Challenges and Strategies for Potency Assay Development in High-Valency Vaccines

Milica Grozdanovic

Merck & Co., Inc., West Point, PA USA

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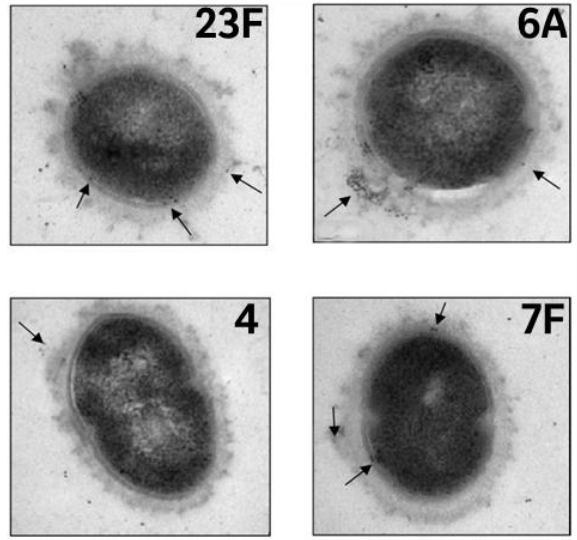
Overview: Multi-valent and Conjugated Vaccines

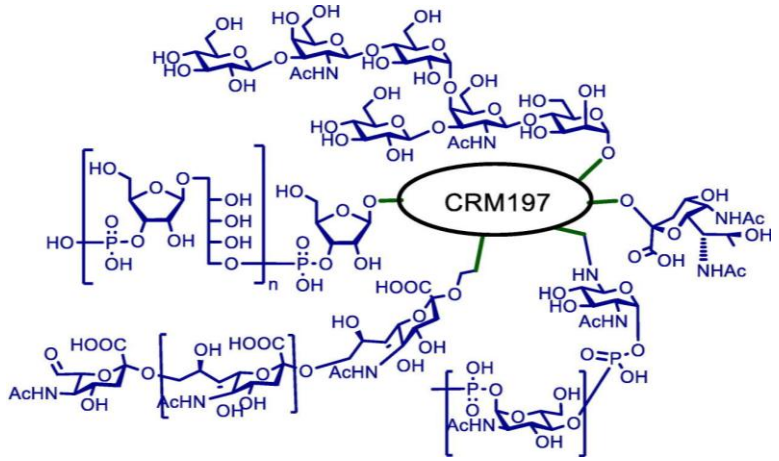
- Successful vaccines arise from not only sound scientific strategy, but also from manufacturing and cold-chain supply chain logistics to meet commercial demand
- Optimization of logistics and improved patient adherence is often achieved by the well-established ***practice of combining individual vaccines*** (e.g., diphtheria/tetanus/pertussis or mumps/measles/rubella)

- Encapsulated bacteria ***contain multiple serotypes*** – distinct variations within the species (classified based on the structure of their capsular polysaccharide)

❖ Conjugated vaccines: ***bacterial polysaccharide antigens are linked to carrier proteins*** to induce strong immunity in children and adults

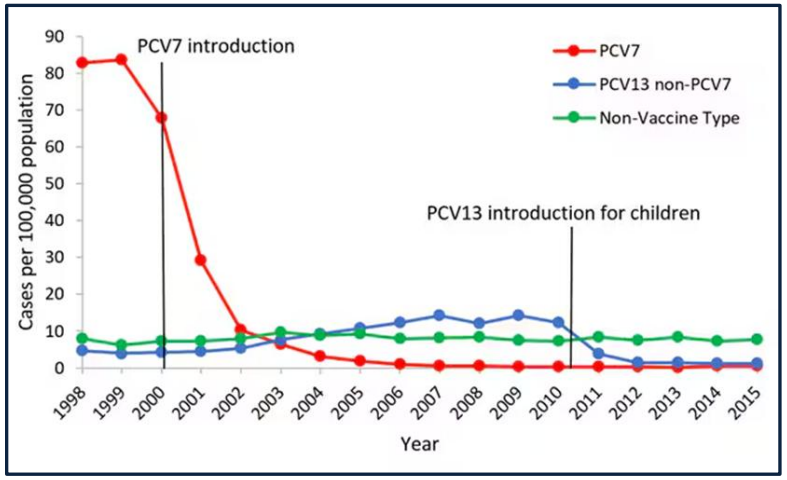
❖ Revolutionized public health, particularly for *H. influenzae,* ***S. pneumoniae****, and N. meningitidis*





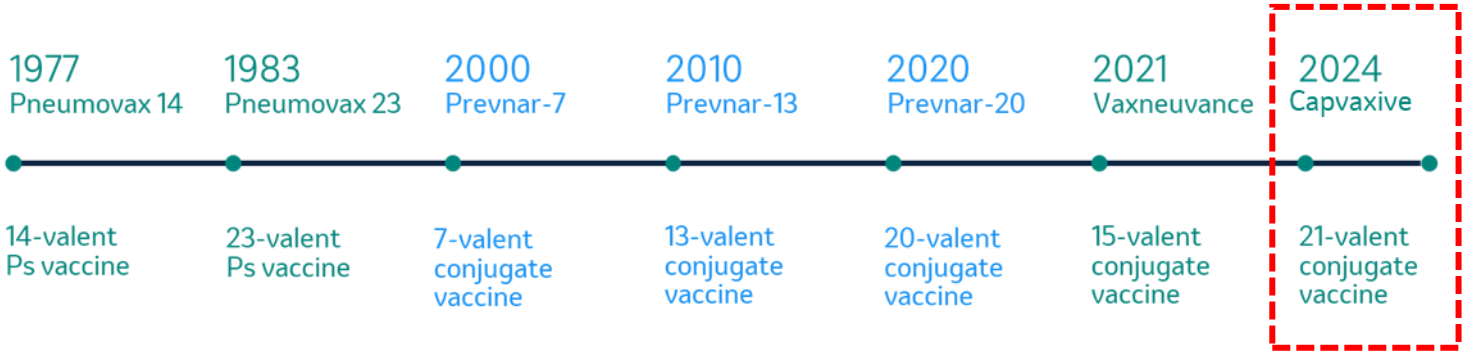
Khatuntseva et al., Int J Biol Macromolecules, 2022

