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| Protocol for the triplex real time PCR - <i>S. pneumoniae</i> serotyping - clinical specimens - United States (US) scheme | Real-time RX1 |
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Purpose: *S. pneumoniae* 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 ₂ O | vary | 20 | vary |
| n/a | Invitrogen-Platinum Quantitative PCR SuperMix-UDG (Cat. No. 11730-025) | 12.5 | 20 | 250 |
| 50mM | MgCl ₂ (50nM) | 1.5 | 20 | 30 |
| 10µM | Fwd Primers | below | | |
| 10µM | Rev Primers | below | | |
| 10µM | Probe | below | | |
| n/a | DNA-clinical specimen | 5 | 20 | |
| Totals | | 25 | 20 | 500 |

| | |
|------------------------|-----------|
| Number of Rxns: | 20 |
|------------------------|-----------|

| 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 | Original conc. (µM) | To get 10µM: | | Primer/probe concentrations (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 |

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

| 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) |
| 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 RX2

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|------------------------|-----------|
| Number of Rxns: | 20 |
|------------------------|-----------|

| 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 3 (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 |

Real-time RX3

| | |
|------------------------|-----------|
| Number of Rxns: | 20 |
|------------------------|-----------|

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

| 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) |
| 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 (819001) | 82.61 | 30.26 | 219.74 | 0.25 | 20 | 5 |
| 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 |
| | | | | 5.25 | | 105 |

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

| Probes set up | |
|---------------|------------|
| Serotypes | Probes |
| 1 | FAM |
| 11A/11D | ROX or CY5 |
| 16F | HEX |

| 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) |
| 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 |

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

| 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 6 | (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 RX6 | |
| Number of Rxns: | 20 |

| 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 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 HEX (02822) | | 69.89 | 35.77 | 214.23 | 0.5 | 20 | 10 |
| 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 FAM (902140) | | 256.05 | 9.76 | 240.24 | 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 |
| | | | | | 6 | | 120 |

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

| Probes set up | |
|---------------|------------|
| Serotypes | Probes |
| 2 | HEX |
| 5 | FAM |
| 23F | ROX or CY5 |