S2 Table: Reaction conditions for the bacterial and viral multiplex real-time PCR assays

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| **Multiplex Targets** | **Primers and probes** | **Reaction concentration** | **Initial denaturation** | **PCR Cycles** |
| ***S. pneumoniae***  ***H. influenzae***  ***N. meningitidis***  ***IAC\**** | *S. pneumoniae-F* | 200nM | 50oC (2mins)95oC (10mins) | 95oC (15s) 60oC(60s)\* (X40) |
| *S. pneumoniae-R* | 200nM |
| *S. pneumoniae Probe* | 200nM |
| *H. influenzae-F* | 300nM |
| *H. influenzae-R* | 100nM |
| *H. influenzae Probe* | 200nM |
| *N. meningitidis-F* | 300nM |
| *N. meningitidis-R* | 900nM |
| *N. meningitidis Probe* | 100nM |
| *IAC-F* | 200nM |
| *IAC-R* | 200nM |
| *IAC Probe* | 200nM |
| ***Herpes simplex***  ***Enterovirus***  ***Mumps*** | *Herpes simplex -F* | 500nM | 50oC (10mins) 95oC (5mins) | 95oC (15s) 62oC(30s)\* (X45) |
| *Herpes simplex -R* | 500nM |
| *Herpes simplex Probe* | 200nM |
| *Enterovirus-F* | 700nM |
| *Enterovirus-R* | 700nM |
| *Enterovirus Probe* | 200nM |
| *Mumps -F* | 300nM |
| *Mumps -R* | 300nM |
| *Mumps Probe* | 200nM |

F- Forward primer; R- Reverse primer; IAC- internal amplification control

\*IAC only included in final stages/final version of bacterial multiplex assay