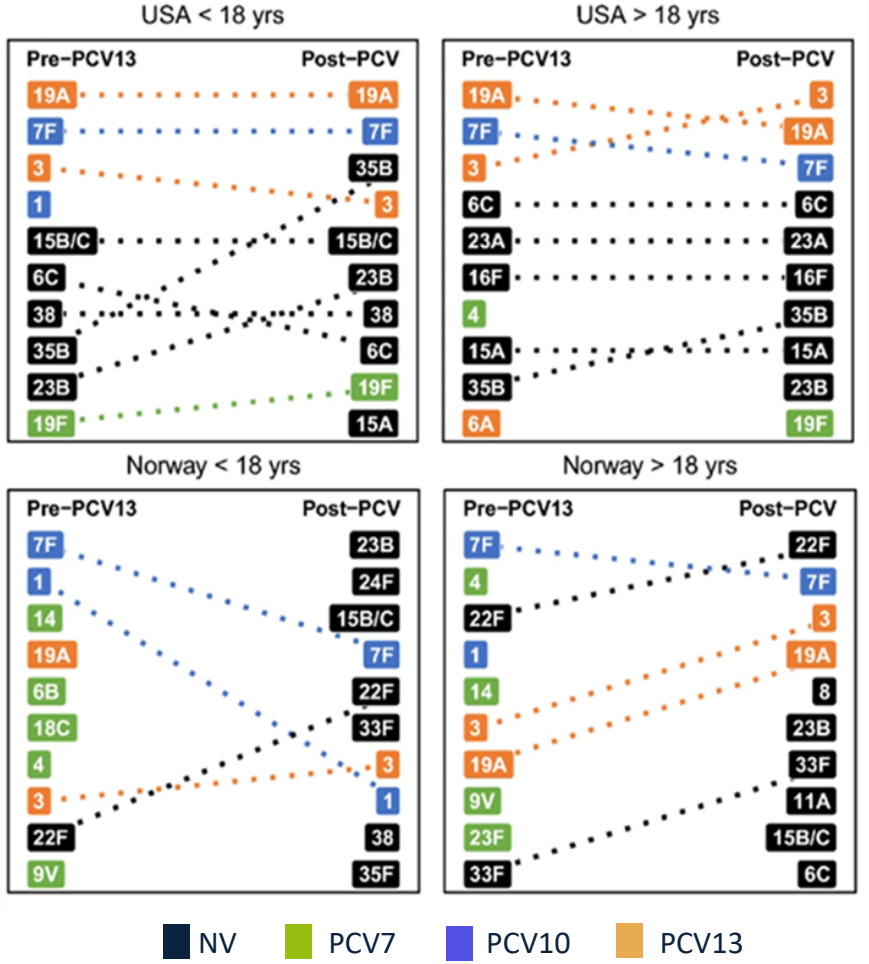
Effect of Pneumococcal vaccines: A game of serotypes



Effect 1: A decrease in the overall rate of pneumococcal pneumonia and associated diseases

Effect 2: Decrease in incidence of vaccine type serotypes but increases in non-vaccine serotypes along with serotype switching.





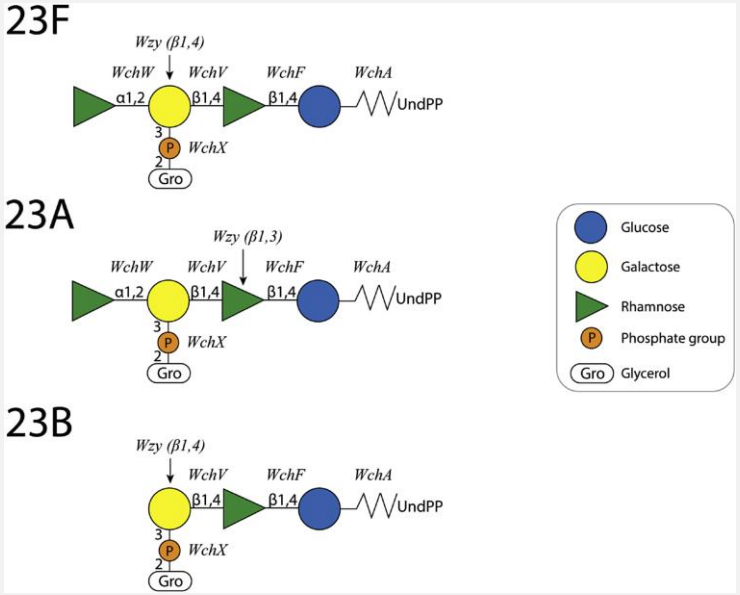
https://www.cdc.gov/vaccines/pubs/surv-manual/chpt11-pneumo.html

Løchen et al. Scientific Reports, vol 10, 2020

Musher et al. Pneumonia, vol 14, 2022

Regulatory Requirements for Release & Stability

Serotype specific assay



1

Immunoassays are routinely used for complex multivalent polysaccharide vaccines

- Generation of highly specific mAbs – capture and detection- against structurally very similar polysaccharides (Ps)
- Responsibility to define clinically relevant antibodies, especially when no animal model exists.
- Challenging to obtain since many Ps tend to be highly-cross reactive and can be poorly immunogenic in animals.
- Dedicated teams to handle critical reagents generation, storage, stability

2 X 21 = 42 critical reagents

Ravenscroft et al., Carbohydrate Research, 2017

Regulatory Requirements for Release & Stability

Compounding risk for lot release

2

- Measured dose must be 70-130% of the label claim
- 80-120% confidence interval around reportable result
- Challenge:
  21 results for release per lot
  10 (timepoints) x 21 = 210 results for stability per lot
- 5% failure rate based on assay variability is acceptable for a monovalent product
- If you have a 21 valent product, chances of failing to release a lot raises to **66%**

