Novel Influenza Vaccines: Fusion of HA with bacterial flagellin drives strong HAI titers and allows for efficient bacterial expression

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Flu-2015

June 8, 2015

VAXINNATE

The VaxInnate Advantage

E. coli Based Manufacturing Supports Unparalleled Pandemic Response

	Egg Based	Cell Culture	Baculovirus	*E. coli*
	Pisano pers comm	CIDRAP June 2014	Press Release June2014	Phase 1 Experience
Efficiency Yield/L of Bulk Vaccine				
	8 mg/L	6 mg/L	70 mg/L	200mg/L
Speed Estimated Time to first and last 600 MM doses (bulk)	30 MM	50 MM	13 MM	120 MM
	Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr	Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar A	Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr	Jan Feb Mar Apr May Jun Jul Aug
	>1 yr	>1 yr	>1 yr	32 wks

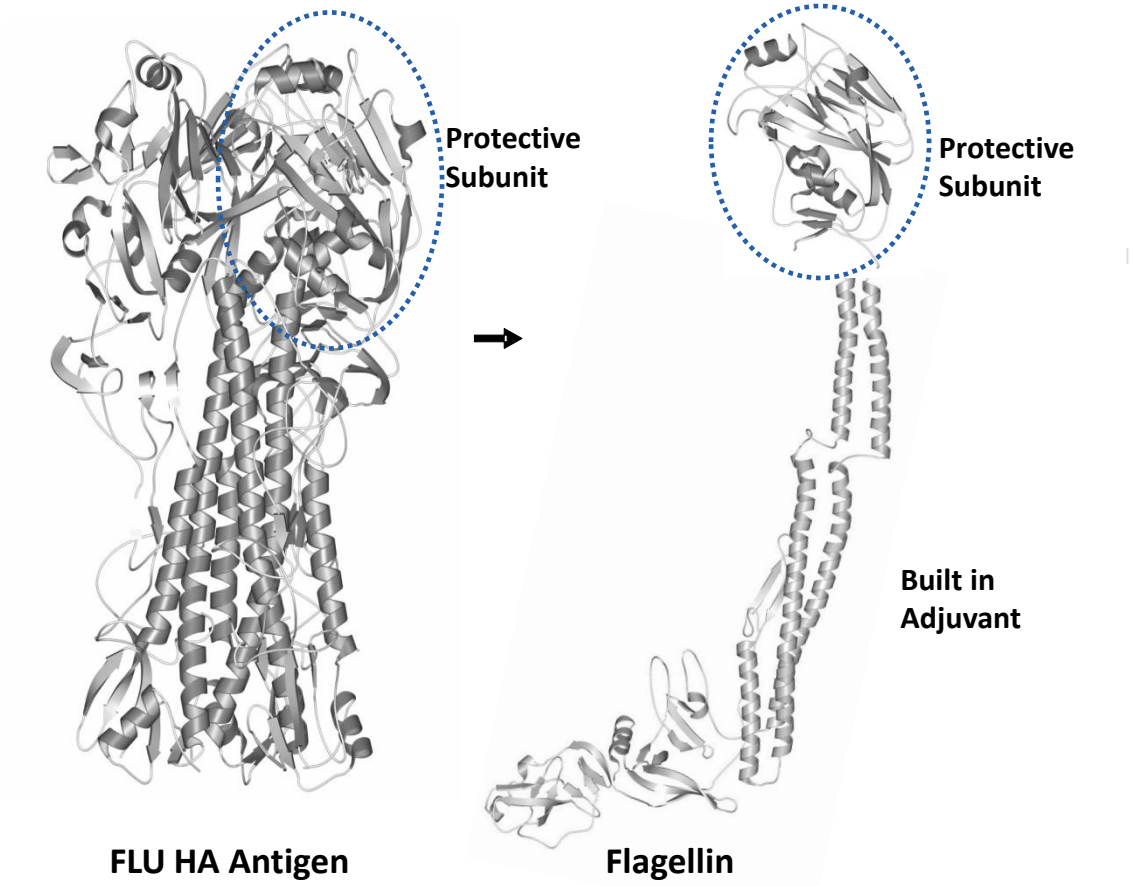
= first doses available

VaxInnate's platform can exceed the RFP objective of delivering 50MM doses in 6 months

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Influenza Vaccine Platform

Highly Immunogenic Fusion Vaccines that can be Manufactured in E. Coli



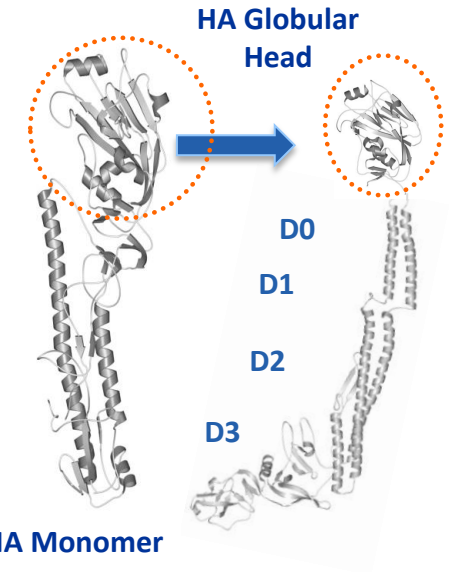
- **FAST** – Rapid response capabilities **LOW COST** – Of goods and low capital investment **HIGH YIELD** – Unsurpassed supply

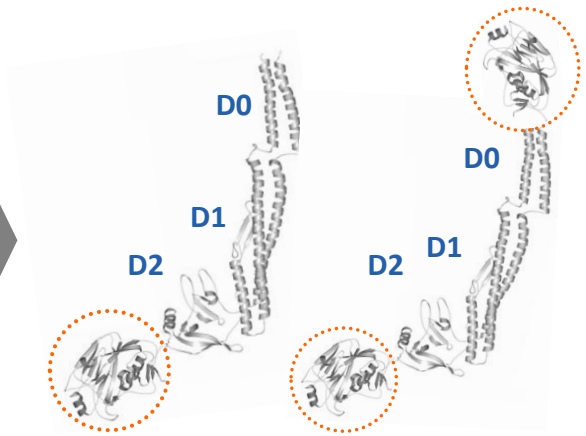
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Influenza Vaccine Platform

Multiple Vaccine Formats Developed: Three Support Influenza Vaccines

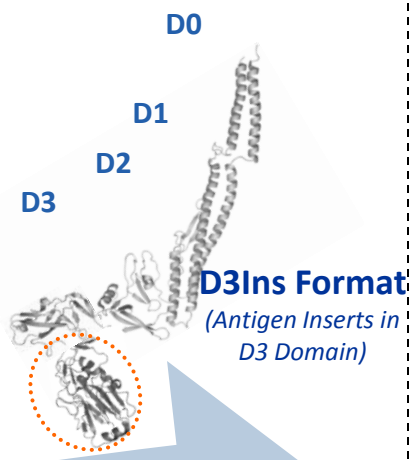
Evolution of the Platform





R3 Format *(Antigen Replaces D3 Domain)*

R3.2x Format *(Antigen Replaces D3 Domain and Fused to C term)*



D3Ins Format *(Antigen Inserts in D3 Domain)*

Inception

- First vaccines fused the HA globular head to C term of flagellin

Breakthrough

- Alternative vaccine formats were found to 'separate' immunogenicity and reactogenicity, thereby widening the therapeutic window
- Clinically demonstrated

Expansion

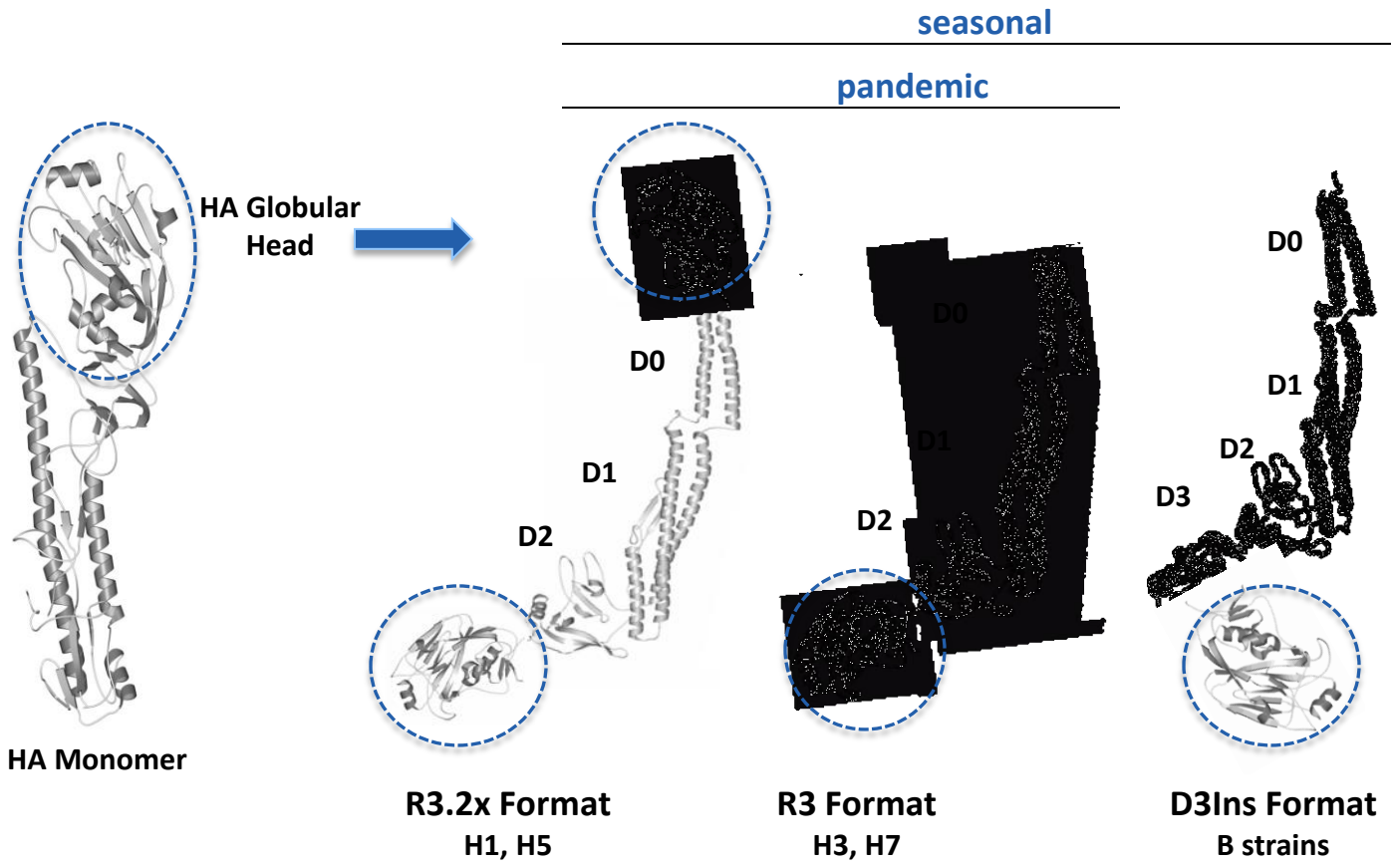
- Subsequent refinements led to the development of an entire repository of vaccine formats
- Using lessons from flu, have extended the platform to support dengue and other infectious disease target designs

