

Oligonucleotides to detect meningitis bacterial pathogens using triplex real-time PCR

| Real Time PCR Target | Primer/ Probe | Sequence (5'–3') | Conc.(nM) | Reference |
|--|---------------|---|-----------|-------------------------------------|
| Triplex Reaction - 1 | | | | |
| <i>Streptococcus pneumoniae</i> ¹ | lytA-F | ACGCAATCTAGCAGATGAAGCA | 200 | Ouattara et al. (2019) ⁶ |
| | lytA-R | TCGTGCGTTTTAATCCAGCT | 200 | |
| | lytA-P | 5'-Cy5-TGCCGAAAACGC"TT"TGATACAGGGAG-3'-SpC6 | "T"=BHQ2 | |
| <i>Neisseria meningitidis</i> ² | sodC-F | GCACACTTAGGTGATTTACCTGCAT | 300 | |
| | sodC-R | CCACCCGTGTGGATCATAATAGA | 300 | |
| | sodC-P | 5'FAM-CATGATGGCACAGCAACAAATCCTGTTT-3'-BHQ1 | 200 | |
| <i>Haemophilus influenzae</i> ² | hpd-F | GGTAAATATGCCGATGGTGTGG | 100 | |
| | hpd-R | TGCATCTTTACGCACGGTGTA | 900 | |
| | hpd-P | 5'HEX-TTGTGTACACTCCGT"TT"GGTAAAAGAAGTGCAC-3'-SpC6 | "T"=BHQ1 | |
| Triplex Reaction – 2 | | | | |
| <i>Streptococcus pyogenes</i> ³ | spy-F | GCACTCGCTACTATTTCTTACCTCAA | 300 | Ouattara et al. (2020) ⁷ |
| | spy-R | GTCACAATGTCTTGGAAACCAGTAAT | 300 | |
| | spy-P | 5'-Cy5-CCGCAAC"TT"CATCAAGGATTTCTGTTACCA-3'-SpC6 | "T"=BHQ2 | |
| <i>Streptococcus agalactiae</i> ⁴ | cfb-F | GGGAACAGATTATGAAAAACCG | 200 | |
| | cfb-R | AAGGCTTCTACACGACTACCAA | 200 | |
| | cfb-P | 5'-HEX-AGACTTCATTGCGTGCCAACCCTGAGAC-3'-BHQ1 | 200 | |
| <i>Streptococcus suis</i> ⁵ | fbpS-F | TCCRATRCTGCTCTGCCATT | 200 | |
| | fbpS-R | TGATAGTAGAAGTCCAGCARACT | 200 | |
| | fbpS-P | 5'FAM-AATAGCCC"TT"GAAAAMCAGCCACWYTTTGARA-3'-SpC6; | "T"=BHQ1 | |

¹Carvalho, 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.

²Vuong, J. *et al.*, 2016. Development of Real-Time PCR Methods for the Detection of Bacterial Meningitis Pathogens without DNA Extraction. <https://doi.org/10.1371/journal.pone.0147765>

³Kodani *et al.*, 2011. Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. J Clin Microbiol. 49(6):2175-82.

⁴Diaz *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.

⁵Srinivasan *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.

⁶Ouattara *et al.*, 2019. Triplex real-time PCR assay for the detection of *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* directly from clinical specimens without extraction of DNA. Diagn Microbiol Infect Dis. 93: 188-190.

⁷Ouattara *et al.*, 2020. Identification of *Streptococcus suis* Meningitis by Direct Triplex Real-Time PCR, Burkina Faso. Emerg Infect Dis 26(9): 2223-2226.