How we meet the Precision & Accuracy Requirements

A **11**

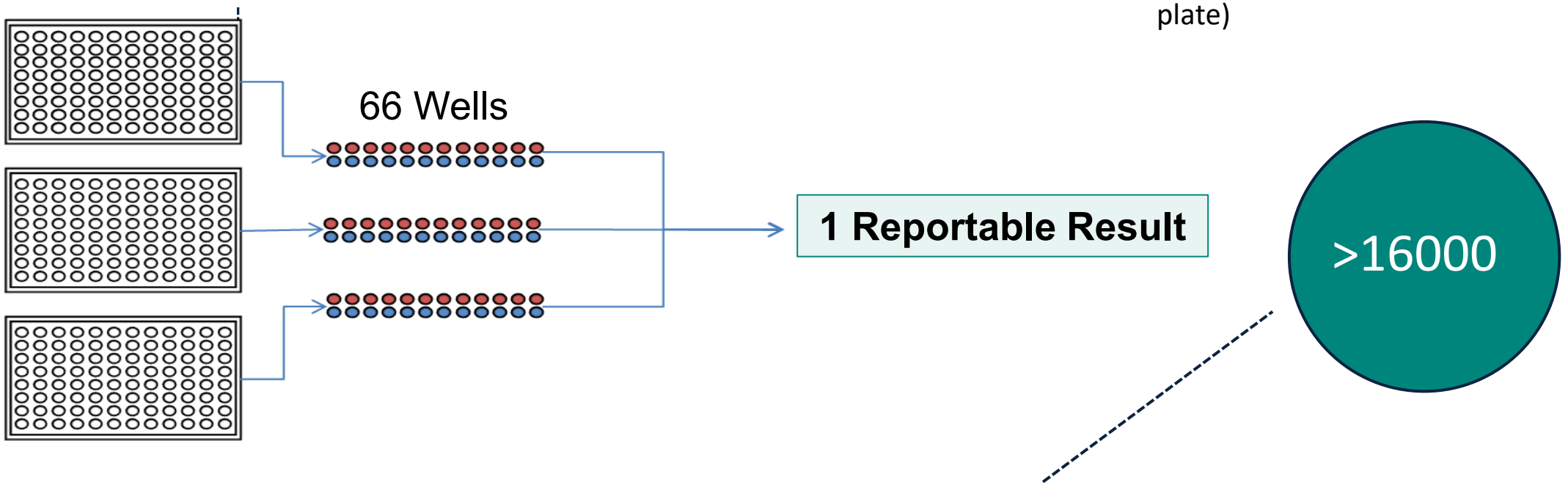
An **11-point serial dilution curve** is plated for both reference and sample

B **22**

Each curve is plated in **duplicate points**

C **66**

3 Plates (a total of 6 curves) are run for each sample (3 samples fit per plate)



Total number of reportable results from ELISA assays in support of Capvaxive development

Capvaxive ELISA by the Numbers

- Number of GMP analysts needed to measure all 21 serotypes in 1 day: **10**
- Time needed to complete assay: **6-7 h**
- Number of reagent solutions prepared**: 42**
- Number of microtiter plates**: 63**
- Number of analyst interactions with each plate: **12 (756 total)**

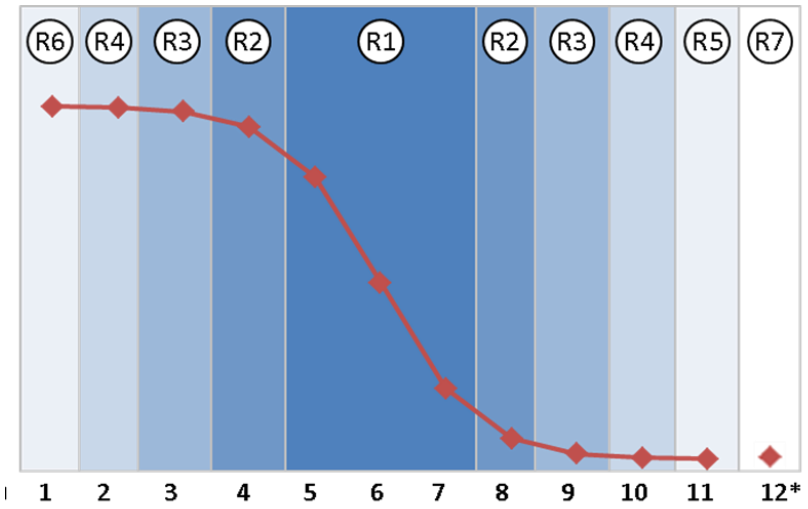
Cumulative number of manual analyst steps that need to be executed correctly = 798

Plate randomization & Liquid Handlers

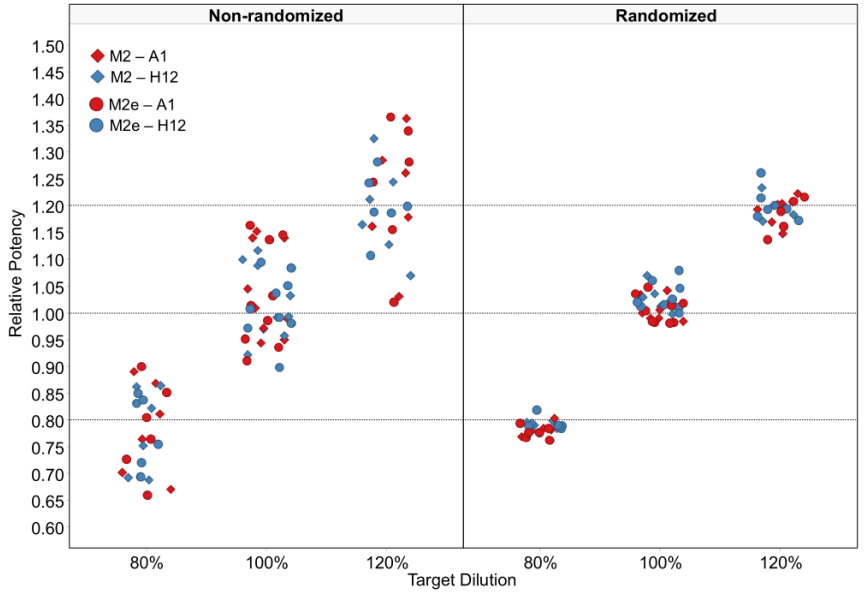
- Plate positioning effects translate into potency bias
- Block randomization mitigates impact of positioning effects on potency