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Influenza Vaccine Platform

Three Vaccine Formats Support Influenza

The Choice of Format is Reliably Determined by the HA Type/Subtype



- **We have tested 5 H1s, including 2 in human; 3 H2s; 3 H5s, including 1 in human; 8 H3s, including 1 in human; 3 H7s; 2 H9s and 7 Bs of both Yamagata and Victoria lineages, including 2 in human.**

Influenza Vaccine Platform

More than 1,700 Subjects Evaluated Over Eight Clinical Campaigns

Study	Initiated	Phase	Indication	Design	Status/Size
VAX102-01 thru 09	Aug 2007	Phase 1/2	Universal flu vaccine (M2e antigen)	Series of studies to determine dose, route of administration and use with TIV in adults 18-49	Complete (N=351)
VAX125-01	Jul 2008	Phase 1/2	H1N1 Solomon Islands	Dose ranging, safety and immunogenicity in adults 18-49	Complete (N=128)
VAX125-02	Jul 2009	Phase 1/2	H1N1 Solomon Islands	Dose ranging, safety and immunogenicity in adults ≥ 65	Complete (N=120)
VAX128-01	Jul 2010	Phase 1	H1N1 California 07	Dose ranging, safety and immunogenicity in adults 18-49	Complete (N=112)
VAX128-01	Aug 2010	Phase 1	H1N1 California 07	Dose ranging, safety and immunogenicity in adults ≥ 65	Complete (N=100)
VAX128-02	Nov 2010	Phase 2	H1N1 California 07	Expanded safety and immunogenicity in adults 18-64	Complete (N=100)
VAX161-01	May 2012	Phase 1/2	H5 Indonesia	Dose ranging, safety and immunogenicity in adults 18-49	Complete (N=499)
VAX2012Q-01	Mar 2014	Phase 1	Seasonal Quadrivalent	Dose ranging, safety and immunogenicity in adults 18-40	Complete (N=316)

Clinical
Rationale for Format and Dose Selection

Influenza Vaccine Platform

Key Observations from the VAX102 Program

- VaxInnate's first clinical experience was based on the conserved influenza antigen, M2
- The vaccine utilized the 'C term' format. Four copies of the M2 ectodomain were fused to the C terminus of flagellin to form STF2.4xM2e, VAX102
- This initial Phase I study was designed as a dose escalating study with dose groups of 10, 33 and 100 μg
- Mid-way into the 10 μg dose group a small number of subjects presented with a cluster of symptoms that were consistent with a pro-inflammatory response due to innate immune stimulation
  - Fever, aches, nausea and chills were among systemic symptoms reported; there was a rapid onset of these symptoms
  - Symptoms were related to rises in the acute phase reactant CRP (regulated by IL6)
- Lower doses were equally immunogenic but well tolerated.
  - Immunogenicity and reactogenicity therefore were 'separable' in that there is a dose window that is immunogenic but not reactogenic

Influenza Vaccine Platform

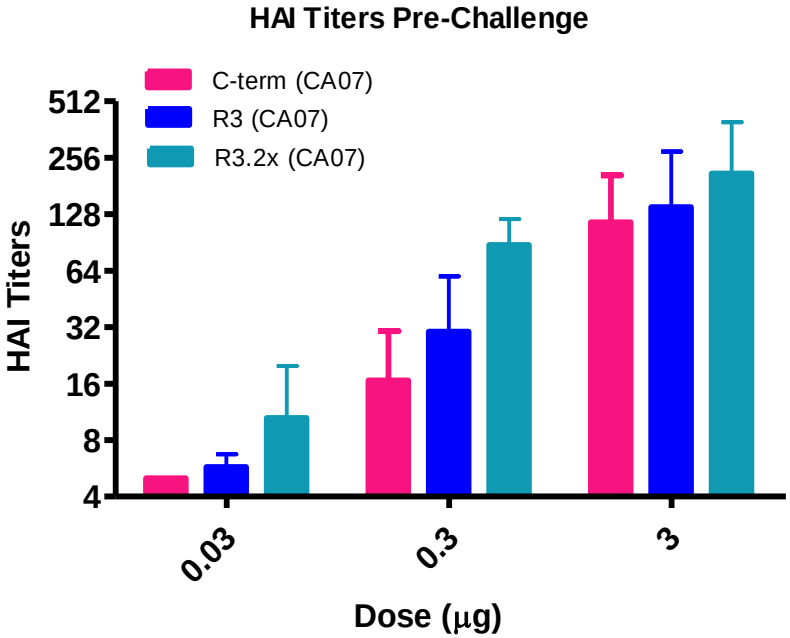
Models for Optimizing the Therapeutic Window

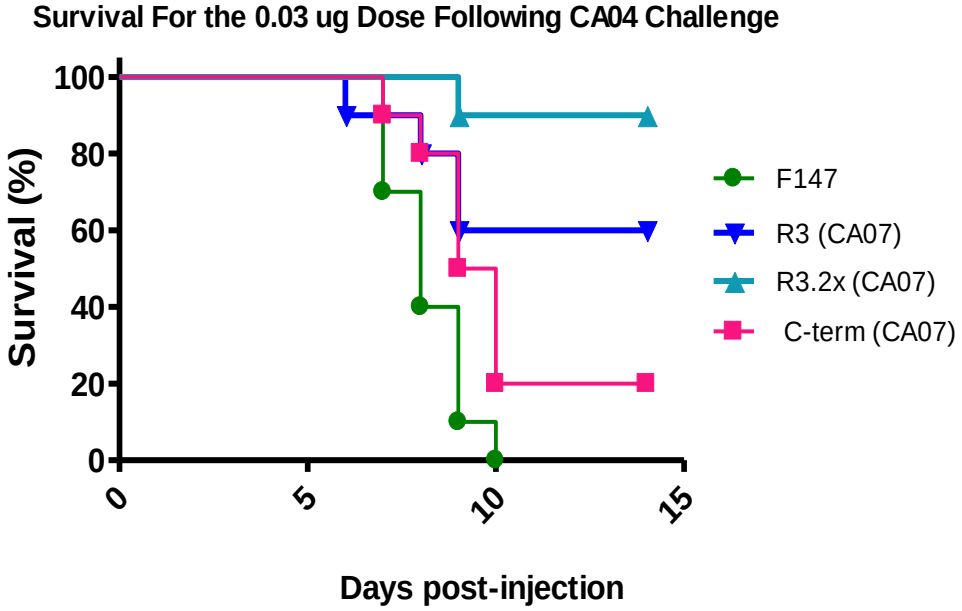
- A rabbit model of reactogenicity was established in which the following measures were evaluated in the first 24 hr post immunization:
  - food consumption
  - Temperature
  - CRP: C reactive protein
- The goal was to use changes in these measures to help establish the dose range for evaluation in the clinic
- The model was consistent with the observed therapeutic window for VAX102, suggesting that it could be used to guide initial clinical dose selection

A rabbit model for predicting the therapeutic window was established

Influenza Vaccine Platform

Optimizing the Therapeutic Window



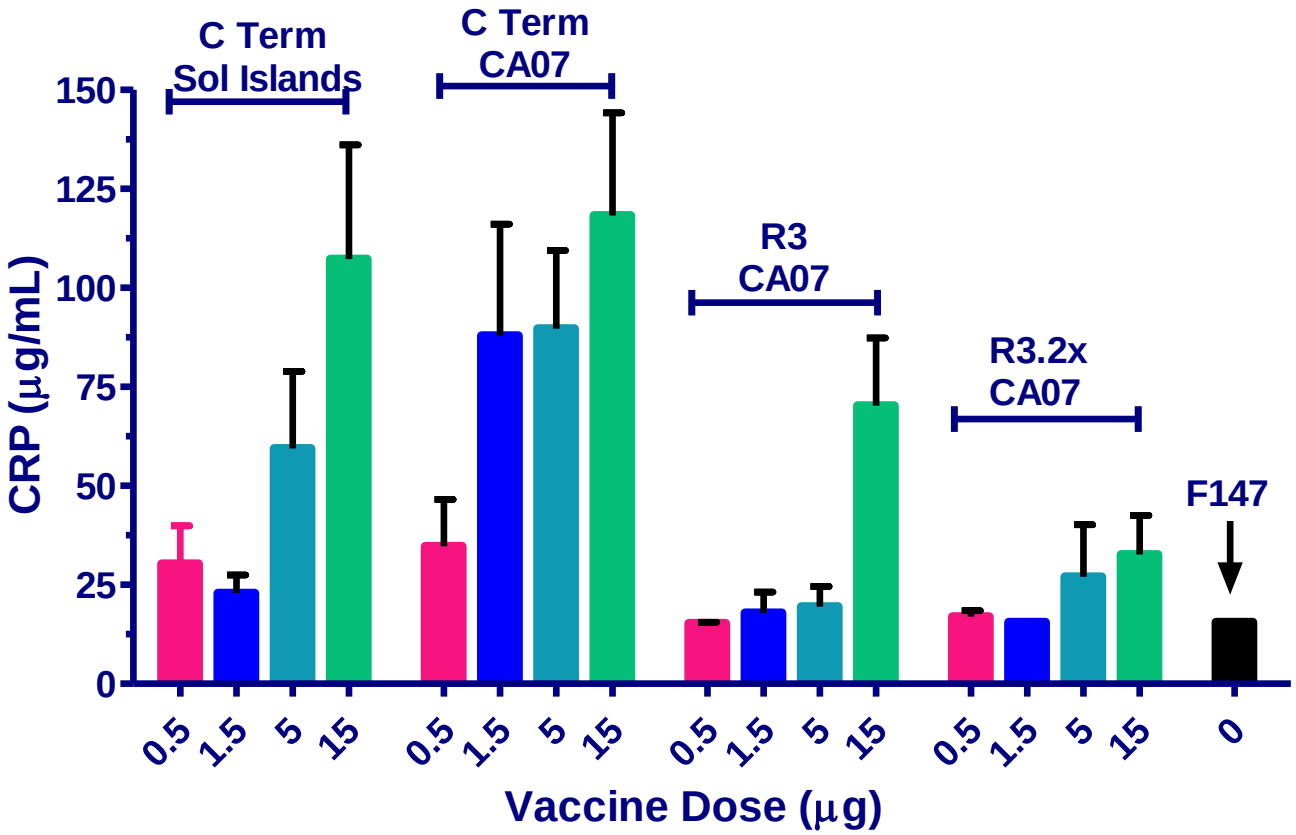


N=15 mice per group. Mice were immunized twice with the indicated doses and infected with a lethal dose of the H1N1 influenza virus.

