

**Table 2: List of oligonucleotide primers used in 21 assays for 37 pneumococcal serotype deduction by real time multiplex*
PCR (Pimenta *et al.* J Clin Microbiol. 2013 Feb;51(2):647-52)**

Coordinates	Primer/Probe ID	Sequence (5'-3')	Probe			nM
			dye	special chemistry	Quencher (3')	
CR931632 (wzy)	1-F	TTTCATCCCATATGTTGGTATAG				300
	1-R	GCTTTAGAAGGTAGAGTTAAACAAC				300
	1-Probe	TGCCAAAGCCAGCCAT	FAM	LNA ²	BHQ1	100
CR931633 (wzy)	2-F	TGTTATCCCATATAAGAACCAGTGT				300
	2-R	AAAATTACCCAAAAGCTATCCAA				300
	2-Probe	TGCAATT"TT"CAATTTTTTGCCCAATCTC	FAM/HEX ³	"T" ⁴ =BHQ1	BHQ1	200
CR931634 (gal U)	3-F	CCACTAAAGCTTTGGCAAAAGAAA				300
	3-R	CCCGAACGTAAAGCTTCTTCA				300
	3-Probe	TTGTAGACGCCCCCAAA"TT"TCATTTTGT	HEX	"T" ⁴ =BHQ1	BHQ1	200
CR931635 (wzy)	4F	GCTTCTGCTGAACCTTTGTGC				300
	4-R	CACCACCATAGTAACCAAGTTC				300
	4-Probe	TCCACAAAAGAAGAGCTACAGTAACCCCA	ROX/CY5 ⁵		BHQ2/BHQ3	100
CR931637 (wzy)	5-F	CATGATTTATGCCCTCTTGCAA				300
	5-R	GACAGTATAAGAAAAGCAAGGCTAA				300
	5-Probe	TCTTCTCTCA"TT"CGTTCCGCATGCTTTT	FAM/HEX ³	"T" ⁴ =BHQ1	BHQ1	200
CR931639 (wci P)	6A/6B/6C/6D-F	GTT TGCAC TAGATATGGGAAGG				200
	6A/6B/6C/6D-R	TAGCCTTTCTGAAAACATTAGCG				200
	6A/6B/6C/6D-Probe	TGTTCTGCC"TT"TGAGCAACTGGTCTGTATC	FAM	"T" ⁴ =BHQ1	BHQ1	200
EF538714 (wci N)	6C/6D-F	TTGGGATGATTGGTCTGATTAG				200
	6C/6D-R	CTCTCAATTAGTTCTCAGTTCC				200
	6C/6D-Probe	CCACGCAATTGCCATC	FAM	LNA ²	BHQ1	100
CR931643 (wzy)	7F/7A-F	ATGAAGGCTTTGGTTTGACAGG				200
	7F/7A-R	ATTCTGCCATCAATTGCATATTC				200
	7F/7A-Probe	ACACCACATAGGCTGTTGAGACTAACGCACA	ROX/CY5 ⁵		BHQ2/BHQ3	100
CR931648 (wzx)	9V/9A-F	AGGTATCTATATACTGCTTTAGG				300
	9V/9A-R	CGAATCTGCCAATATCTGAAAG				300
	9V/9A-Probe	ACAATTGACAACCGCT	HEX	LNA ²	BHQ1	100
CR931653 (wzy)	11A/11D-F	AAATGGTTTGGATATGGTTTGTGG				300
	11A/11D-R	AGTGCTAACTGTAAAACCTGATTATGAG				300
	11A/11D-Probe	ATTCCAACTCTCCAATTCTGCCACGG	ROX/CY5 ⁵		BHQ2/BHQ3	100
CR931660 (wzx)	12F/12A/12B/44/46-F	GCACCCACGGGTAATATTCTAC				300
	12F/12A/12B/44/46-R	CAACTAAGAACCAAGGATCCACAG				300
	12F/12A/12B/44/46-Probe	TGCCCAACCAACCCAGGTCCAGGT	ROX/CY5 ⁵		BHQ2/BHQ3	200
CR931662 (wzy)	14-F	AGAGTGATGAGGAATCC				300
	14-R	ATATATCTACTGTAGAGGGAAT				300
	14-Probe	CGCCAAGTAACA"TT"TTCCATCCATT	FAM	"T" ⁴ =BHQ1	BHQ1	100
CR931663 (wzy)	15A/15F-F	AATTGCCTATAAACCATTGAGATAG				200
	15A/15F-R	CCATAGGAAGGAAATAGTATTTGTTCC				200
	15A/15F-Probe	CCGC CA AACTCTG CT CT	FAM	LNA ²	BHQ1	100
CR931668 (wzy)	16F-F	TAATGTTATGACCTTGGTAATCTCCC				300
	16F-R	TCCCAAAGGATAATCAATACTTTAGAAG				300
	16F-Probe	AGCCATAAGTCT"TT"CCAAATGCTTAACCGCT	HEX	"T" ⁴ =BHQ1	BHQ1	100
CR931673 (wzy)	18C/18F/18B//18A-F	TCGATGGCTAGAACAGATTTATGG				200
	18C/18F/18B//18A-R	CCATTGCTCCCTGAAGACCATTG				200
	18C/18F/18B//18A-Probe	AGGGAGTTGAATCAACCTATAATTCGCCCC	HEX		BHQ1	100
CR931675 (wzy)	19A-F	CGCCTAGTCTAAATACCA				200
	19A-R	GAGGTCAACTATAATAGTAAGAG				200
	19A-Probe	TATCAATGAGCCGATCCGTCACCT	FAM		BHQ1	100
CR931678 (wzy)	19F-F	TGAGGTTAAGATTGCTGATCG				300
	19F-R	CACGAATGAGAAGCTCGAATAAAAG				300
	19F-Probe	CGCACTGTC CA ATTCACCTTC	ROX/CY5 ⁵	LNA ¹	BHQ2/BHQ3	100
CR931682 (wcv V)	22F/22A-F	TCTATTAATAAACCATTGGAATTGAAACG				200
	22F/22A-R	TCGCAATTGAAGACCACATAAACTG				200
	22F/22A-Probe	TCCGTAAT"TT"CGCTTATGGGCACATCTCCA	HEX	"T" ⁴ =BHQ1	BHQ1	200

