

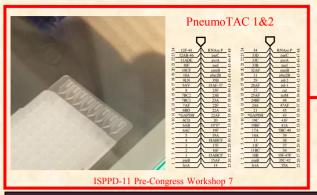
DEVELOPMENT OF A SYNTHETIC DNA, NUVERSA, TO BE USED

AS A STANDARD IN QUANTITATIVE PCR REACTIONS FOR

MOLECULAR PNEUMOCOCCAL DETECTION AND SEROTYPING

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Background

- Emergence of non-vaccine serotype pneumococcal disease is a new concern
- Absolute quantification methods that identify vaccine serotypes as well as non-vaccine serotypes, such as quantitative (q)PCR, will be useful for evaluation and prediction of vaccine efficacy.
- Obstacles for serotype population research with qPCR.
 - qPCRs for most of non-vaccine serotypes have not been reported.
 - Single-plex reaction is more efficient for serotype screening, but time-consuming and sample wasting.
 - The strain library for quantification standard, potentially 90s strains, will be required.

Goal

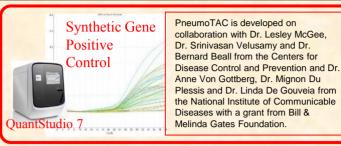
- Develop single-plex qPCR reactions covering most pneumococcal serotypes.
- II. Improve the utility of single-plex reaction with TaqMan Array Card technology.
- III. Engineer single synthetic DNA, Pneumococcal Universal Sequence Plasmid, for quantification standard of all qPCR reactions.

I. Single-plex qPCRs for Serotyping

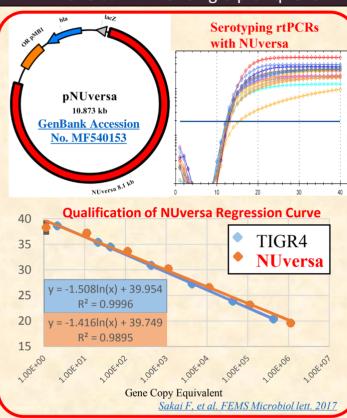
Ne'	wly	developed	assa	ays	(Hig	hli	ghted C	Cells)	
	Serotype Reference		Serotype		Reference		Serotype	Reference	
	1	(1)	16F		(1)		33B		(4)
	2	(1)	164		This Stu	ıdy	33D		(4)
	3	(1)	174		This Stu	ıdy	33C	This	Study
	4	(1)	17F		This Stu	ıdy	34		(4)
	5	(1)	18ABCF		(1)		35AC/42	(4)	
	6ABCD (1)		19A		(1)		35F/47F		(4)
	6CD (1)		19BF		(2)		35B		(2)
7AF		(1)	19F		(1)		36		(4)
	7BC/40	(4)	19"F		(4)		38		(2)
7C		(4)	19C 20		This Study		39		(4)
	8 (2)		20		(2)		41A 41F		(4)
	9VA (1) 9LN (4)		21 22A	-			411		(4)
	9LN (4) 10A (4)		22AF 23A		(1)		43 45		(4) (4)
	10A	(4)	23R		(3)		46		(4)
10CF		This Study	23F		(1)		47AF		(4)
11AD'E'		(1)	24/		(4)		48	This	Study
	11F	(4)	24B		This Stu	ıdız			July
11BC 12ABF/44/46 12B		This Study	25AF		(4)				
		16 (1)	27		(4)				
		(4)	28AF		This Study				
	13	(4)	29 31 32AF		(3) (4) This Study		(1) Pimenta FC, et al. JCM. 2013 (2) Azzari C, et al. PLOS ONE 2011 (3) Azzari C, et al. Vaccine 2012		
	14	(1)							
	15ABCF	(2)							
_	15AF	(1)	33AF/	37	(1)		(4) Sakai F, et	al. PLOS ON	E 2015
Serotype(s)		Serguanno	Target region	Accession No.	Position	Sine (ke)	Limit of detection genome equivalence (g)	Concentration (sAf)	Reaction efficiency (ev
SOCT	Porward Reverse Probe	OGAGTERFOGATGTTCTTATTGGC OCCAMICOCCACTCTGTATTC ACADDOCAAGACTGTGAATRTTGTTCCA	unjū	CE001651	4808-4831 4925-4944 4837-4864	197	21.4 (50)	400 400 300	223.4
118C	Forward Reverse Probe	TEAASTTERESSIATIESTEREA TEAFTRICAGGASAGTTGRIDGEU TOOGTGGGAAGATTCTGGTGCTAAG	sery	CREETES4	10 996-11 021 11 109-11 126 11 079-11 100	105	2.1(5)	400 700	307.8
16A	Forward Reverse Probe	OCTAGGAGGAACTTTTCTAGGG TOGCTGTGGAAATGGGAAAG GCCAGGGGATGAATGGATTATGGGG	work	CR991667	6675 6696 6787-6806 6703-6727	192	21.4 (50)	200 200 200	92.9
1/A	Forward Revenue Probe	TUAPTRIBITEATTECHTEGG AASTECTAAARTTECTGTTTGAAAAGC ATTRIBGGCGTGGGGTTACCGTAGG	Mily	CHROTON	13 895-13 938 13 983-14 006 13 941-13 964	112	21(0)	400 400 700	99.5
17F	Forward Reverse Probe	TOCTTTTCTGGGTAGCAGAG TTATGGCATAAAGCTGAGGGG TGCAGCTGATRTGGCGAGCGART	WZX	CB991670	17 361 17 381 17 473-17 490 17 683 17 662	130	21.4 (50)	400 400 200	97.8
	Comment	ARREGITTTICAGRITIACTTGATAGCTC	weht/	CR9916/7	19 294-19 320 19 386-19 408 19 383-19 367	115	2.1(5)	400 400 200	95.7
19C	Reverse Probe	DETTOCTENTGAGAGTEGTCAAG TETTOCTEGCCOCACATAATGAACT							
19C 24BF	Revenue		uzy	CR991688	15 038-15 067 15 146-15 170 15 085 15 110	133	2.1(5)	400 400 200	201.5

<u> Sakai F, et al. FEMS Microbiol lett. 2017</u>

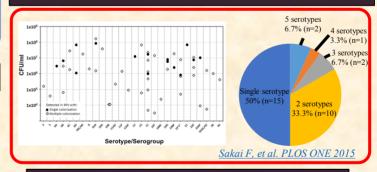
II. TaqMan Array Card: 94 Serotypes/groups



III. NUversa DNA for Single-plex qPCRs



IV. Profiling Individual Serotype Quantify of Multiple-Serotype Carriage in the Nasopharynx



Conclusion

- A total of 67 single-plex qPCR reactions, which altogether detect 94 different pneumococcal serotypes/serogroups were developed.
- ➤ 44 reactions target a unique serotype while 23 detect serotypes/serogroups.
- Utilizing a PCR product amplified from a synthetic vector, the linearity and efficiency of all qPCR reactions tested were similar to those utilizing chromosomal DNA.

Acknowledgement

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BILL & MELINDA GATES foundation