Alternative formats developed for vaccines targeting H5 or H1 pandemic strains were more immunogenic/protective in preclinical models

Influenza Vaccine Platform

Optimizing the Therapeutic Window



These formats were found to have wider dose windows in the rabbit model. Thus, similar to dose, the different formats can separate reactogenicity and immunogenicity

VAX128-01

A Phase I Study to Evaluate the Safety and Immunogenicity of the Different Formats of VAX128 in Healthy Adults 18-49 Years of Age and in Community Living Adults ≥65 Years of Age

Study Design

- Dose escalation study (adaptive, 3 subjects per group initially with a max of 12)
- Compares general tolerability and immunogenicity of VAX128A (C term), VAX128B (R3) and VAX128C (R3.2x) constructs
- Age groups 18-49 years and ≥65 years evaluated
- Vaccine dose was formulated at the clinical site
- All doses given in 0.5 ml IM
- Vaccine buffer used as control
- Safety recorded for first 7 days by clinic visit and diary
- Serum specimen for CRP 24 hrs post-vaccination
- Immune response (HAI) measured on day 0, 7, 14 and 28
- Long term follow up at 6 months and one year

VAX128 targets the A/California/07/2009 H1 strain

VAX128-01

Number of Subjects Evaluated by Dose and Construct

	Age 18-49 years					Age ≥ 65 years				
Dose	VAX128					VAX128				
(µg)	A	B	C	Plac.	Total	A	B	C	Plac.	Total
0				10	10	0			10	10
0.5	3	3	3	0	9	0	0	0		0
1.25	3	3	3	0	9	3	3	3		9
2.5	12	3	3	0	18	3	3	3		9
4	12	3	3	0	18	3	3	3		9
8	6	3	3	0	12	21	6	3		30
12		3	3	0	6		3	3		6
16		12	12	0	24		12	3		15
20			6	0	6			12		12
Total	36	30	36	10	112	30	30	30	10	100

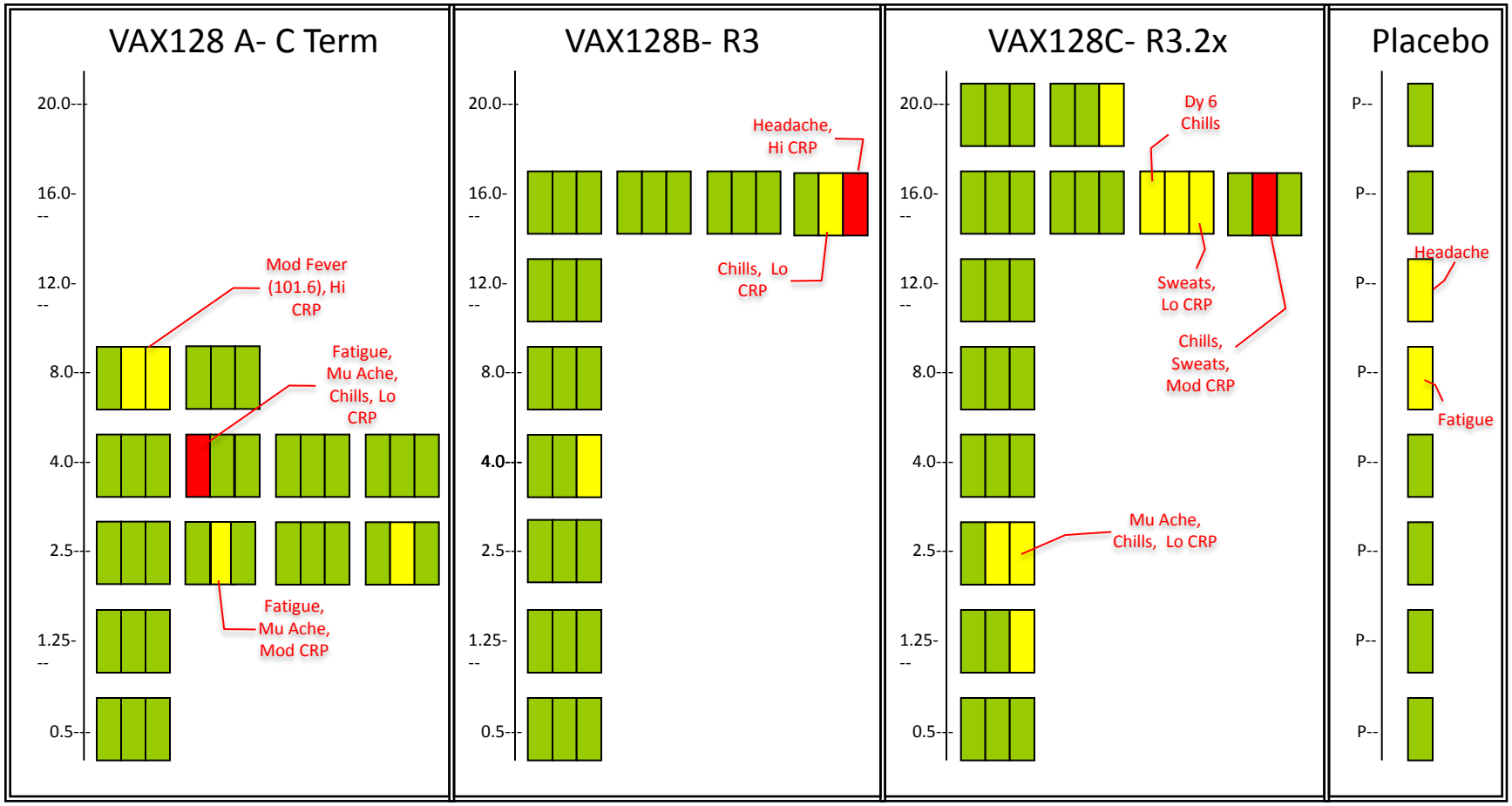
A= C term
B= R3
C= R3.2x

Evaluation of the R3 and R3.2x formats escalated to higher dose levels

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VAX128-01

Through Day 7 Systemic Safety in Young Adults, n=110



= *Participants With No or Mild Adverse Reaction*

= *Participants With Moderate Symptoms*

= *Participants With Severe Symptoms*

The R3 and R3.2x formats were generally well tolerated at higher dose levels than C term

VAXINNATE

VAX128-01

Immunogenicity in Healthy and Elderly Adults

VAX128 construct	Age Group	No. of Subjects (PP)	HAI GMT (95% CI)		MFR	Percent (95% CI)	
			Day 0	Day 28	Day 28	SC	SP
C term	Y	35	15 (9,25)	229 (117, 446)	17	83 (67, 94)	86 (71, 95)
R3	Y	29	13 (7, 22)	246 (110, 548)	19	83 (64, 94)	83 (64, 94)
R3.2x	Y	36	10 (7,15)	320 (177, 578)	32	89 (74, 97)	92 (78, 98)
Placebo	Y	10	9 (5, 18)	10 (5, 21)	1	0 (0, 31)	20 (3, 56)
C term	E	29	13 (9, 20)	57 (35, 95)	4	52 (32,71)	72 (53, 87)
R3	E	29	12 (8, 18)	99 (40, 244)	8	59 (39,77)	69 (49, 85)
R3.2x	E	30	15 (9, 26)	130 (57, 299)	9	53 (34,72)	70 (51, 85)
Placebo	E	10	20 (11, 35)	21 (11, 41)	1	0 (0, 31)	30 (7, 65)

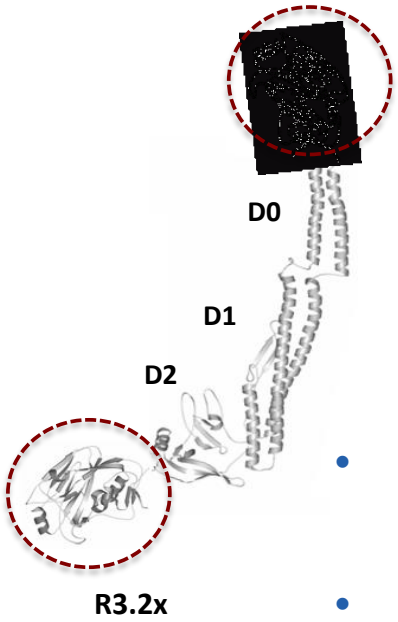
*Note that the data was pooled for the different dose levels

All VAX128 Formats Elicited Good Titers, Sero-Conversion and Sero-Protection Rates in Healthy and Elderly Adults

VAXINNATE

VAX128-01

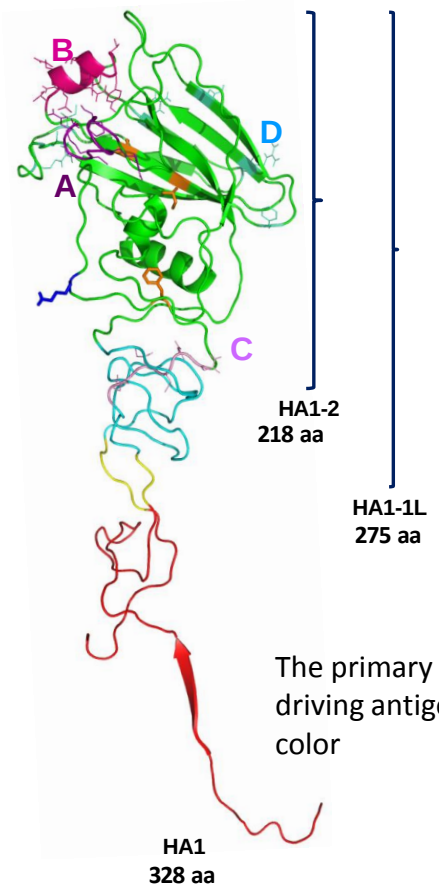
Comparative Safety and Immunogenicity of VAX128A, B and C: Conclusions



- All three formats elicit robust immune responses in healthy and elderly adults
- VAX128B and C have apparent improved safety windows relative to VAX128A
- VAX128C was well tolerated to the highest dose level and was therefore selected for further development

Influenza Vaccine Platform

Building a Quadrivalent Product: The R3L Format



- **R3 or R3.2x formats of H3 HAs aggregate during production**
- **Aggregation is alleviated and yields improve more than 10 fold with incorporation of:**
  - A longer globular head with additional secondary structure
  - Key amino acid substitutions in the head domain (outside of antigenic region) and in the linking region

