

Comparison of traditional culture and molecular qPCR for detection of simultaneous carriage of multiple pneumococcal serotypes in African children

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Supplementary table S1: Performance of the quantitative real-time PCR assay

Bacterial target	Efficiency (%)	Correlation coefficient (r²)	Limit of detection (copies/PCR)	Intra-assay variation	Accuracy	Inter-assay variation
<i>Streptococcus pneumoniae</i> (lytA)	97	0.99	10	0.02	0.043	0.025
<i>Streptococcus pneumoniae</i> serotype 1	105	0.99	10	0.011	0.025	0.032
<i>Streptococcus pneumoniae</i> serotype 3	94	0.99	10	0.025	-0.013	0.025
<i>Streptococcus pneumoniae</i> serotype 4	91	0.99	10	0.074	-0.01	0.015
<i>Streptococcus pneumoniae</i> serotype 5	95	0.99	100	0.018	0.048	0.047
<i>Streptococcus pneumoniae</i> serogroup 6A/B/C/D	97	0.99	100	0.013	-0.26	0.023
<i>Streptococcus pneumoniae</i> serogroup 6C/D	92	0.99	10	0.011	-0.078	0.041
<i>Streptococcus pneumoniae</i> serotype 7C	102	0.99	10	0.008	-0.076	0.041
<i>Streptococcus pneumoniae</i> serogroup 9A/L/N/V	97	0.99	10	0.019	-0.059	0.023
<i>Streptococcus pneumoniae</i> serotype 10A/B	91	0.99	10	0.006	0.001	0.008
<i>Streptococcus pneumoniae</i> serogroup 11A/B/C/D/F	98	0.99	10	0.027	0.023	0.053
<i>Streptococcus pneumoniae</i> serogroup 12A/B/F	94	0.99	10	0.018	0.068	0.009
<i>Streptococcus pneumoniae</i> serotype 13	94	0.99	10	0.003	-0.023	0.003
<i>Streptococcus pneumoniae</i> serotype 14	91	0.99	100	0.078	-0.041	0.056
<i>Streptococcus pneumoniae</i> serogroup 15A/B/C/F	98	0.99	10	0.056	-0.059	0.038
<i>Streptococcus pneumoniae</i> serogroup 16F	92	0.99	10	0.03	-0.013	0.029
<i>Streptococcus pneumoniae</i> serotype 17F	99	0.99	10	0.054	-0.026	0.018
<i>Streptococcus pneumoniae</i> serogroup 18A/B/C	91	0.99	10	0.023	0.021	0.047
<i>Streptococcus pneumoniae</i> serotype 19A	92	0.99	10	0.001	0.054	0.005
<i>Streptococcus pneumoniae</i> serogroup 19B/F	93	0.99	10	0.006	0.001	0.003
<i>Streptococcus pneumoniae</i> serotype 20	102	0.99	10	0.015	0.054	0.05
<i>Streptococcus pneumoniae</i> serotype 21	101	0.99	10	0.098	0.075	0.11
<i>Streptococcus pneumoniae</i> serogroup 23A/B/F	96	0.99	10	0.013	-0.056	0.021
<i>Streptococcus pneumoniae</i> serotype 23F	90	0.99	100	0.002	-0.073	0.038
<i>Streptococcus pneumoniae</i> serogroup 34/37/17A	92	0.99	10	0.002	0.09	0.013

Supplementary table S3: Detection of overall pneumococcal carriage by culture, stratified by colonization density measured by quantitative PCR (qPCR)

Density (CFU/ml)	Number culture positive/ number in density category by PCR (%)
Undetected	8/67 (12)
0-10 ²	1/4 (25)
> 10 ² -10 ³	22/37 (59)
>10 ³ -10 ⁴	64/81 (79)
>10 ⁴ -10 ⁵	85/97 (88)
>10 ⁵ -10 ⁶	65/67 (97)
>10 ⁶ -10 ⁷	18/18 (100)
>10 ⁷ -10 ⁸	3/3 (100)
Total	266/374 (71)

CFU/ml, colony forming units per millimetre as measured by qPCR. Values are number culture positive/number in density category by qPCR (%).

