

## 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"TC ATC AAG GAT TTC TGT TAC CA-3'-SpC6 "T" = BHQ1			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 "T"TG ATA CAG GGA G -3'-SpC6 "T" = BHQ1			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
<i>Streptococcus salivarius</i> <sup>4,5</sup>	Forward	CAC GCC ATG CTG GAA GTG	<i>gtfP</i>	CP013216	756384	200
	Reverse	GCG ATG AGC CAA GCT GAA G			756405	200
	Probe	5'-FAM-TTA GCT GCT GCG TAG ACT TCG TCT-3'-BHQ1			756433	200
<i>Streptococcus suis</i> <sup>6</sup>	Forward	TCC RAT RCT GCT CTG CCA TT	<i>fbpS</i>	CP003993	1333350	200
	Reverse	TGA TAG TAG AAG TCC AGC ARA CT			1333374	200
	Probe	FAM-AA TAG CCC "T"GA AAA MCA GCC ACWYTT TGA RA-6SpC; "T" = BHQ1			1333435	100
SPN*† Detection and antibiotic susceptibility <sup>7</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 U83667	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			333	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 quadruplex 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>Seow et al. 2009. Oral Streptococcus species in pre-term and full-term children – a longitudinal study. Int J Peadiatr Dent 19: 406–411.

<sup>5</sup>Srinivasan et al. 2012. Using PCR-based detection and genotyping to trace *Streptococcus salivarius* meningitis outbreak strain to oral flora of radiology physician assistant. PLoS ONE 7(2): e32169. doi:10.1371/journal.pone.0032169

<sup>6</sup>Srinivasan et al. 2016. Species-specific real-time PCR assay for the detection of *Streptococcus suis* from clinical specimens. Diagn Microbiol Infect Dis. 85(2): 131-132.

<sup>7</sup>Srinivasan et al. 2011. Quadruplex 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.