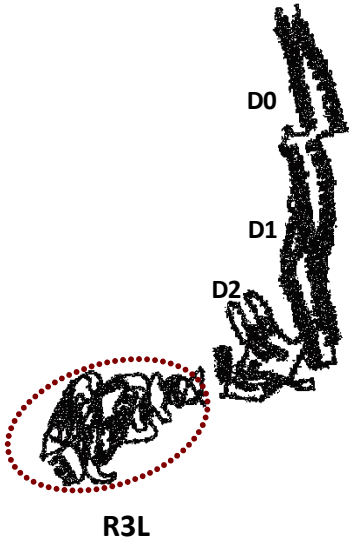
The primary antigenic regions in the HA head are depicted by **A**, **B**, **C**, D. Residues driving antigenic variation in each region are highlighted by the corresponding color

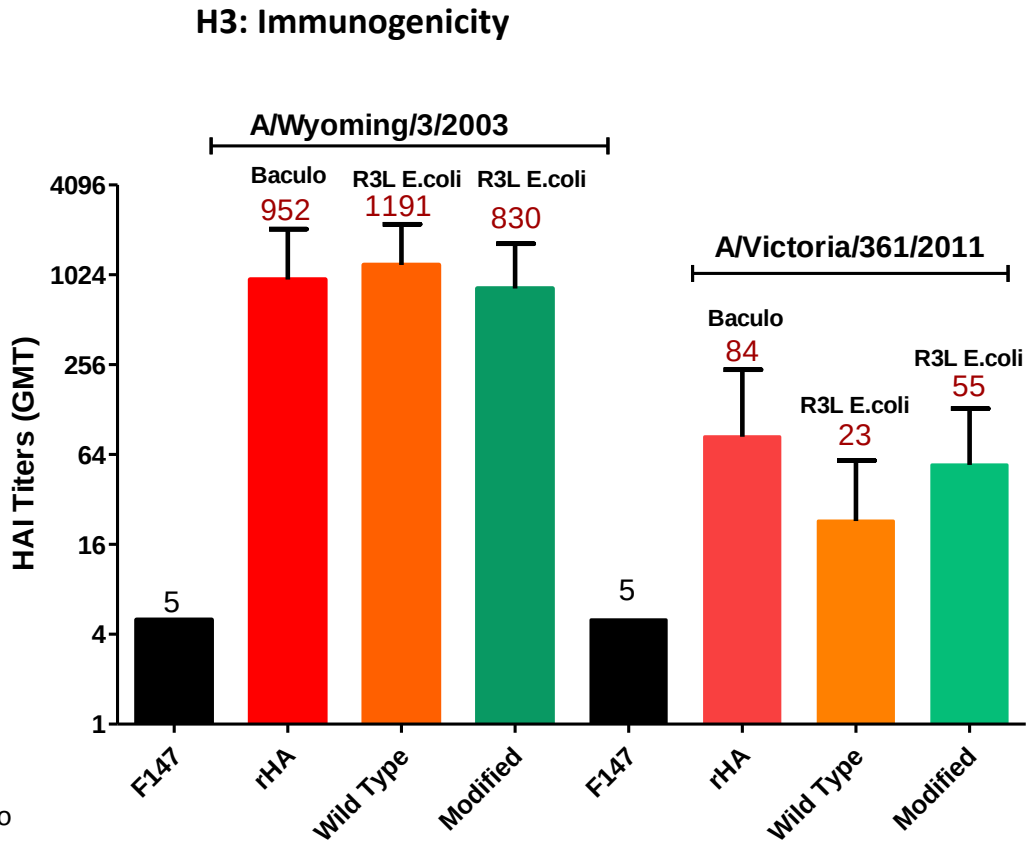
Additional HA sequence and key amino acid substitutions, outside of the antigenic determining regions, assist refolding and prevent apparent intra-molecular interactions between the flagellin and HA moieties

VAXINNATE

Influenza Vaccine Platform

Building a Quadrivalent Product





Study carried out in mice. Two doses of 5 μg delivered at a 3 week interval

Ferret anti-A/Wyoming: 5120

Ferret anti-A/Victoria: 2560

The modified R3L format of H3 improves purification yields without negatively impacting antigenicity and immunogenicity (n=4 H3 HAs).

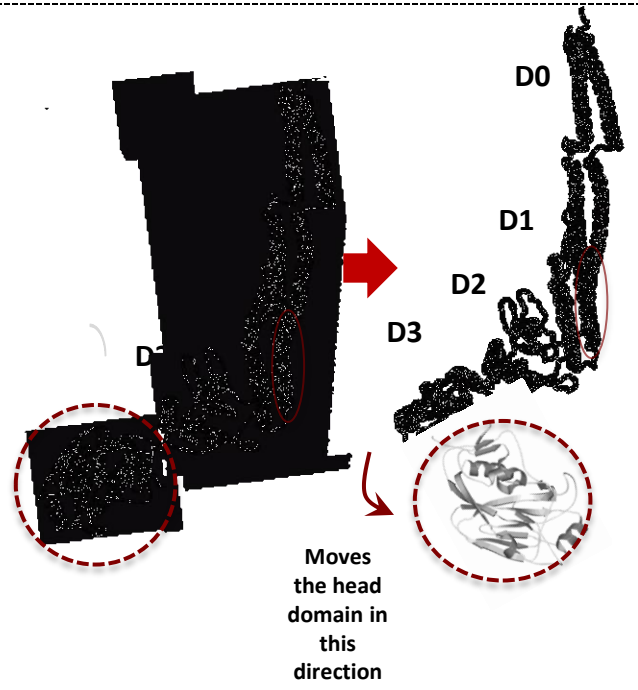
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Influenza Vaccine Platform

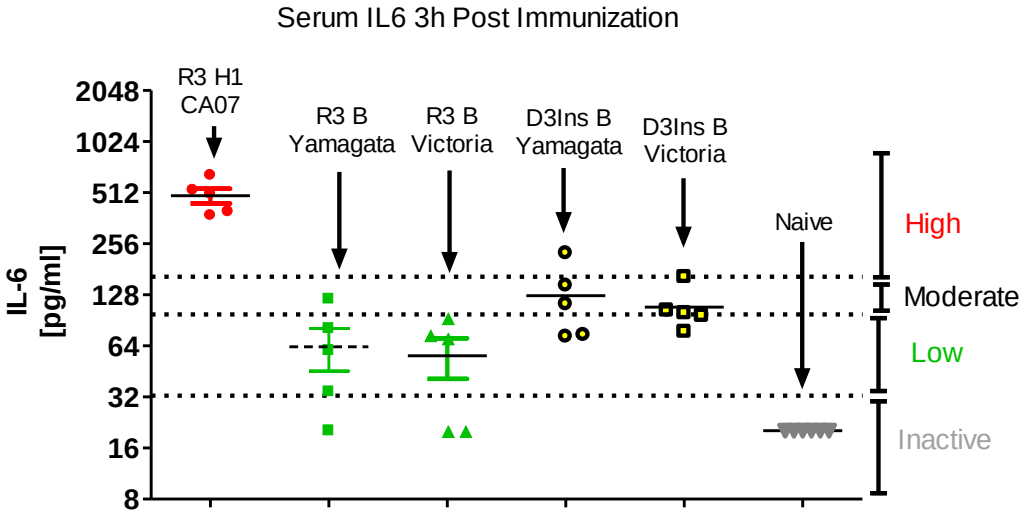
Building a Quadrivalent Product: The D3Ins Format

! See Appendix E for Description of In Vivo Model

FLU B Vaccines Utilize the D3Ins Format



- **R3 formats of B vaccines are poor triggers of TLR5**



Mice were immunized with 1 µg

- **Use of a D3Ins Format Improves TLR5 signaling**

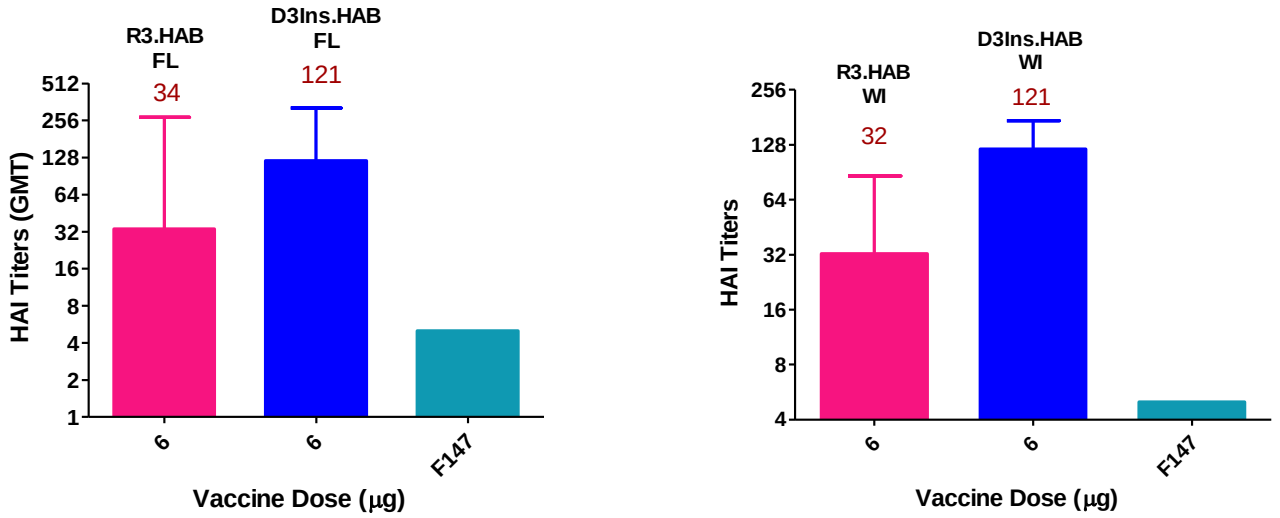
Inserting the globular head domain in Domain 3, extends the head away from flagellin, improves TLR5 signaling and enhances immunogenicity

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Influenza Vaccine Platform

Building a Quadrivalent Product: The D3Ins Format

FLU B: Immunogenicity



Study carried out in mice. Two doses of 6 µg delivered at a 3 week interval

The D3Ins format is superior to the R3 format in preclinical immunogenicity studies of B vaccines

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Influenza Vaccine Platform

Basis of Format Selection

HA Subtype	Phylogenetic group	R3.2x	R3L	D3Ins
H1	1	☑		
H5	1	☑		
H2	1	☑		
H9	1	☑		
H3	2		☑	
H7	2		☑	
FLU B	B			☑

Choice of format trends with HA group and actually is related to the pI of the HA head. HAs with higher pIs use the R3L format, HAs with the highest pIs use the D3Ins

Seasonal Influenza

VAX2012Q: A Quadrivalent Seasonal Vaccine

Blend	Strains	Monovalent Products	Format
VAX2012(Q)	A/California/07/2009 (H1N1)	VAX128	R3.2x
	A/Perth/16/2009 (H3N2)	VAX181	R3L
	B/Wisconsin/01/2010 (B Yam)	VAX173	D3 Ins
	B/Bangladesh/5945/2009 (B Vic)	VAX172	D3 Ins