Supplementary table S4: Yield of culture and molecular qPCR for the detection of *Streptococcus pneumoniae* serotypes/groups, including for those specific serotypes which were individually identifiable on qPCR in nasopharyngeal swabs (n = 374)

	All serotypes/serogroups					Serotypes individually identifiable by qPCR				
	qPCR (-)	qPCR (+)	Total	<i>p</i> -value	Concordance (<i>kappa</i>)	qPCR (-)	qPCR (+)	Total	<i>p</i> -value	Concordance (<i>kappa</i>)
Culture (+)	239	21	260			86	11	97		
Culture (-)	150	8566	8562	<0.001	0.73	69	5070	4985	<0.001	0.68
Total	389	8586	8976			155	5080	5236		

Supplementary table S5: Detection of pneumococcal serotypes by culture and quantitative PCR (qPCR)

Serotype	Culture	Total (N=374)			
		qPCR	p-value	sensitivity	specificity
1	0	3(0.8)	0.08	0	100
3	4(1.1)	5(1.4)	0.56	60	99.8
4	1(0.3)	3(0.8)	0.16	33.3	100
5	0	23(6.1)	<0.001	0	100
6A/B	54(14.4)	55(14.7)	0.99	93	99
A	19(5.1)	-	-	-	-
B	35(9.4)	-	-	-	-
6C/D	1(0.3)	5(1.3)	0.046	20	100
C	1(0.3)	-	-	-	-
D	0	-	-	-	-
7C	1(0.3)	2(1)	0.32	50	100
9A/L/N/V	15(4)	40(10.7)	<0.001	35	99.7
A	0	-	-	-	-
L	0	-	-	-	-
N	2(0.53)	-	-	-	-
V	14(3.7)	-	-	-	-
10A	1(0.3)	4(1.1)	0.08	25	100
11A/B/C/D/F	4(1.1)	13(3.5)	0.003	31	100
A	4(1.1)	-	-	-	-
B	0	-	-	-	-
C	0	-	-	-	-
D	0	-	-	-	-
F	0	-	-	-	100
12A/B/F	0	0	-	-	-
A	0	-	-	-	-
B	0	-	-	-	-
F	0	-	-	-	-
13	1(0.3)	8(2.1)	0.008	12.5	100
14	18(4.8)	26(7)	0.01	65.4	99.7
15A/B/C/F	14(3.7)	18(4.8)	0.16	66.7	99.4
A	3(0.8)	-	-	-	-
B	9(2.4)	-	-	-	-
C	2(0.5)	-	-	-	-
F	0	-	-	-	-
16F	7(1.9)	8(2.1)	0.32	87.5	100
17F	2(0.53)	3(0.8)	0.56	33.3	99.7
18A/B/C	9(2.4)	17(4.5)	0.01	47.1	99.7
A	1(0.3)	-	-	-	-
B	0	-	-	-	-
C	8(2.1)	-	-	-	-
F	0	-	-	-	-
19A	10(2.7)	14(3.7)	0.1	64.3	99.7
19B/F	57(21.9)	70(18.7)	0.034	81.4	100
B	5(0.8)	-	-	-	-
F	52(20)	-	-	-	-
20	3(0.8)	2(0.53)	0.32	100	99.7
21	5(0.8)	4(1.1)	0.32	100	99.7
23AB	5(0.8)	8(2.1)	0.32	25	99.2
A	3(0.8)	-	-	-	-
B	2(0.53)	-	-	-	-
23F	44(11.8)	50(13.4)	0.13	78	98.5
34/37/17F	4(1.1)	8(2.1)	0.046	50	100
34	4(1.1)	-	-	-	-
37	0	-	-	-	-
17A	0	-	-	-	-

Supplementary table S6: Detection of pneumococcal serotype carriage by culture, stratified by colonization density measured by quantitative PCR (qPCR)

