

|  |                      |
|--|----------------------|
| <b>Protocol for the triplex real time PCR - <i>S. pneumoniae</i> serotyping - clinical specimens - Africa scheme</b>               | <b>Real-time RX1</b> |
| Purpose: <i>S. pneumoniae</i> serotyping directly from clinical specimens (Pimenta et al. J Clin Microbiol. 2013 Feb;51(2):647-52) | Date: ____, 2014     |

| Initial Conc. | Reagent  | Volume (μL) | Total Rxns | Final Volume |
|---------------|--|-------------|------------|--------------|
| n/a           | H <sub>2</sub> O   | vary        | 20         | vary         |
| n/a           | Invitrogen-Platinum Quantitative PCR SuperMix-UDG (Cat. No. 11730-025) | 12.5        | 20         | 250          |
| 50mM          | MgCl <sub>2</sub> (50nM)   | 1.5         | 20         | 30           |
| 10μM          | Fwd Primers  | below       |            |              |
| 10μM          | Rev Primers  | below       |            |              |
| 10μM          | Probe  | below       |            |              |
| n/a           | DNA-clinical specimen  | 5           | 20         |              |
|               | <b>Totals</b>  | <b>25</b>   | <b>20</b>  | <b>500</b>   |

|                        |           |
|------------------------|-----------|
| <b>Number of Rxns:</b> | <b>20</b> |
|------------------------|-----------|

| Thermal Cycling Parameters for all RXs |                |
|--|----------------|
| 1X                                     | 50°C for 2min  |
| 1X                                     | 95°C for 10min |
| 40X                                    | 95°C for 15sec |
|  | 60°C for 1min  |

| Primer Info                  |                     | To get 10μM:       |            | Primer/probe concentrations (μM) |                 |                    |
|------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 1 (primer/probe ID) | Original conc. (μM) | Primer/ probe (μL) | Water (μL) | Conc. (μM) 1 rxn                 | Per X Rxns (μM) | Amount to add (μL) |
| 1-F (817279)                 | 504.03              | 4.96               | 245.04     | 0.75                             | 20              | 15                 |
| 1-R (817280)                 | 434.58              | 5.75               | 244.25     | 0.75                             | 20              | 15                 |
| 1-pb FAM (11654)             | 82.61               | 30.26              | 219.74     | 0.25                             | 20              | 5                  |
| 5-F (817287)                 | 415.41              | 6.02               | 243.98     | 0.75                             | 20              | 15                 |
| 5-R (817288)                 | 364.46              | 6.86               | 243.14     | 0.75                             | 20              | 15                 |
| 5-pb HEX (00286)             | 146.33              | 17.08              | 232.92     | 0.5                              | 20              | 10                 |
| 23F-F (817307)               | 456.37              | 5.48               | 244.52     | 0.75                             | 20              | 15                 |
| 23F-R (817308)               | 487.42              | 5.13               | 244.87     | 0.75                             | 20              | 15                 |
| 23F-pb ROX (1005206)         | 105.99              | 23.59              | 226.41     | 0.5                              | 20              | 10                 |
|                              |                     |                    |            | 5.75                             |                 | 115                |

| Probes set up |            |
|---------------|------------|
| Serotypes     | Probes     |
| 1             | FAM        |
| 5             | HEX        |
| 23F           | ROX or CY5 |

| Primer Info                  |                     | To get 10µM:       |            | Primer/probe concentrations (µM) |                 |                    |
|------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 2 (primer/probe ID) | Original conc. (µM) | Primer/ probe (µL) | Water (µL) | Conc. (µM) 1 rxn                 | Per X Rxns (µM) | Amount to add (µL) |
| 4-F (817285)                 | 455.26              | 5.49               | 244.51     | 0.75                             | 20              | 15                 |
| 4-R (817286)                 | 408.90              | 6.11               | 243.89     | 0.75                             | 20              | 15                 |
| 4-pb ROX (1004311)           | 153.25              | 16.31              | 233.69     | 0.25                             | 20              | 5                  |
| 6A/6B/6C/6D-F (1005960)      | 419.17              | 5.96               | 244.04     | 0.5                              | 20              | 10                 |
| 6A/6B/6C/6D-R (1005961)      | 431.49              | 5.79               | 244.21     | 0.5                              | 20              | 10                 |
| 6A/6B/6C/6D-pb FAM (1006435) | 328.83              | 7.60               | 242.40     | 0.5                              | 20              | 10                 |
| 9V/9A-F (817295)             | 463.81              | 5.39               | 244.61     | 0.75                             | 20              | 15                 |
| 9V/9A-R (817296)             | 411.12              | 6.08               | 243.92     | 0.75                             | 20              | 15                 |
| 9V/9A-pb HEX (819002)        | 252.35              | 9.91               | 240.09     | 0.25                             | 20              | 5                  |
|                              |                     |                    |            | 5                                |                 | 100                |