➢ Liquid handlers for dilution curve plating remove analyst errors and can perform block randomization:
- Dilution points in the R1-R3 curve zone (A)
are prioritized for dispensing in the center of the plate (B)

A



Block-randomization Results

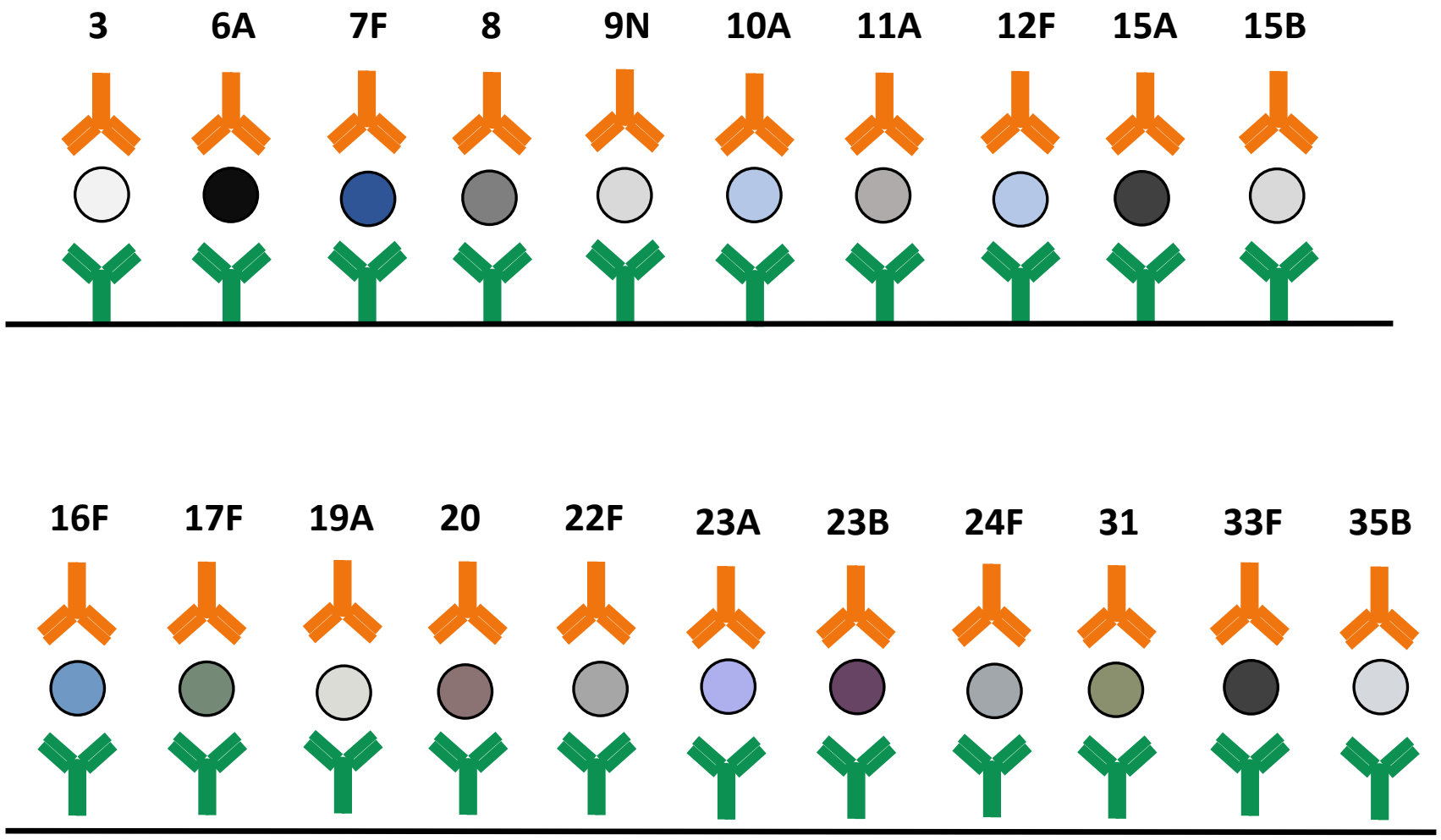


B

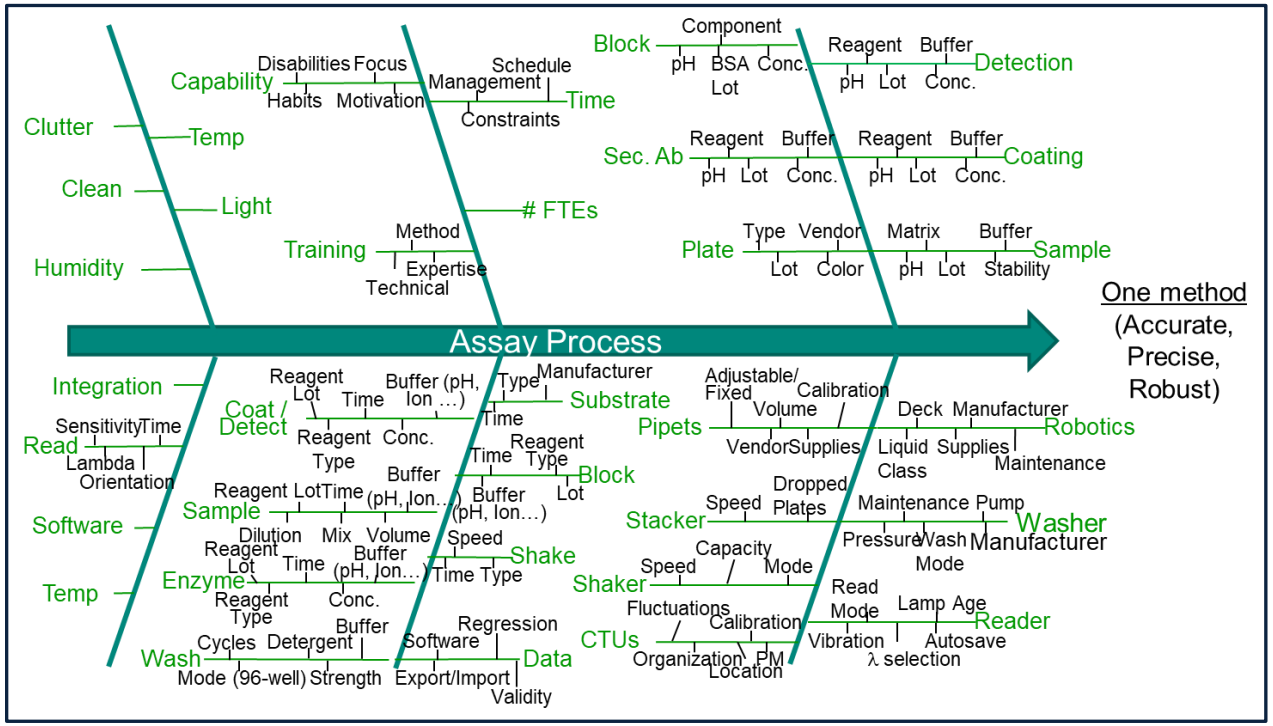
	1	2	3	4	5	6	7	8	9	10	11	12
A	7	7	6	5	5	4	4	5	5	6	7	7
B	6	4	3	3	3	3	3	3	3	3	4	6
C	4	2	2	1	1	1	1	1	1	2	2	4
D	4	2	2	1	1	1	1	1	1	2	2	4
E	4	2	2	1	1	1	1	1	1	2	2	4
F	4	2	2	1	1	1	1	1	1	2	2	4
G	6	4	3	3	3	3	3	3	3	3	4	6
H	7	7	6	5	5	4	4	5	5	6	7	7

Roselle et al., J Immunol Methods, 2017

“The Potency assay” is an umbrella term for 21 different assays



All choices are made for 21 curves



- Potency testing with 21 serotype-specific assays is a very resource-heavy approach.
- Methods need to be simplified and unified
- Differences between serotypes introduce opportunities for errors

Aligning all method parameters to work for 21 different curves is very challenging

Aligned:

All common buffers (dilution, wash, blocking buffers)
Incubation times, temperatures
Sample prep (share between STs)
Equipment settings (wash volumes, λ, shaker speed)

Not-Aligned:

Curve starting concentrations (mAb sensitivity)
Working mAb concentrations
Detection reagent concentrations

Examples

Case study #1 Signal to Noise Background

- Blocking buffer containing BSA chosen during development