Pneumococcal density by qPCR (CFU/ml)	Number of culture positive/number in density category by qPCR (%)											
	1	3	4	5	6A/B	6C/D	7C	9A/L/N/V	10A	11A/B/C/D/F	12A/B/F	13
Undetected	0/371	1/369(0.3)	0/371	0/351	3/319(0.9)	0/369	0/372	1/333(0.3)	0/370	0/361	0/374	0/366
0- 10 ²	0	0	0/1	0	0	0	0/1	0/19	0	0/7	0	0
> 10 ² -10 ³	0/2	0/1	0/1	0/23	7/7(100)	0/2	0	1/8(13)	0	0/2	0	0/5
>10 ³ -10 ⁴	0	1/1(100)	1/1(100)	0	12/16(100)	0/2	1/1(100)	7/7(100)	0/1	2/2(100)	0	0/1
>10 ⁴ -10 ⁵	0/1	1/2(50)	0	0	18/18(100)	0	0	5/6(83)	1/1(100)	2/2(100)	0	0/1
>10 ⁵ -10 ⁶	0	1/1(100)	0	0	9/9(100)	0	0	1/1(100)	0/2	0	0	0
>10 ⁶ -10 ⁷	0	0	0	0	5/5(100)	1/1(100)	0	0	0	0	0	1/1(100)
>10 ⁷ -10 ⁸	0	0	0	0	0	0	0	0	0	0	0	0
>10 ⁸	0	0	0	0	0	0	0	0	0	0	0	0
Total	0/374	4/374(1.1)	1/374(0.3)	0/374	54/374(14.4)	1/374(0.3)	1/374(0.3)	15/374(0.3)	1/374(0.3)	4/374(1.1)	0/374	1/374(0.3)
Pneumococcal density by qPCR (CFU/ml)	14	15A/B/C/F	16F	17F	18A/B/C	19A	19B/F	20	21	23A/B	23F	34/37/17A
	Undetected	1/348(0.9)	2/356(0.6)	0/366	1/371(0.3)	1/357(0.3)	1/360(0.3)	0/304	1/372(0.3)	1/370(0.3)	3/365(0.8)	5/324(1.5)
0- 10 ²	0	0	0	0/1	0/1	0/2	10/12(83.3)	0	0	0	0	1/2(50)
> 10 ² -10 ³	2/11(18)	1/3(33.3)	3/3(100)	0/1	1/7(14.3)	1/3(33.3)	8/12(66.7)	0	2/2(100)	2/8(25)	17/26(65.4)	1/4(25)
>10 ³ -10 ⁴	10/10(100)	5/9(55.6)	3/3(100)	1/1(100)	4/5(80)	8/9(88.9)	21/23(91.3)	0	0	0/1	15/16(93.8)	1/1(100)
>10 ⁴ -10 ⁵	4/4(100)	6/6(100)	0/1	0	3/4(75)	0	15/18(83.3)	2/2(100)	0	0	7/8(87.5)	1/1(100)
>10 ⁵ -10 ⁶	1/1(100)	0	0	0	0	0	3/4(75)	0	2/2(100)	0	0	0
>10 ⁶ -10 ⁷	0	0	0	0	0	0	1/1(100)	0	0	0	0	0
>10 ⁷ -10 ⁸	0	0	1/1(100)	0	0	0	0	0	0	0	0	0
>10 ⁸	0	0	0	0	0	0	0	0	0	0	0	0
Total	18/374(4.8)	14/374(3.7)	7/374(1.9)	2/374(0.5)	9/374(2.4)	10/374(2.7)	58/374(15.5)	3/374(0.8)	5/374(1.3)	5/374(1.3)	44/374(13.4)	4/374(1.1)

Supplementary table S7: Yield of additional serotypes detected by quantitative PCR (qPCR) in relation to being co-carriage with other pneumococcal serotypes

Serotype/group	Number of serotypes co-carried/additional serotypes detected by qPCR (%)	Number of dominating colonizing serotype/number of serotypes co-carried (%) [*]
1	2/3(75)	1/2(50)
3	2/2(100)	2/2(100)
4	2/2(100)	0/2
5	23/23(100)	1/23(4.3)
6A/B	3/4(75)	2/3(66.7)
6C/D	4/4(100)	0/4
7C	0/1	0
9A/L/N/V	19/26(73)	0/19
10A	2/3(75)	1/2(50)
11A/B/C/D/F	7/9(77.8)	2/7(28.6)
12A/B/F	0	0
13	6/7(85.7)	0/6
14	7/9(77.8)	1/7(14.3)
15A/B/C/F	5/6(83.3)	1/5(25)
16F	0/1	0
17F	1/2(50)	0/1
18A/B/C	7/9(77.8)	1/7(14.3)
19A	3/5(60)	0/3
19B/F	12/13(92.3)	2/12(16.7)
20	0	0
21	0	0
23A/B	5/6(83.3)	1/5(20)
23F	6/11(54.5)	0/8
34/37/17A	1/4(25)	0/1
Total	117/150	15/117(12.8)

^{*}Density of pneumococcal carriage was used to determine if the additional serotypes detected only by quantitative PCR (qPCR) were dominant colonizing serotypes (highest density colonizer).

Supplementary table S8: Primers and probe sequences for the qPCR detection of pneumococcal serotypes