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|-----------------|---------------|----|
|                 | Real-time RX2 |    |
|                 |               | 20 |
| Number of Rxns: |               |    |

| Probes set up |            |
|---------------|------------|
| Serotypes     | Probes     |
| 4             | ROX or CY5 |
| 6A/6B/6C/6D   | FAM        |
| 9V/9A         | HEX        |

| Primer Info                     |                     | To get 10µM:       |            | Primer/probe concentrations (µM) |                 |                    |
|---------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 3 (primer/probe ID)    | Original conc. (µM) | Primer/ probe (µL) | Water (µL) | Conc. (µM) 1 rxn                 | Per X Rxns (µM) | Amount to add (µL) |
| 14-F (817299)                   | 458.60              | 5.45               | 244.55     | 0.75                             | 20              | 15                 |
| 14-R (817300)                   | 486.64              | 5.14               | 244.86     | 0.75                             | 20              | 15                 |
| 14-pb FAM (819003)              | 164.25              | 15.22              | 234.78     | 0.25                             | 20              | 5                  |
| 18C/18F/18B/18A-F (703368)      | 401.92              | 6.22               | 243.78     | 0.5                              | 20              | 10                 |
| 18C/18F/18B/18A-R (703369)      | 369.36              | 6.77               | 243.23     | 0.5                              | 20              | 10                 |
| 18C/18F/18B/18A-pb HEX (817317) | 291.31              | 8.58               | 241.42     | 0.25                             | 20              | 5                  |
| 19F-F (1004362)                 | 426.43              | 5.86               | 244.14     | 0.75                             | 20              | 15                 |
| 19F-R (1004363)                 | 402.51              | 6.21               | 243.79     | 0.75                             | 20              | 15                 |
| 19F-pb ROX (1004938)            | 127.19              | 19.66              | 230.34     | 0.25                             | 20              | 5                  |
|                                 |                     |                    |            | 4.75                             |                 | 95                 |

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|-----------------|---------------|----|
|                 | Real-time RX3 |    |
|                 |               | 20 |
| Number of Rxns: |               |    |

| Probes set up   |            |
|-----------------|------------|
| Serotypes       | Probes     |
| 14              | FAM        |
| 18C/18F/18B/18A | HEX        |
| 19F             | ROX or CY5 |

| Primer Info                  |                     | To get 10µM:       |            | Primer/probe concentrations (µM) |                 |                    |
|------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 4 (primer/probe ID) | Original conc. (µM) | Primer/ probe (µL) | Water (µL) | Conc. (µM) 1 rxn                 | Per X Rxns (µM) | Amount to add (µL) |
| 3-F (817283)                 | 377.24              | 6.63               | 243.37     | 0.75                             | 20              | 15                 |
| 3-R (817284)                 | 414.93              | 6.03               | 243.97     | 0.75                             | 20              | 15                 |
| 3-pb HEX (02823)             | 93.47               | 26.75              | 223.25     | 0.5                              | 20              | 10                 |
| 7F/7A-F (817293)             | 448.12              | 5.58               | 244.42     | 0.5                              | 20              | 10                 |
| 7F/7A-R (817294)             | 458.83              | 5.45               | 244.55     | 0.5                              | 20              | 10                 |
| 7F/7A-pb ROX (817315)        | 188.41              | 13.27              | 236.73     | 0.25                             | 20              | 5                  |
| 19A-F (1019212)              | 469.98              | 5.32               | 244.68     | 0.5                              | 20              | 10                 |
| 19A-R (1019213)              | 367.08              | 6.81               | 243.19     | 0.5                              | 20              | 10                 |
| 19A-pb FAM (1019183)         | 362.50              | 6.90               | 243.10     | 0.25                             | 20              | 5                  |
|                              |                     |                    |            | 4.5                              |                 | 90                 |

|                 |               |    |
|-----------------|---------------|----|
|                 | Real-time RX4 |    |
|                 |               | 20 |
| Number of Rxns: |               |    |

| Probes set up |            |
|---------------|------------|
| Serotypes     | Probes     |
| 3             | HEX        |
| 7F/7A         | ROX or CY5 |
| 19A           | FAM        |

| Primer Info                       |                     | To get 10µM:       |            | Primer/probe concentrations (µM) |                 |                    |
|-----------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 5 (primer/probe ID)      | Original conc. (µM) | Primer/ probe (µL) | Water (µL) | Conc. (µM) 1 rxn                 | Per X Rxns (µM) | Amount to add (µL) |
| 6C/6D-F (1001589)                 | 446.81              | 5.60               | 244.40     | 0.5                              | 20              | 10                 |
| 6C/6D-R (1001590)                 | 459.25              | 5.44               | 244.56     | 0.5                              | 20              | 10                 |
| 6C/6D-pb FAM (1002498)            | 186.88              | 13.38              | 236.62     | 0.25                             | 20              | 5                  |
| 12F/12A/12B/44/46-F (902281)      | 422.52              | 5.92               | 244.08     | 0.75                             | 20              | 15                 |
| 12F/12A/12B/44/46-R (902282)      | 469.69              | 5.32               | 244.68     | 0.75                             | 20              | 15                 |
| 12F/12A/12B/44/46-pb ROX (817319) | 238.59              | 10.48              | 239.52     | 0.5                              | 20              | 10                 |
| 22F/22A-F (821384)                | 419.17              | 5.96               | 244.04     | 0.5                              | 20              | 10                 |
| 22F/22A-R (821385)                | 447.67              | 5.58               | 244.42     | 0.5                              | 20              | 10                 |
| 22F/22A-pb HEX (02824)            | 107.35              | 23.29              | 226.71     | 0.5                              | 20              | 10                 |
|                                   |                     |                    |            | 4.75                             |                 | 95                 |