- Robustness performed for incubation time, BSA concentration, volume/well
- 2 labs reported high Signal/Noise for only one of 21 Serotypes (STs)
- Investigation initially focused on mAbs and samples due to serotype-specific issues

❖ New BSA lot caused issues only for one ST!

Case study #2 Low reportable result

- 1 ST reported with results below target
- Investigation initially focused on mAbs and samples due to serotype-specific issues
- mAb lots compared, lab to lab confirmatory testing, plate readers compared

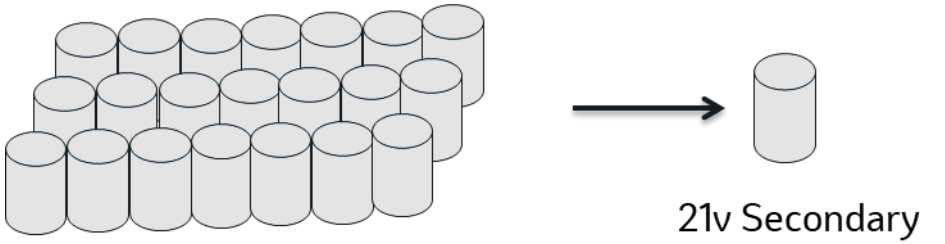
❖ Catalog number of solution changed in method accidentally yielded 10X lower BSA conc

(examples are different STs ☺)

Reference Stability: challenges of multi-valent DP

- Concentration of each serotype is often very low
- Very few methods provide type specific information on the multivalent formulation
- Multi-valent formulations can mask drift in individual components
- Variability usually taken from most variable ST

2-Tier Approach - Primary Reference Standard (PRS) and Secondary Reference Standard (SRS)

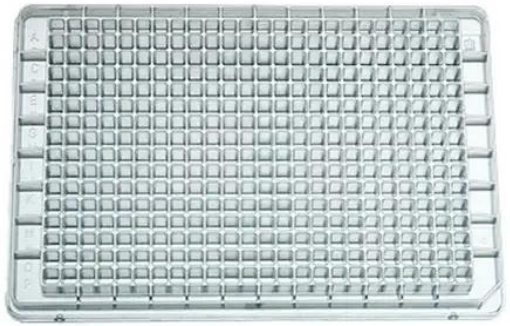


21 PRS: From pivotal clinical trial batches

21v Secondary Reference Standard (SRS)

DS is monovalent and formulated at high concentrations
Monitored using traditional methods (e.g., HPLC, Biophysical methods)
Stored at -70^{o}C and compared against multi-valent Ref Std on stability

Automation: ideal solution for high-valency?