Primers/Probe ^a name	Sequence (5' - 3')	serotype/serogroup	Source
lytA-Fwd lytA-Rev lytA-Probe	TCTTACGCAATCTAGCAGATGAAGC GTTGTTTGGTTGGTTATTCGTGC (VIC)-TTTGCCGAAAACGCTTGATACAGGG-(MGB)	<i>Streptococcus pneumoniae</i>	McAvin <i>et al</i> , 2001
1-Fwd 1-Rev 1-Probe	CGTGCGGTAATTGAAGCTATGA TGTGGCCCCAGCAACTCT (FAM)-TGCTTGCCCTTGTATAGGGT-(MGB)	<i>Streptococcus pneumoniae</i> _serotype 1	Azzari <i>et al</i> , 2010
3-Fwd 3-Rev 3-Probe	GGTCAGCAGAAAGTATGCATTGG TCGTTTATCCAGGGTCTGATGA (VIC)-TATTGGATGTGGTTTATCGTGAAGA-(MGB)	<i>Streptococcus pneumoniae</i> _serotype 3	Azzari <i>et al</i> , 2010
4-Fwd 4-Rev 4-Probe	TGGGATGACATTTCTACGCACTA CCGTCGCTGATGCTTTATCA (FAM)-TCCTATTGGATGGTTAGTTGGTGA-(MGB)	<i>Streptococcus pneumoniae</i> _serotype 4	Azzari <i>et al</i> , 2010
5-Fwd 5-Rev 5-Probe	TTACGGGAGTATCTTATGTCTTTAATGG CAGCATTCCAGTAGCCTAAAAGTAGA (VIC)-TTGTCTCAGCAACTCTATTTGGCTGTGGG-(MGB)	<i>Streptococcus pneumoniae</i> _serotype 5	Azzari <i>et al</i> , 2010
6A/B/C/D-Fwd 6A/B/C/D-Rev 6A/B/C/D-Probe	AAGTTTGCCTAGAGTATGGGAAGGT ACATTATGTCCRTGTCTTCGATACAAG (VIC)-TGTTCTGCCCTGAGCAACTGG-(MGB)	<i>Streptococcus pneumoniae</i> _serogroup 6A/B/C/D	Azzari <i>et al</i> , 2010
6C/D-Fwd 6C/D-Rev 6C/D-Probe	TTGGGATGATTGGTCGTATTAG CTCTTCAATTAGTTCTTCAGTTCG (FAM)-CCACGCAATTCGCCATC-(MGB)	<i>Streptococcus pneumoniae</i> _serogroup 6C/D	Azzari <i>et al</i> , 2010
7C-Fwd 7C-Rev 7C-Probe	CGTCAGGAATAGGTGCAATCTCT TGAAATTCCAAGCGAAGCAA (VIC)-TTCATCTATTGGTTCTTATGGTGTT-(MGB)	<i>Streptococcus pneumoniae</i> _serotype 7C	This Study
9A/L/N/V-Fwd 9A/L/N/V-Rev 9A/L/N/V-Probe	TGGAATGGGCAAAGGGTAGTA TCGGTTCCCAAGATTTTCTC (FAM)-TTAATCATGCTAACGGCTCATCGA-(MGB)	<i>Streptococcus pneumoniae</i> _serogroup 9A/L/N/V	Azzari <i>et al</i> , 2010
10A/B-Fwd 10A/B-Rev	CCTCTCCTATCAACTATTACTCATTATACTACCT AATAACCATAAGTCCCTAGATCATTCAAAG	<i>Streptococcus pneumoniae</i> _serotype 10A/B	Azzari <i>et al</i> , 2010