Current and recent strains are the active components of the VAX2012 quadrivalent seasonal product

Seasonal Influenza Program

Analytical Assays: Drug Product Potency

- VaxInnate has developed a potency assay strategy in consultation with CBER
- The strategy employs biophysical and biological methods to determine the concentration, potency, and proper conformation of the vaccine components
- A highly sensitive, accurate and precise reversed phase ultra performance liquid chromatography (RP-UPLC) method has been developed to determine the concentration of the components
  - The precision of the method can support a $\pm$ 20% specification for formulation, product fill and release of product in the low (2-3 mcg) range
- A Capture ELISA which detects HA and the integrity of the flagellin fusion has been developed
  - Reference serum has significant HAI titers to matched virus
  - The method is stability indicating and precise
- Properly conformed HA epitopes are measured with a neutralization inhibition assay (NIA)
  - The method uses the same neutralizing serum used in the ELISA, is highly sensitive to proper folding of neutralizing epitopes and can serve as a surrogate for *in vivo* potency
- *In vivo* potency has been used to demonstrate the vaccine's ability to generate HAI titers and link/validate the results to those obtained from *in vitro* potency assays
- With the benefit of clinical data, we will begin to bridge to use of the RP-UPLC, ELISA and NIA as our potency assay suite post-Phase 2

Seasonal Program

Potency Assays Overview

Potency Assay Strategy

- The strategy was developed following discussions with CBER. It is based on a suite of assays that measure:
  - HA content determined by physico-chemical release assays
    - RP-HPLC (mass)
    - Capture ELISA (antigenicity)
  - Functional antigenicity (proper folding of HA head) verified by characterization assays
    - NIA
    - *In vivo* Potency

Seasonal Influenza Program

Analytics: VAX2012Q Phase 1 Specs

BDS Release Tests and Specifications

Release Assay	Specification
Physical Appearance	A clear solution free of particulates by visual inspection (CS)
pH	6.8 - 7.2
Purity by RP-HPLC (%)	Purity ≥ 90
	RT ± 2% of RS
Aggregates by SEC-HPLC (%)	Aggregates ≤ 2
	RT ± 2% of RS
Identity and antigenicity by ELISA	C- Value: 14.0 to 23.3
	C Value Ratio (RS/TA)
TLR5 bioactivity	TLR5 activity ratio of 0.6 to 1.4
Concentration by UV_{280} (g/L)	Report
Endotoxin (EU/mg)	< 50
Residual DNA (ng/mg)	≤ 40
Residual RNA (ng/mg)	≤ 80
Residual host cell protein (ng/mg)	≤ 100
Bioburden	Total microbial aerobic count ≤10 CFU/mL
	Total combined yeasts/ moulds ≤10 CFU/mL

Seasonal Influenza Program

Analytics: VAX2012Q Phase 1 Specs

DP Release Tests and Specifications

Assay	Specification
pH	6.8 – 7.2
Physical Appearance	A clear solution free of particulates by visual inspection (CS)
Osmolality	500 to 600 mOs/Kg
Purity & Concentration by RP-HPLC	Purity ≥ 90% Retention time ± 2% of Reference Concentration at Release - 48 mcg/mL ± 10 mcg/mL Concentration on Stability ± 15% of Release
Identity and Antigenicity by ELISA	C-Value – 14.0 to 23.2 RS/TA Ratio – Report
TLR5 Bioactivity	TLR5 Activity Ratio of 0.6 to 1.4 (test article/reference standard)
Sterility	No Growth
Bacteriostasis/Fungistasis	Negative
Endotoxin	< 40 EU/mL
General Safety Test	Pass

Seasonal Influenza Program

Analytics: VAX2012Q Phase 1 Characterization Assays

BDS Characterization Tests

Assay	Specification
Molecular Mass By Electrospray Ionization MS	Report
Identity by SDS-PAGE	Report
Rabbit immuno- potency – HAI	Report
NIA	Report C Value and RS/TA ratio
Residual IPTG	Report
Res. Triton X-100	Report
Residual Urea	Report

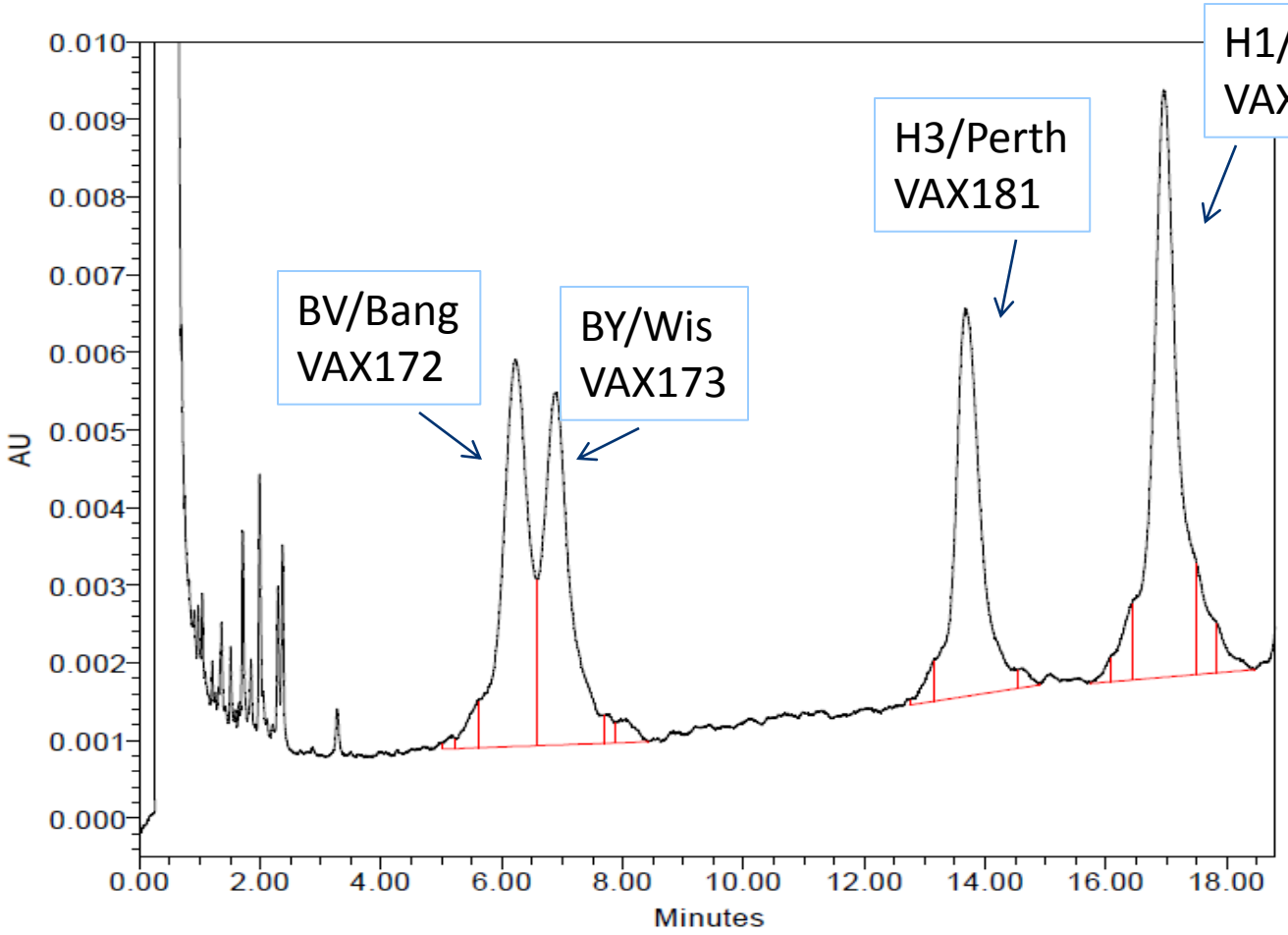
DP Characterization Tests

Assay	Specification
SDS-PAGE silver stain	Report
In vivo potency: HAI	Report
Neutralization Inhibition Assay (NIA)	Report
Polysorbate 80 Concentration	Pending

Seasonal Influenza

Potency Assay Development: Detection of Quadrivalent Product by UPLC

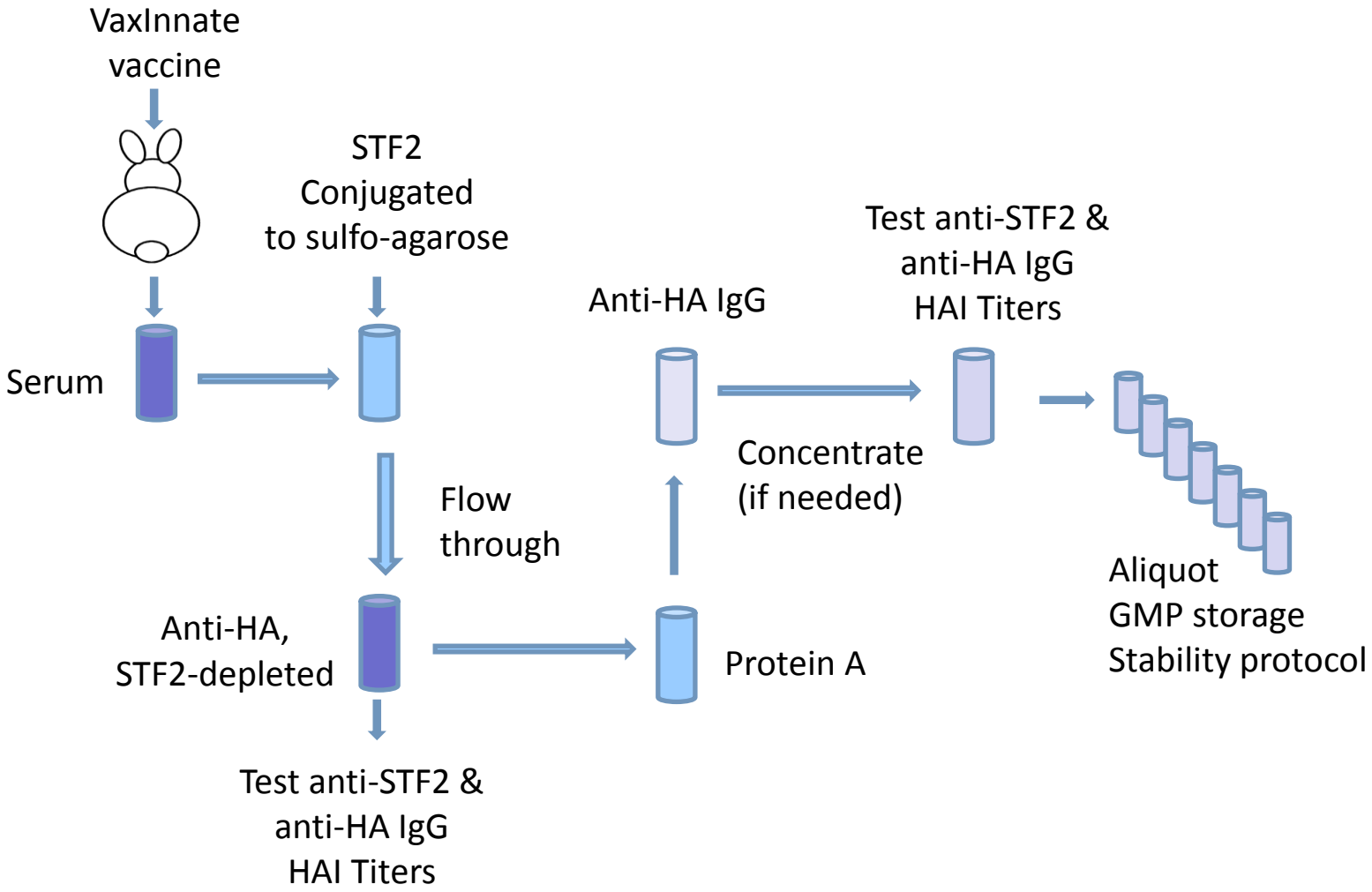
VAX2012Q vaccine components at 5 µg/mL each in a quadrivalent mixture. The vaccine components were VAX172 (BV/Bangladesh), VAX173 (BY/Wisconsin), VAX181 (H3/Perth) and VAX128C (H1/CA07). Separation occurred on a Waters Acquity UPLC H-Class Bio System with a Waters BEH Phenyl column.