Fully automated system:

- Reagents loaded onto the deck of a system equipped with a robotic manipulator arm
- 384-well multi-channel arm (MCA)
- 8-channel liquid-handling arm (Tecan EVO).
- KiNEDx robotic arm with hoteling stations for storage of plates and tips.
- KiNEDx arm transferred plates to the Tecan EVO for each step.
- MCA picks up the tips, aspirates and dispenses reagents to plates, washes the tips, and returns them to the box

Challenges:

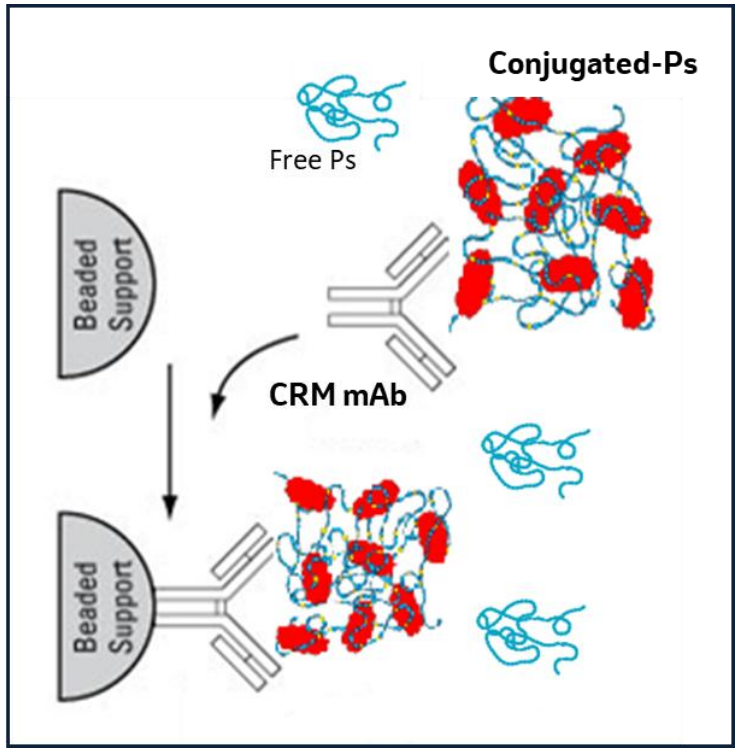
- Not QC lab friendly (Vaccine life-cycle duration, testing on import)
- Automation is more friendly for “parallel” applications and not “serial”

  (i.e. same assay 20 times, not 20 assays 1 time)
- Multi-hour setup does not pay off for < 5 samples

Pedrazzi et al., SLAS Technology, 2023
Image: Fleischer et al., J Lab Automation, 2016

Conjugated vaccines: Multi-attribute meets Multi-valent

- ❑ Product is a complex mixture of different molecules, total serotype-specific Ps dose is not enough
- ✓ Need to develop assays that measure only Conjugated-Ps, Free Ps, or Free protein



Challenge 1: *Complete removal* of CRM-conjugated Ps prior to measuring Free Ps

- ➢ 21 STs have different MW, charges, chemical properties

Challenge 2: Assay has to be very sensitive and serotype specific to measure remaining low Free Ps levels in DP

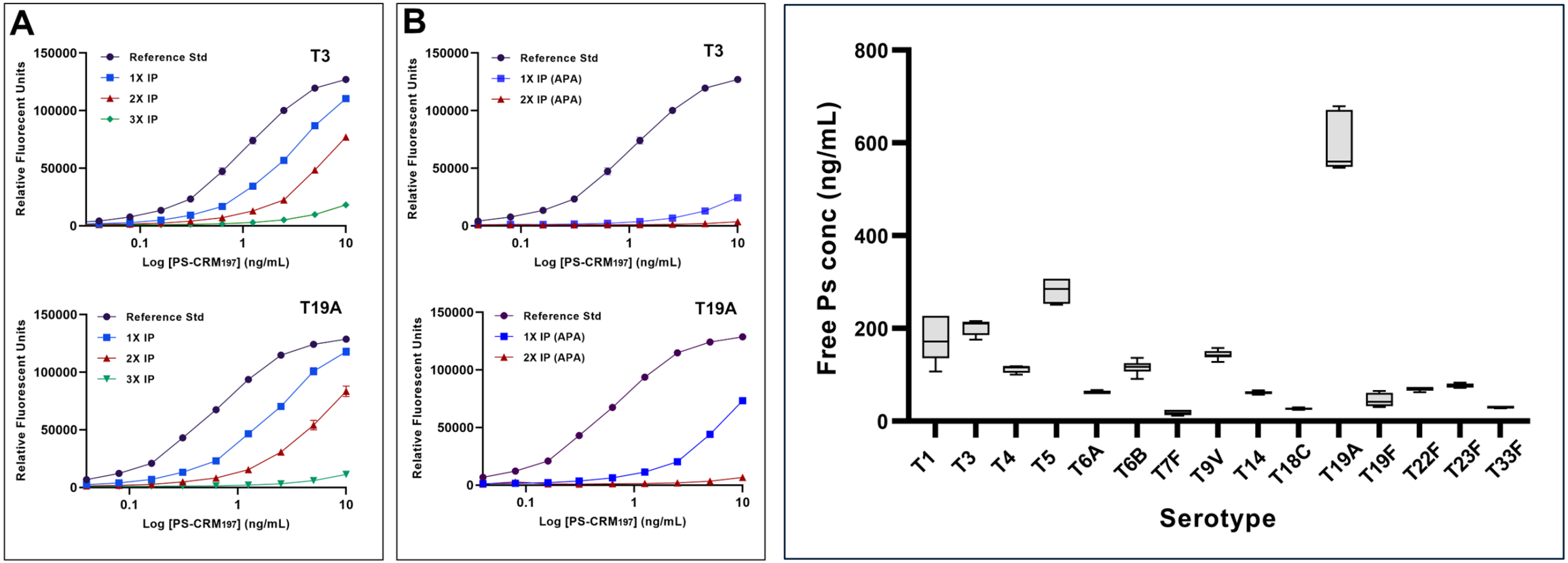
- ➢ < 400 ng/mL for each individual serotype

Magnetic bead immunoprecipitation of CRM-conjugated Ps

- ✓ Our goal: *complete depletion* of the antigen from the sample.
- ✓ Utilizes 3 clones of anti-CRM mAbs to pull down Conjugated-Ps