10A/B-Probe	(VIC)-TCATTACAACCTCCCTATGTGACACGGGTCTTTT-(MGB)		
11A/B/C/D/F-Fwd	ACCGCATTTCCTTATCGCACTATATT	<i>Streptococcus pneumoniae</i> _serogroup 11A/B/C/D/F	This Study
11A/B/C/D/F-Rev	TCTCCTTACCATCAAACATGTTAATCA		
11A/B/C/D/F-Probe	(FAM)-TGAATCAGTCTGACCGTTT-(MGB)		
12A/B/F-Fwd	GATTATTCGCTTGCCTCTTCATG	<i>Streptococcus pneumoniae</i> _serogroup 12A/B/F	Azzari <i>et al</i> , 2010*
12A/B/F-Rev	ATAGCCGAAATAAGCTTTCCAGAA		
12A/B/F-Probe	(FAM)-ATTTGTAAGCGGACGTGCGATT-(MGB)		
13-Fwd	TCGGATTTAGTAGTAACCCCATTTGA	<i>Streptococcus pneumoniae</i> _serotype 13	This Study
13-Rev	TTCTTGATTGAGGATGCATTTCC		
13-Probe	(VIC)-AGTAGTAAGAGATCATATTCAAG-(MGB)		
14-Fwd	CGACTGAAATGTCCTAGGAGAAGAT	<i>Streptococcus pneumoniae</i> _serotype 14	Azzari <i>et al</i> , 2010
14-Rev	AATACAGTCCATCAATTACTGCAATACTC		
14-Probe	(VIC)-TGTCATTCGTTTGCCAATACTTGATGGTCTC-(MGB)		
15A/B/C/F-Fwd	TTGAATCAGGTAGATTGATTTCTGCTA	<i>Streptococcus pneumoniae</i> _serogroup 15A/B/C/F	Azzari <i>et al</i> , 2010
15A/B/C/F-Rev	CTCTAGGAATCAAATACTGAGTCCTAATGA		
15A/B/C/F-Probe	(FAM)-CTCCGGCTTTTGTCTTCTCTGT-(MGB)		
16F-Fwd	GCAACTGGTATTTTTGATATTGGAGAA	<i>Streptococcus pneumoniae</i> _serotype 16F	This Study
16F-Rev	CAAAGGAATGCCATGCCATA		
16F-Probe	(NED)-AAAATGCTAACTTCGTTGGAGG-(MGB)		
17F-Fwd	GTAAAGATTTTCATGTCCTATAAGGGAGAA	<i>Streptococcus pneumoniae</i> _serotype 17F	This Study
17F-Rev	AGGCGTCCCTGTTTATGAGAAG		
17F-Probe	(FAM)-TTGTACATGGTCTGGATTT-(MGB)		
18A/B/C-Fwd	CCTGTTGTTATTCACGCCTTACG	<i>Streptococcus pneumoniae</i> _serogroup 18A/B/C	Azzari <i>et al</i> , 2010
18A/B/C-Rev	TTGCACTTCTCGAATAGCCTTACTC		
18A/B/C-Probe	(FAM)-AACCGTTGGCCCTTGTGGTGGGA-(MGB)		
19A-Fwd	TTCGACGACGTATCAGCTTCA	<i>Streptococcus pneumoniae</i> _serotype 19A	Azzari <i>et al</i> , 2010
19A-Rev	TCATTGAGAGCCTTAACCTCTTCA		
19A-Probe	(VIC)-ACCCAAAACGGTTGACGCATTATACT-(MGB)		
19B/F-Fwd	GGTCATGCGAGATACGACAGAA	<i>Streptococcus pneumoniae</i> _serogroup 19B/F	Azzari <i>et al</i> , 2010
19B/F-Rev	TCCTCATCAGTCCCAACCAATT		
19B/F-Probe	(VIC)-ACCTGAAGGAGTAGCTGCTGGAACGTTG-(MGB)		

20-Fwd	AAAGATACTGGCTGAGGAGCTATCTATT	<i>Streptococcus pneumoniae</i> _serotype 20	Azzari <i>et al</i> , 2010
20-Rev	AGTCAAAAGTACTCAACCATTCTGATATATTC		
20-Probe	(VIC)-AGGATAAGGTCTACTTTGTGGGAGTTC-(MGB)		
21-Fwd	CCATTTGAAGGACCAGTTGTTG	<i>Streptococcus pneumoniae</i> _serotype 21	This Study
21-Rev	AAAAAGCCACTATCAGGAATACCAA		
21-Probe	(FAM)-AATGGCATTGCTTCGTAAA-(MGB)		
23A/B/F-Fwd	GGTGGACTTTCCGATGCAA	<i>Streptococcus pneumoniae</i> _serogroup 23A/B/F	This Study
23A/B/F-Rev	CACTGTCAACAAAAATGAGGTAATCTC		
23A/B/F-Probe	(VIC)-AAATGTCCGTATAGATAAAG-(MGB)		
23F-Fwd	TGCTATTTGCGATCCTGTTCAT	<i>Streptococcus pneumoniae</i> _serotype 23F	Azzari <i>et al</i> , 2010
23F-Rev	AGAGCCTCCGTTGTTTCGTAAA		
23F-Probe	(FAM)-TTTCTCCGCATCAAACGTTAAG-(MGB)		
34/37/17A-Fwd	GGATACTATGTACGAACAGATGGACTTG	<i>Streptococcus pneumoniae</i> _serogroup 34/37/17A	This Study
34/37/17A-Rev	CTCACTAACTCGCCCGAATAAAC		
34/37/17A-Probe	(FAM)-CCGACTATACTCCATTTGA-(MGB)		

Probe^a: minor groove binding (MGB) dye labelled TaqMan probe. * probe sequence modified.

Supplementary table S9: Primer/probe duplex combinations

Primmer/probe combinations	Duplex reaction
5 & 21	A
20 & 23F	B
4 & 14	C
3 & 17F	D
1 & 23A/B/F	E
6A/B/C/D & 6C/D	F
10A/B & 15A/B/C/F	G
13 & 9A/L/N/V	H
19A & 18A/B/C	I
19B/F & 12A/B/F	J
11A/B/C/D/F & 16F	K
7C & 34/37/17A	L