The RP-UPLC is capable of quantitating each component of a quadrivalent influenza vaccine

Potency Assay Development

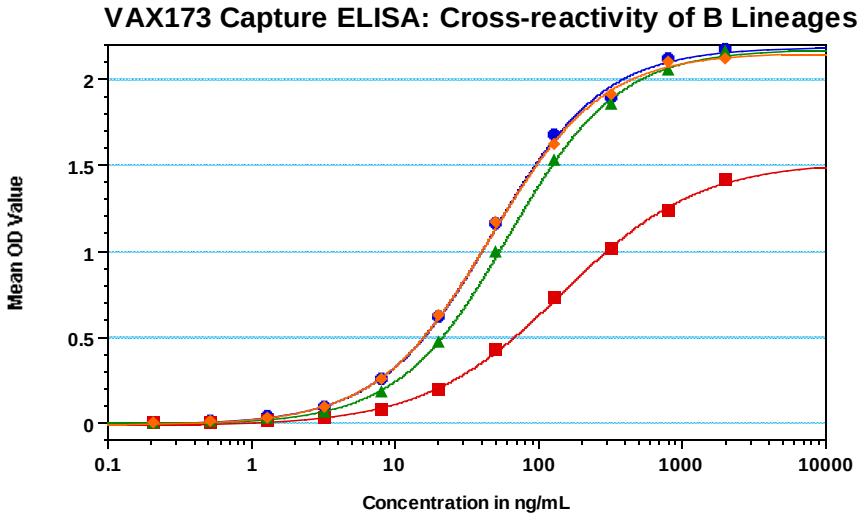
Reference Serum Generation



Rabbit serum is depleted of anti-flagellin activity, purified over Protein A column and concentrated., if needed After testing for anti-STF2, anti-HA and HAI titers, serum will be used to develop, qualify and perform Capture ELISA and NIA assays

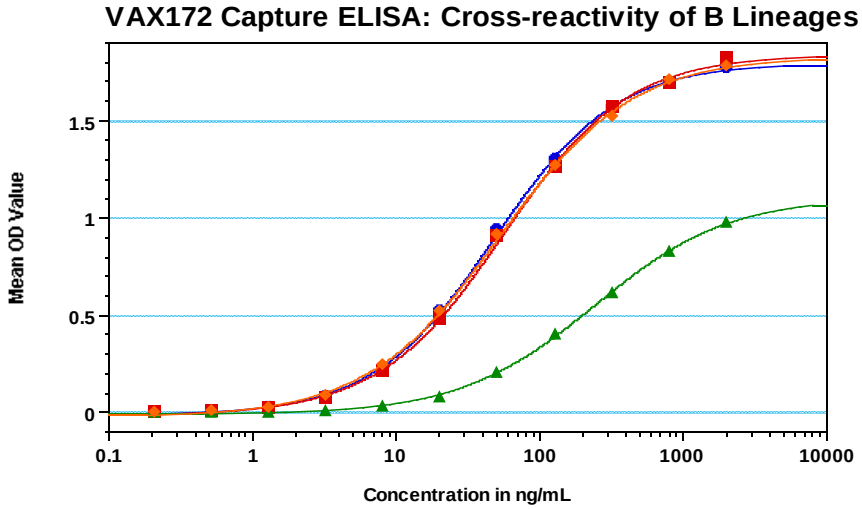
Seasonal Influenza

Potency Assay Development: ELISA Specificity



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
VAX2012Q (1:1:1:1) (VAX2012 Quadrivalent (...	-0.00994	1.12	46.5	2.19	0.999
VAX172 (172712005-1: Concentration vs Me...	-0.0173	0.912	143	1.52	0.999
VAX173 (173712004-1: Concentraiton vs Me...	-0.00945	1.13	60.9	2.18	1
VAX172:VAX173 (1:1) (VAX172:VAX173 (1:1): ...	-0.0129	1.11	45.3	2.16	1

Weighting: Fixed



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
VAX2012 (1:1:1:1) (VAX2012 Quadrivalent (1:...	-0.0129	1.03	47.7	1.8	1
VAX172 (172712005-1: Concentration vs Me...	-0.0149	0.999	55.2	1.84	0.999
VAX173 (173712004-1: Concentraiton vs Me...	-0.00886	0.927	239	1.11	0.999
VAX172:VAX173 (1:1) (VAX172:VAX173 (1:1): ...	-0.0226	0.942	52.1	1.83	1

Weighting: Fixed

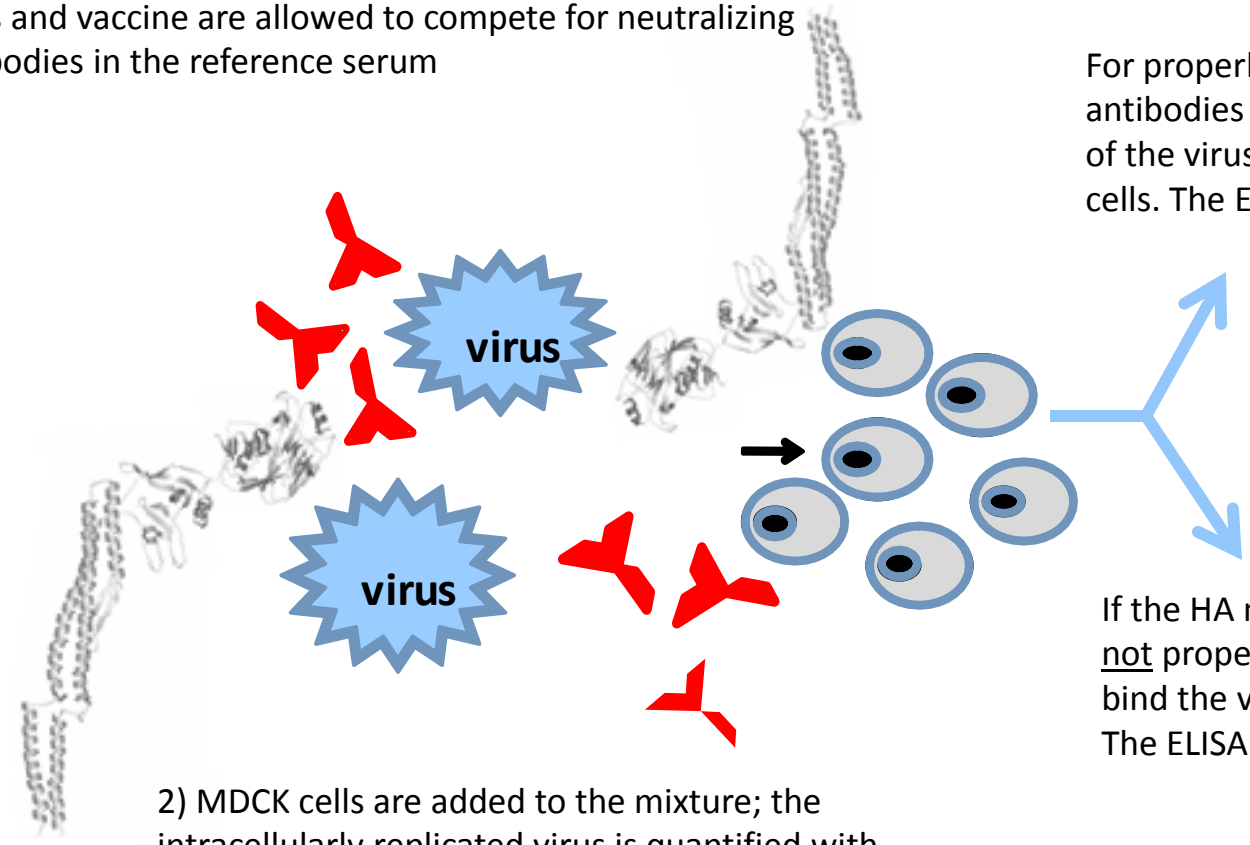
VAX173 (B Yamagata) serum shows some cross-reactivity to VAX172 (B Victoria), the effect is smaller in the reverse direction. The cross reactivity is similar to what can occur in the SRID of traditional QIV. VaxInnate is developing a method to use a mixed reference standard in release testing in order to verify the antigenicity of the two B strains

VAXINNATE

Seasonal Influenza

Potency Assay Development: NIA Schematic

1) Virus and vaccine are allowed to compete for neutralizing antibodies in the reference serum



For properly folded vaccine, the antibodies bind the HA moiety. The HA of the virus is free to attach and infect cells. The ELISA readout will be **positive**

If the HA moiety of the vaccine is <u>not</u> properly folded, antibody will bind the virus and prevent infection. The ELISA readout will be **negative**

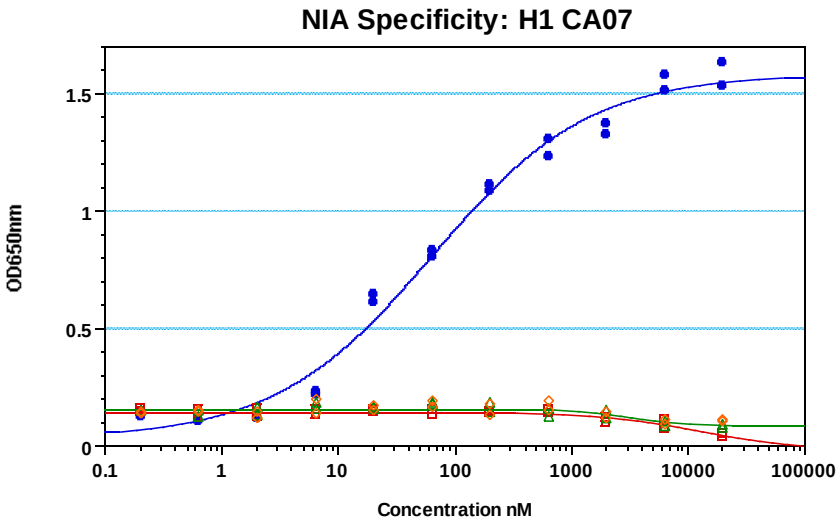
2) MDCK cells are added to the mixture; the intracellularly replicated virus is quantified with an ELISA using NP MAbs

The NIA is based on the microneutralization assay (MN). Only properly folded vaccine can inhibit neutralization in a dose-dependent fashion resulting in virus infection and a positive signal in the assay.