Free Ps remains in the supernatant

Free Polysaccharide ELISA



Data from: Grozdanovic, M., Samuel, R., Grau, B. et al. Serotype-specific quantification of residual free polysaccharide in multivalent pneumococcal conjugate vaccines. Glycoconj J 41, 47–55 (2024). https://doi.org/10.1007/s10719-023-10143-6

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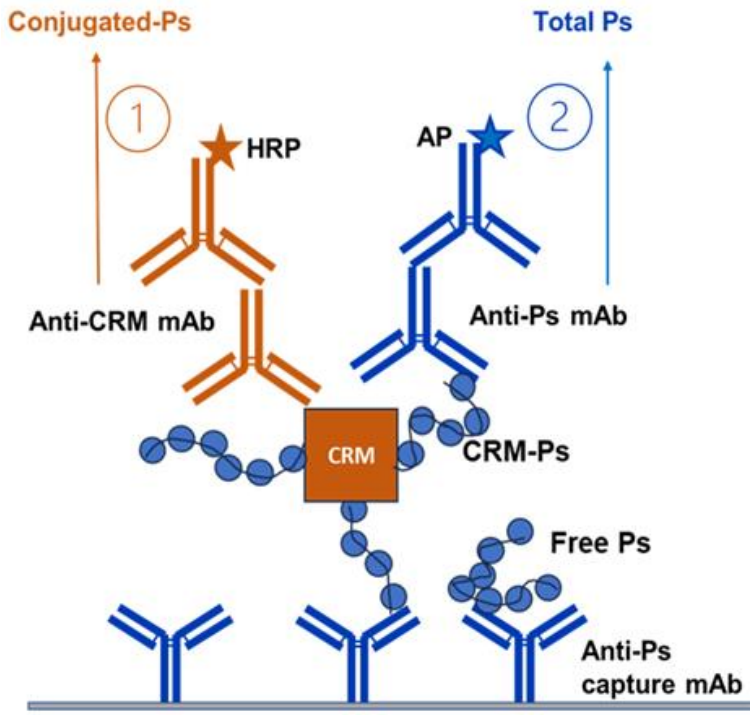


