 200        |
|   | Reverse       | TCG TGC GTT TTA ATT CCA GCT                               |              |               | 1840961  | 200        |
|   | Probe         | 5'-FAM-TGC CGA AAA CGC "TT"TG ATA CAG GGA G -3'-SpC6      |              |               | 1840985  | 200        |
| <i>Streptococcus agalactiae</i> <sup>3</sup>                  | Forward       | GGG AAC AGA TTA TGA AAA ACC G                             | <i>cfb</i>   | JQ289578      | 207      | 200        |
|   | Reverse       | AAG GCT TCT ACA CGA CTA CCA A                             |              |               | 311      | 200        |
|   | Probe         | 5'-FAM-AG ACT TCA TTG CGT GCC AAC CCT GAG AC-3'-BHQ1      |              |               | 263      | 200        |
| SPN*†<br>Detection and antibiotic susceptibility <sup>4</sup> | Forward       | CTT GGA TAT TCA CCG AAC AC                                | <i>ermB</i>  | AB111455      | 766      | 300        |
|   | Reverse       | TTG GTT TAG GAT GAA AGC AT                                |              |               | 844      | 300        |
|   | Probe         | 5'-ROX-AA GTC TCG ATT CAG CAA TTG CTT AAG-3'-BHQ2         |              |               | 807      | 100        |
|   | Forward       | TAT GGA GCT ACC TGT CTG GA                                | <i>mef</i>   | AF227520      | 291      | 200        |
|   | Reverse       | GGT ACT AAA AGT GGC GTA ACC                               |              |               | 375      | 200        |
|   | Probe         | 5'-HEX-CC GTA GCA TTG GAA CAG CTT TTC-3'- BHQ1            |              |               | U83667   | 100        |
|   | Forward       | CTG TTT GGA CCA TAT AGG TAT TT                            | <i>pbp2b</i> | AE007317      | 1494906  | 300        |
|   | Reverse       | CAA TTC TTG GTA TAC TCA GGC T                             |              |               | 1494676  | 300        |
|   | Probe         | 5'-Cy5-TC CAG AGC TTG GAC CGC TGT GAT A-3'-BHQ3           |              |               | 1494938  | 100        |

\* SPN : *S. pneumoniae*; †- This quadriplex assay (as mentioned in reference 4) should be run together with *lytA* real-time PCR oligos

<sup>1</sup>Kodani et al., 2011. Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. J Clin Microbiol. 49(6):2175-82.

<sup>2</sup>Carvalho, Mda G. et al 2007. Evaluation and improvement of real-time PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection of pneumococcal DNA. J Clin Microbiol. 45:2460-6.

<sup>3</sup>Diaz et al. 2013. Optimization of Multiple Pathogen Detection Using the TaqMan Array Card: Application for a Population-Based Study of Neonatal Infection. PLoS One. 21;8(6):e66183.

<sup>4</sup>Srinivasan et al. 2011. Quadriplex real-time polymerase chain reaction (*lytA*, *mef*, *erm*, *pbp2b*(wt)) for pneumococcal detection and assessment of antibiotic susceptibility. Diagn Microbiol Infect Dis. 71(4):453-6.