Seasonal Influenza

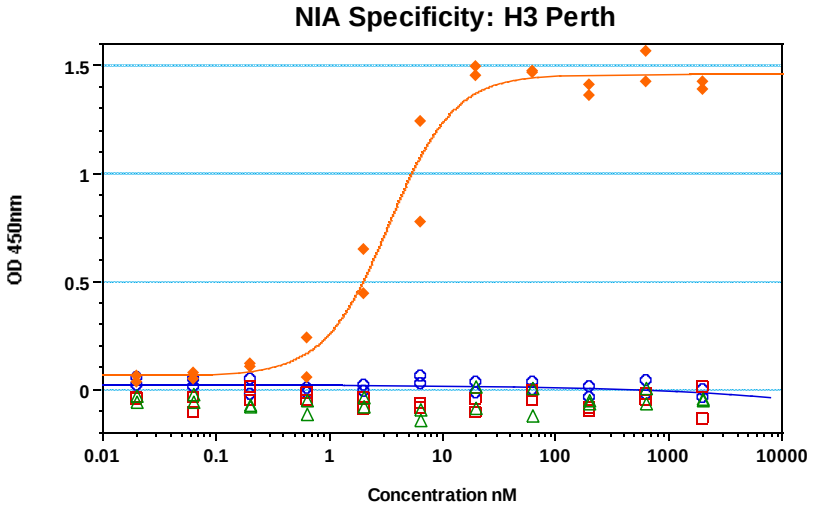
Potency Assay Development: NIA Status

HA-specific rabbit serum has been used to develop the NIA for the VAX2012Q (and other) strains



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
VAX128C (HL186: Concentration vs Values)	0.0307	0.643	62.8	1.58	0.988
VAX181 (HL490: Concentration vs Values)	0.145	0.962	1.26e+04	-0.0201	0.85
VAX173 (HL772: Concentration vs Values)	0.16	1.63	3e+03	0.0843	0.687
VAX787 (HL787: Concentration vs Values)					

Weighting: Fixed



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
VAX173 (HL772: Concentration vs Values)	0.022	0.338	3.15e+19	-1.12e+04	0.167
VAX172 (HL787: Concentration vs Values)					
VAX128C (HL186: Concentration vs Values)					
VAX181 (HL490: Concentration vs Values)	0.0617	1.51	3.33	1.46	0.975

Weighting: Fixed

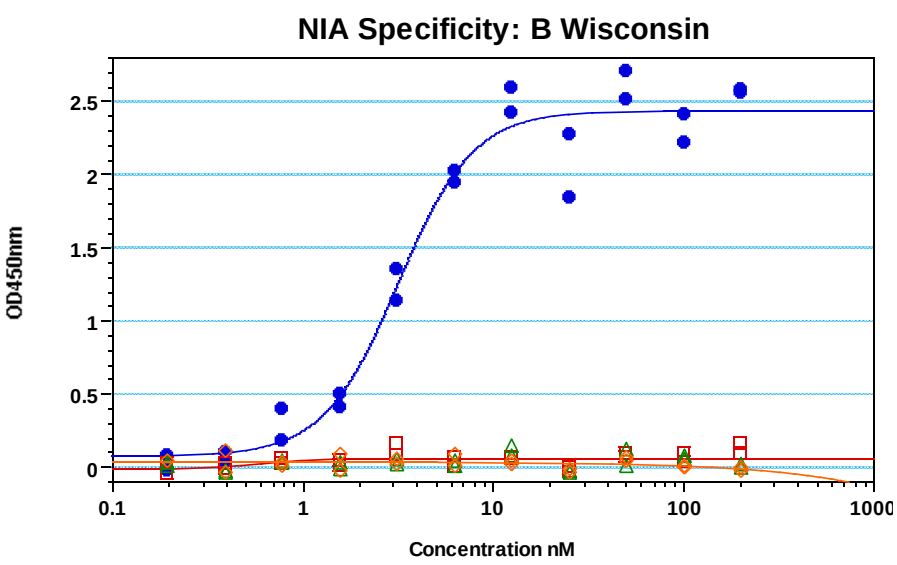
The VAX181 (H3 Perth) and VAX128C (H1 CA07) assays are highly specific

VAXINNATE

Seasonal Influenza

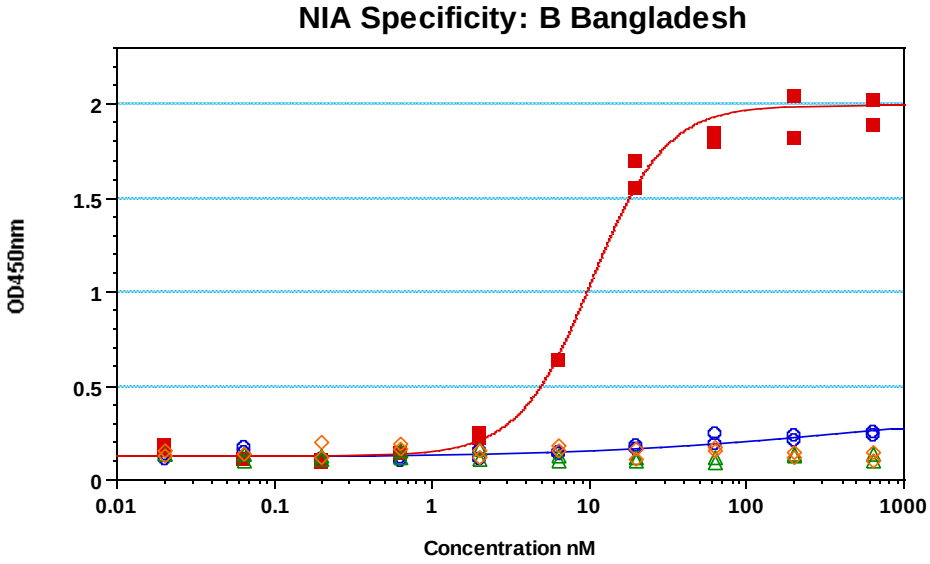
Potency Assay Development: NIA Status

HA-specific rabbit serum has been used to develop the NIA for the VAX2012Q (and other) strains



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
VAX173 (HL772: Concentration vs Values)	0.0768	2.21	3.15	2.44	0.971
VAX172 (HL787: Concentration vs Values)	-0.0139	2.53	0.609	0.0664	0.247
VAX128C (HL186: Concentration vs Values)					
VAX181 (HL490: Concentration vs Values)	0.039	0.803	1.37e+09	-1.5e+04	0.134

Weighting: Fixed



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
VAX173 (HL772: Concentration vs Values)	0.118	0.495	470	0.385	0.848
VAX172 (HL787: Concentration vs Values)	0.125	1.86	10.3	1.99	0.99
VAX128C (HL186: Concentration vs Values)					
VAX181 (HL490: Concentration vs Values)	0.146	23.8	2.35e+03	0.979	0.0432

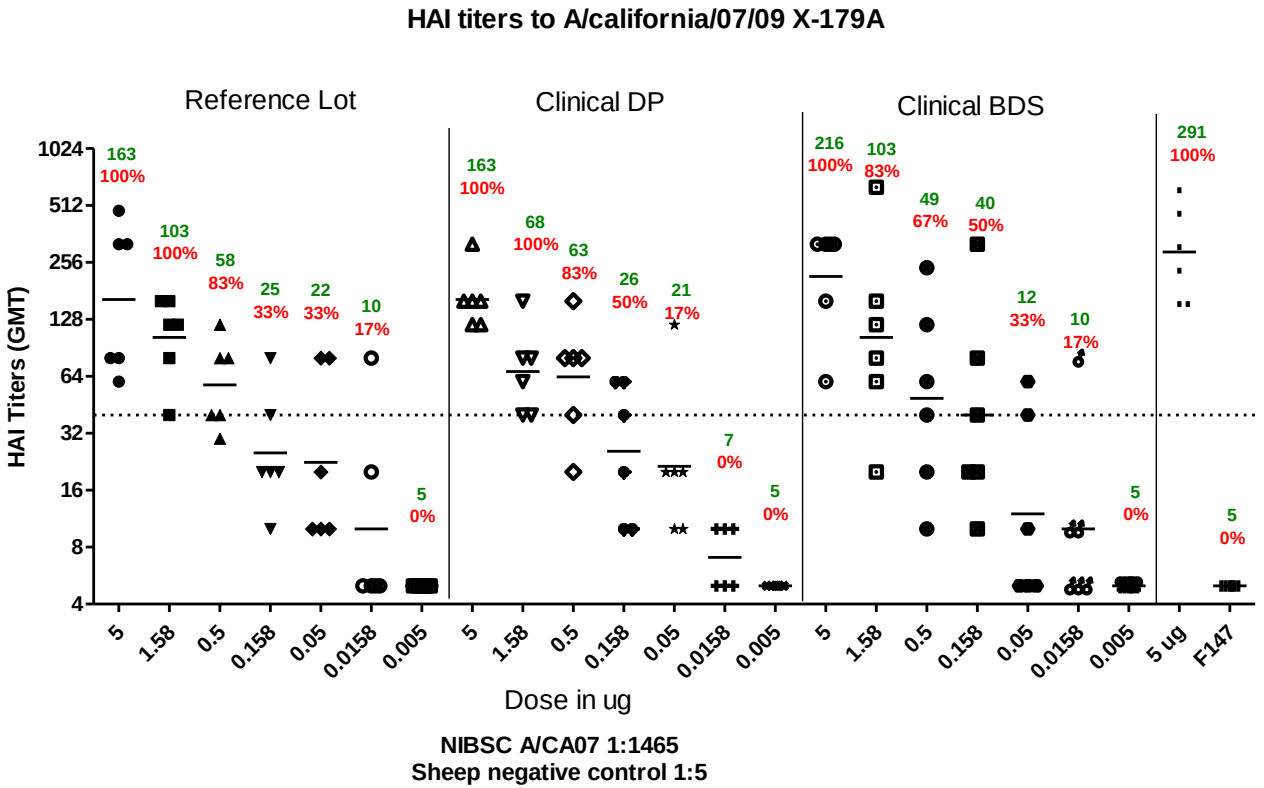
Weighting: Fixed

The NIA for VAX173 (B Yamagata) and VAX172 (B Victoria) shows lineage specificity