The Multi-attribute ELISA combines 2 separate assays into one assay

Coat: Ps-specific capture mAbs

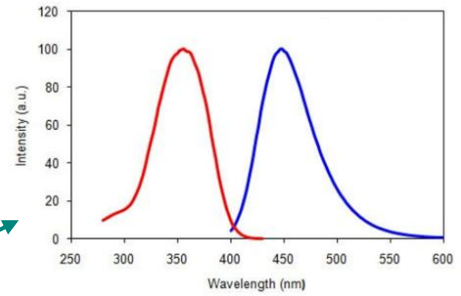
Load: 11-point DP dilution curve

Detection 1: Anti-CRM mAbs + HRP-IgG

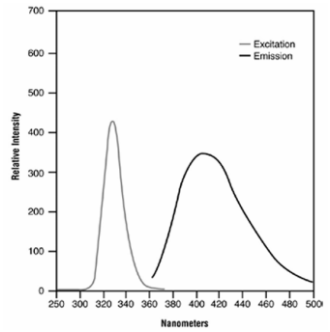
HRP fluorescent substrate

Detection 2: Anti-Ps mAbs + AP-IgG

AP fluorescent substrate



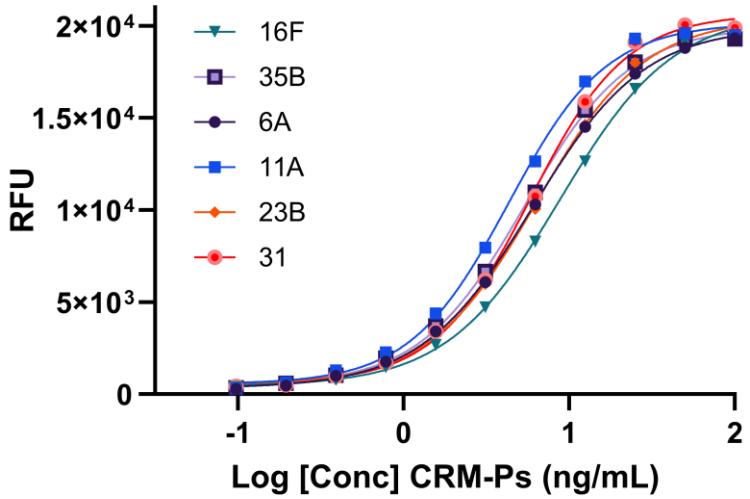
Measures Conjugated-Ps

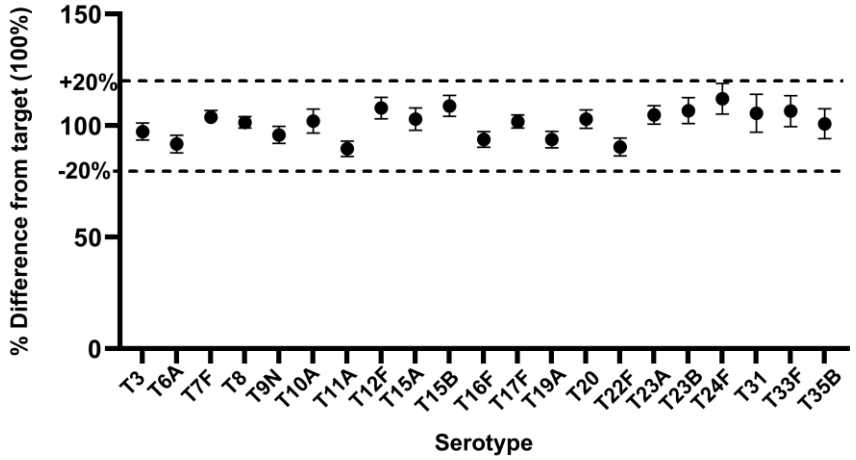


Measures Total-Ps

Multi-attribute ELISA

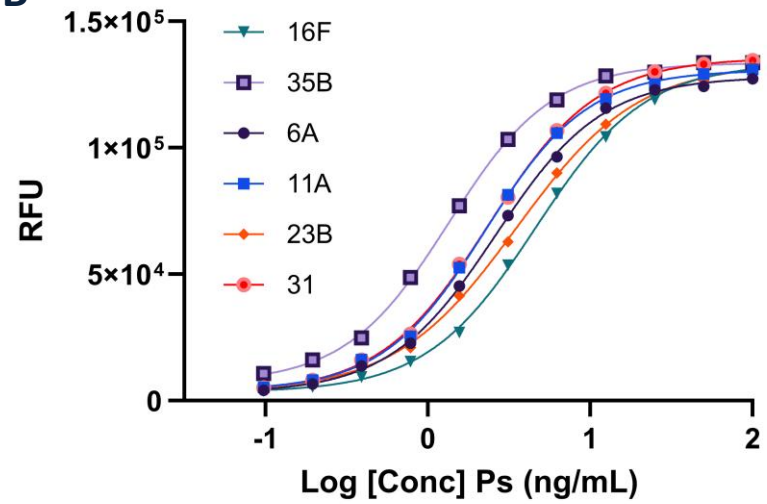
A

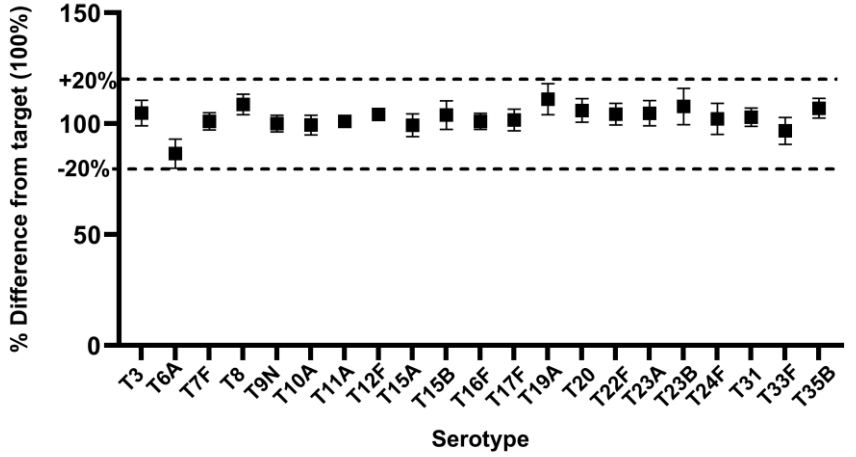




Left: Representative reference standard curves from serotypes 6A, 11A, 16F, 23B, 31, 35B

B

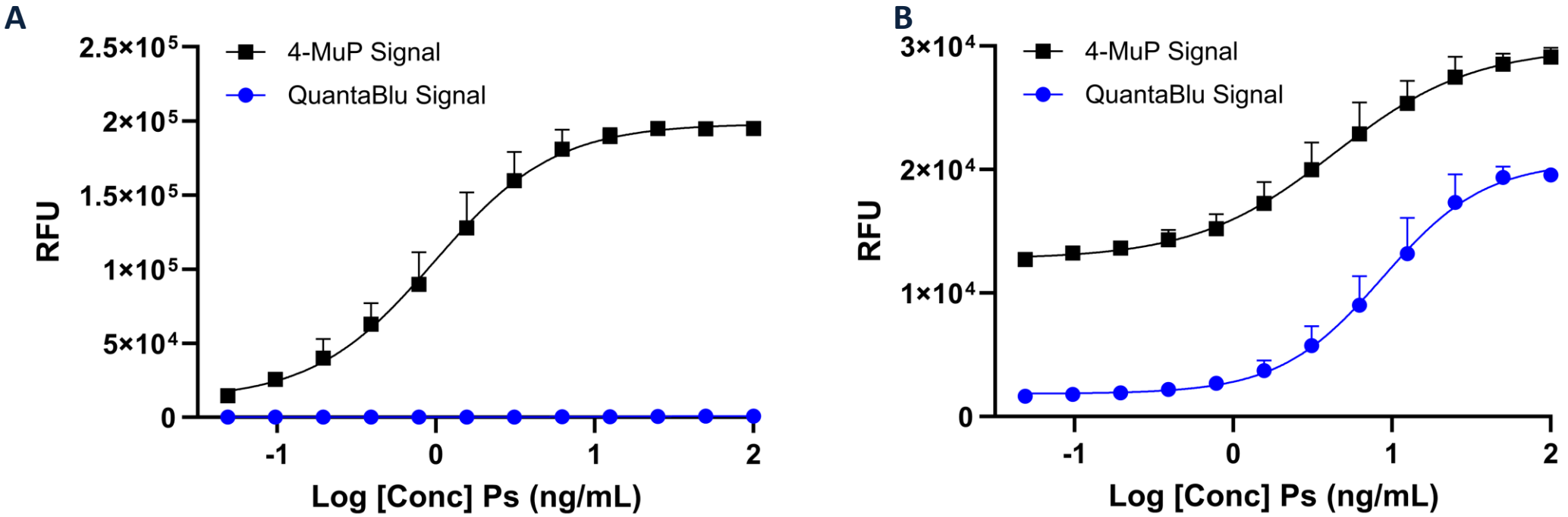




A. CRM-conjugated Ps
B. Total Ps

Right: Average accuracy across 3 dose levels

Multi-attribute ELISA

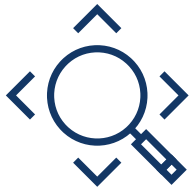


Order of detection steps matters:

A) The plate was first developed using QB fluorescent substrate followed by 4-MuP

B) The plate was developed first with 4-MuP as substrate, washed, and then developed with QB as substrate.

Conclusions & Acknowledgements



High-valency will compound any issues, is resource intensive, and requires adapting with scale of operations in mind

Would not be possible without support & effort from the whole Merck CMC team

Special thanks to the Capvaxive potency team & friends of the team!!!

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