|                 |               |    |
|-----------------|---------------|----|
|                 | Real-time RX5 |    |
|                 |               | 20 |
| Number of Rxns: |               |    |

| Probes set up     |            |
|-------------------|------------|
| Serotypes         | Probes     |
| 6C/6D             | FAM        |
| 12F/12A/12B/44/46 | ROX or CY5 |
| 22F/22A           | HEX        |

| Primer Info                  |                     | To get 10µM:       |            | Primer/probe concentrations (µM) |                 |                    |
|------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 6 (primer/probe ID) | Original conc. (µM) | Primer/ probe (µL) | Water (µL) | Conc. (µM) 1 rxn                 | Per X Rxns (µM) | Amount to add (µL) |
| 15A/15F-F (1011034)          | 455.56              | 5.49               | 244.51     | 0.5                              | 20              | 10                 |
| 15A/15F-R (1011035)          | 469.62              | 5.32               | 244.68     | 0.5                              | 20              | 10                 |
| 15A/15F-pb FAM (1013070)     | 206.53              | 12.10              | 237.90     | 0.25                             | 20              | 5                  |
| 23A-F (1011015)              | 475.44              | 5.26               | 244.74     | 0.5                              | 20              | 10                 |
| 23A-R (1011016)              | 428.52              | 5.83               | 244.17     | 0.5                              | 20              | 10                 |
| 23A-pb ROX (1013071)         | 128.79              | 19.41              | 230.59     | 0.25                             | 20              | 5                  |
| 33F/33A/37-F (1010951)       | 409.52              | 6.10               | 243.90     | 0.5                              | 20              | 10                 |
| 33F/33A/37-R (1010952)       | 464.72              | 5.38               | 244.62     | 0.5                              | 20              | 10                 |
| 33F/33A/37-pb HEX (1013072)  | 50.97               | 49.05              | 200.95     | 0.5                              | 20              | 10                 |
|                              |                     |                    |            | 4                                |                 | 80                 |

|                 |               |    |
|-----------------|---------------|----|
| Number of Rxns: | Real-time RX6 |    |
|                 |               | 20 |

| Probes set up |            |
|---------------|------------|
| Serotypes     | Probes     |
| 15A/15F       | FAM        |
| 23A           | ROX or CY5 |
| 33F/33A/37    | HEX        |

| Primer Info                  |                     | To get 10µM:       |            | Primer/probe concentrations (µM) |                 |                    |
|------------------------------|---------------------|--------------------|------------|----------------------------------|-----------------|--------------------|
| Reaction 7 (primer/probe ID) | Original conc. (µM) | Primer/ probe (µL) | Water (µL) | Conc. (µM) 1 rxn                 | Per X Rxns (µM) | Amount to add (µL) |
| 2-F (817281)                 | 509.62              | 4.91               | 245.09     | 0.75                             | 20              | 15                 |
| 2-R (817282)                 | 426.12              | 5.87               | 244.13     | 0.75                             | 20              | 15                 |
| 2-pb FAM (07806)             | 69.89               | 35.77              | 214.23     | 0.5                              | 20              | 10                 |
| 11A/11D-F (821382)           | 442.15              | 5.65               | 244.35     | 0.75                             | 20              | 15                 |
| 11A/11D-R (821383)           | 411.25              | 6.08               | 243.92     | 0.75                             | 20              | 15                 |
| 11A/11D-pb ROX (1004313)     | 277.98              | 8.99               | 241.01     | 0.25                             | 20              | 5                  |
| 16F-F (821386)               | 493.33              | 5.07               | 244.93     | 0.75                             | 20              | 15                 |
| 16F-R (821387)               | 422.08              | 5.92               | 244.08     | 0.75                             | 20              | 15                 |
| 16F-pb HEX (1005203)         | 135.61              | 18.44              | 231.56     | 0.25                             | 20              | 5                  |
|                              |                     |                    |            | 5.5                              |                 | 110                |

|                 |               |    |
|-----------------|---------------|----|
| Number of Rxns: | Real-time RX7 |    |
|                 |               | 20 |

| Probes set up |            |
|---------------|------------|
| Serotypes     | Probes     |
| 2             | FAM        |
| 11A/11D       | ROX or CY5 |
| 16F           | HEX        |