CR931683 (wzy)	8626-8711	23A-F	CTCCCTCCATTACCCATTGG				200
		23A-R	TGAAGAAAGTGCTGTTTGTGAACC				200
		23A-Probe	AGCTAGAAC"TT"CCACACTCCCTACTCCCA	ROX/CY5 ⁵	"T" ⁴ =BHQ2	BHQ2/BHQ3	100
CR931685 (wzy)	9049-9274	23F-F	GACAGCAACGACAATAGTCATCTC				300
		23F-R	TCCATCCCAACCTAACACACTTC				300
		23F-Probe	ATTGTGTCCA"TT"AACTTCGTCGTATTCCAAAG	ROX/CY5 ⁵	"T" ⁴ =BHQ2	BHQ2/BHQ3	200
CR931702 (wzy)	11778-11882	33F/33A/37-F	GGAAGTGGTTCAGCAACTATACG				200
		33F/33A/37-R	GGTTCTAAGACCGTCTGAAATACC				200
		33F/33A/37-Probe	CCCCAAATAGGAC"TT"TTCTGCCATGCCAAA	HEX	"T" ⁴ =BHQ1	BHQ1	200

*** All individual assay used in the multiplex PCR hold equal or better efficiency when used in monoplex fashion**

1- Abbreviations: FAM, 6-carboxyfluorescein; HEX, hexachloro-6-carboxyfluorescein; ROX, 6-carboxy-X-rhodamine; CY5, indodicarbocyanine; BHQ, black hole quencher.

2- Locked nucleic acid nucleotides are underlined

3- The probe for serotype 2 is labeled with FAM for the triplex reaction formulated for the Africa and Latin America region(s) and HEX for the triplex reaction formulated for the USA and Asia regions; the probe for serotype 5 is labeled with FAM for the triplex reaction formulated for the USA and Asia regions and HEX for the triplex reaction formulated for the Africa and Latin America region(s) reaction set.

4-"T"⁴= black hole quencher placed internally on the thymidine base

5- Probe is labeled with CY5 if ROX is used as a reference dye