

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

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 2 (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

Real-time RX2
Number of Rxns: 20

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

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)
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 RX3
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 10 μ M:		Primer/probe concentrations (μ M)		
Reaction 4 (primer/probe ID)	Original conc. (μ M)	Primer/ probe (μ L)	Water (μ L)	Conc. (μ M) 1 rxn	Per X Rxns (μ M)	Amount to add (μ L)
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

Number of Rxns:	20
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Probes set up	
Serotypes	Probes
3	HEX
7F/7A	ROX or CY5
19A	FAM

Primer Info		To get 10 μ M:		Primer/probe concentrations (μ M)		
Reaction 5 (primer/probe ID)	Original conc. (μ M)	Primer/ probe (μ L)	Water (μ L)	Conc. (μ M) 1 rxn	Per X Rxns (μ M)	Amount to add (μ L)
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 (817315)	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

Number of Rxns:	20
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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)
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 RX6

Number of Rxns:	20
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Probes set up	
Serotypes	Probes
1	FAM
11A/11D	ROX or CY5
16F	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)
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 RX7

Number of Rxns:	20
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Probes set up	
Serotypes	Probes
15A/15F	FAM
23A	ROX or CY5
33F/33A/37	HEX