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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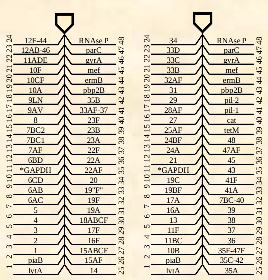

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Background

- 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

I. Develop **single-plex qPCR** reactions covering most pneumococcal serotypes.

II. Improve the utility of single-plex reaction with **TaqMan Array Card** technology.

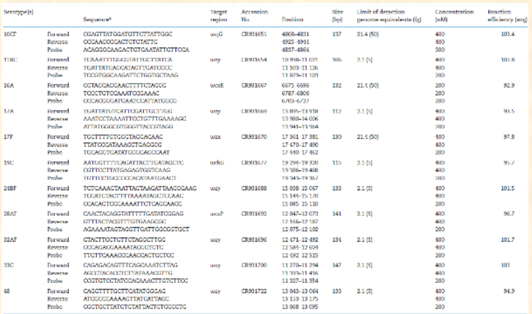
III. Engineer single synthetic DNA, **Pneumococcal Universal Sequence Plasmid**, for quantification standard of all qPCR reactions.

I. Single-plex qPCRs for Serotyping

Newly developed assays (Highlighted Cells)

Serotype	Reference	Serotype	Reference	Serotype	Reference
1	(1)	16F	(1)	33B	(4)
2	(1)	16A	This Study	33D	(4)
3	(1)	17A	This Study	33C	This Study
4	(1)	17F	This Study	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	This Study	39	(4)
8	(2)	20	(2)	41A	(4)
9VA	(1)	21	(3)	41F	(4)
9LN	(4)	22AF	(1)	43	(4)
10A	(4)	23A	(1)	45	(4)
10B	(4)	23B	(3)	46	(4)
10CF	This Study	23F	(1)	47AF	(4)
11AD'E'	(1)	24A	(4)	48	This Study
11F	(4)	24BF	This Study		
11BC	This Study	25AF	(4)		
12ABF/44/46	(1)	27	(4)		
12B	(4)	28AF	This Study		
13	(4)	29	(3)		
14	(1)	31	(4)		
15ABCF	(2)	32AF	This Study		
15AF	(1)	33AF/37	(1)		

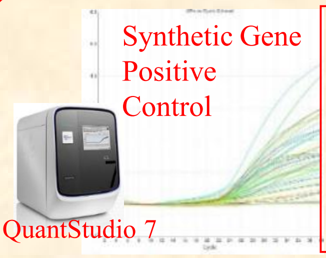
(1) Pimenta FC, et al. JCM. 2013
(2) Azzari C, et al. PLOS ONE 2011
(3) Azzari C, et al. Vaccine 2012
(4) Sakai F, et al. PLOS ONE 2015



Serotype(s)		Sequence	Target region	Accession No.	Position	Size (bp)	Limit of detection genome equivalents (fg)	Concentration (nM)	Reaction efficiency (avg)
10CF	Forward	GGAGTTATGGATGTTCTTATTGGC	wcjG	CR931651	4808–4831	137	21.4 (50)	400	103.4

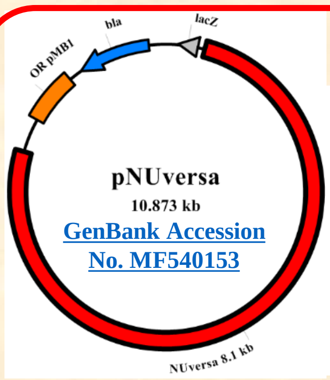
Sakai F, et al. FEMS Microbiol lett. 2017

II. TaqMan Array Card: 94 Serotypes/groups

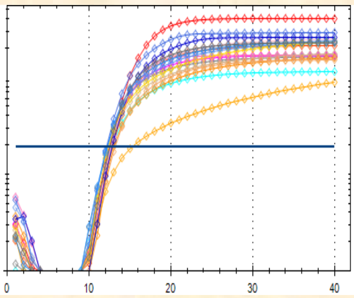


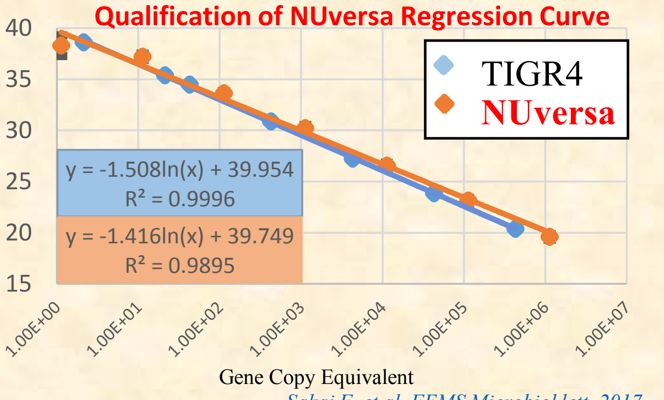
PneumoTAC is developed on collaboration with Dr. Lesley McGee, Dr. Srinivasan Velusamy and Dr. Bernard Beall from the Centers for Disease Control and Prevention and Dr. Anne Von Gottberg, Dr. Mignon Du Plessis and Dr. Linda De Gouveia from the National Institute of Communicable Diseases with a grant from Bill & Melinda Gates Foundation.

III. NUversa DNA for Single-plex qPCRs



Serotyping rtPCRs with NUversa





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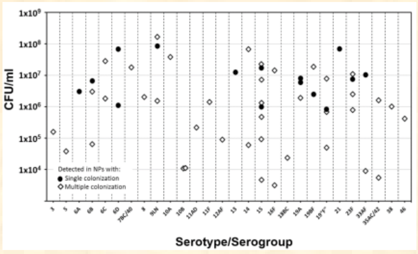
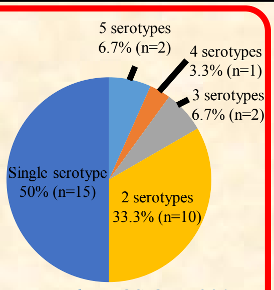
IV. Profiling Individual Serotype Quantify of Multiple-Serotype Carriage in the Nasopharynx





Sakai F, et al. PLOS ONE 2015

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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