VAXINNATE

Potency Assay Package: In vivo Potency

In Vivo Potency: Clinical BDS > 12 months and DP > 6 months HAI Results

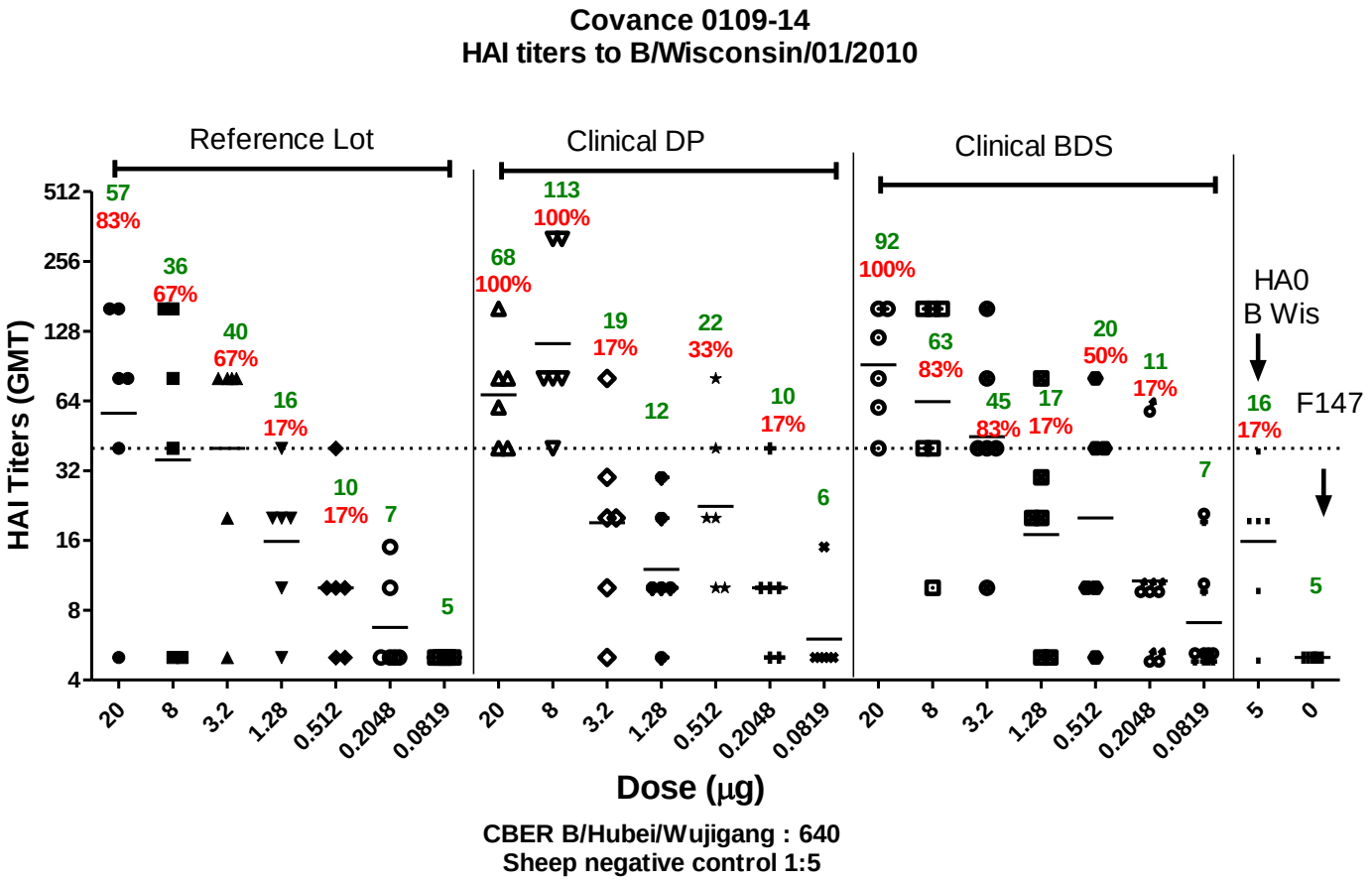


Strong dose response curves were seen for all H1 CA07 lots in HAI. Statistical analysis (ANOVA) found clinical BDS (stored at -60°C for > 12 months) and DP lots (stored at -60°C for > 6 months) comparable to reference

VAXINNATE

Potency Assay Package: In vivo Potency

In Vivo Potency: Clinical BDS > 12 months and DP > 6 months HAI Results

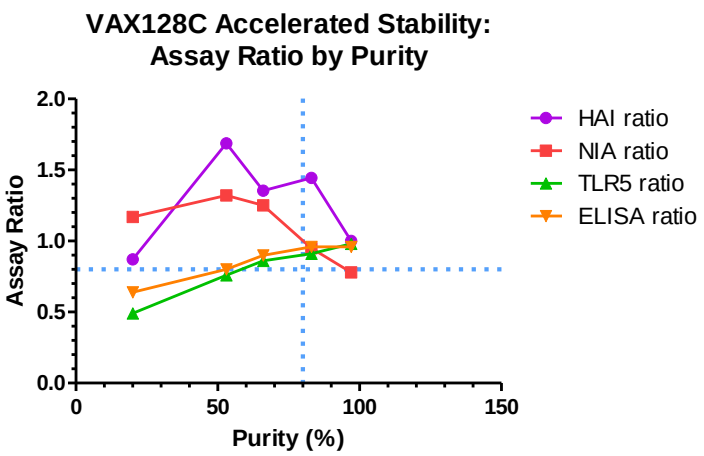


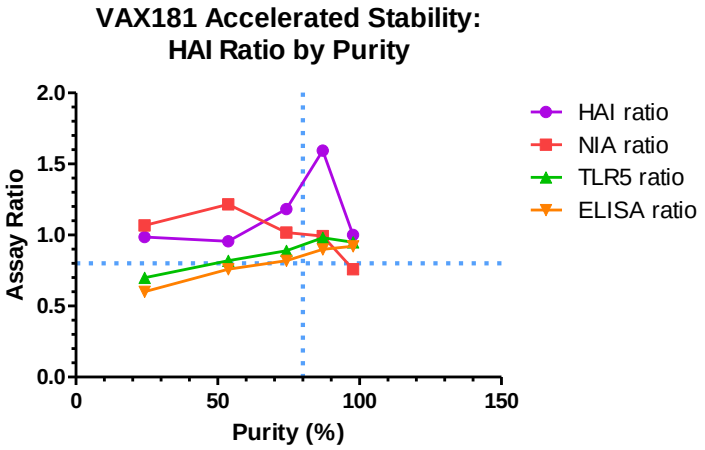
Suitable dose response curves seen for VAX173 reference standard, BDS and DP. All lots are equivalent by 2 way ANOVA

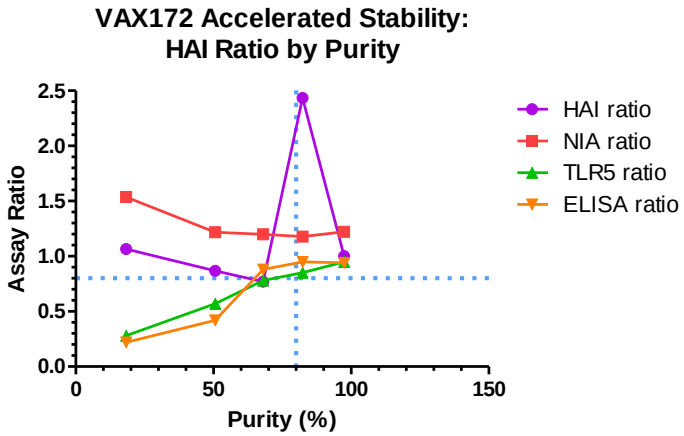
VAXINNATE

Potency Assay Package: Accelerated Stability Samples

In Vivo Potency (HAI) vs. NIA, Capture ELISA and TLR5







Comparisons of in vitro and in vivo potency assays on accelerated stability studies shows that Capture ELISA and TLR5 trend best with decreasing purity, while in vivo potency (HAI) and NIA are less sensitive assays

Seasonal Program

Phase 2 & Beyond Drug Product Analytical Assays

VaxInnate has a streamlined suite of Phase 2 assays supporting release & stability

Purpose	Assays	Drug Product Release	Drug Product Stability	Status
Appearance/ General Tests	Physical Appearance	✓	✓	Compendial methods to be used for all phases
	pH	✓	✓	
	Osmolality	✓		
Quantity/Potency	*Concentration by RP UPLC*	✓	✓	Format suitable for Phase 2 & beyond
Potency and Identity	*Identity and Antigenicity by ELISA**	✓	✓	Format suitable for Phase 2 & beyond
	Neutralization Inhibition (NIA)*	✓	✓	Currently under development
Sterility and Safety	Endotoxin	✓		Compendial methods to be used for all phases
	General Safety Test	✓		
	Sterility	✓	✓	Compendial method suitable for Phase 2 & beyond

***ELISA and NIA use reference serum with significant HAI titers to the matched virus**

Our potency assays are based on the measure of HA content by a sensitive and precise RP UPLC method AND the measure of functional antigenicity by ELISA and NIA

Seasonal Program

Drug Product Potency Assays Overview: Assay Precision

Assay	Readout	Release Specs	Precision (%RSD)
Concentration by RP HPLC	Concentration	± 20% of target*	≤ 3.3%
Capture ELISA	Ratio to reference	0.8-1.2	≤ 6.3%
NIA	Ratio to reference	0.6-1.4	≤25%

*Specification based on formulation rather than assay precision

The determination of concentration by RP UPLC has excellent precision, as expected for a biophysical measurement. The Capture ELISA method has good precision for an assay using polyclonal sera. The NIA ratio shows reasonable precision for an assay involving cells and virus.

Seasonal Program

VAX2012Q-01: A Phase 1 Dose Escalating Study in Healthy Adults 18-40 Years Old

Study Objectives:

1) To evaluate the safety and tolerability of VAX2012Q for up to 7 dose levels
2) To evaluate the immunogenicity of VAX2012Q for up to 7 dose levels
   - 5 different dose levels and 3 different component ratios were evaluated
   - A single dose was delivered IM
   - Safety assessments took place 2 h, 24 h, and 7 days after immunization
   - Sera for HAI evaluation was obtained on days 0 and 21
   - Interim immunogenicity and safety results were used to adjust the dose and component ratio

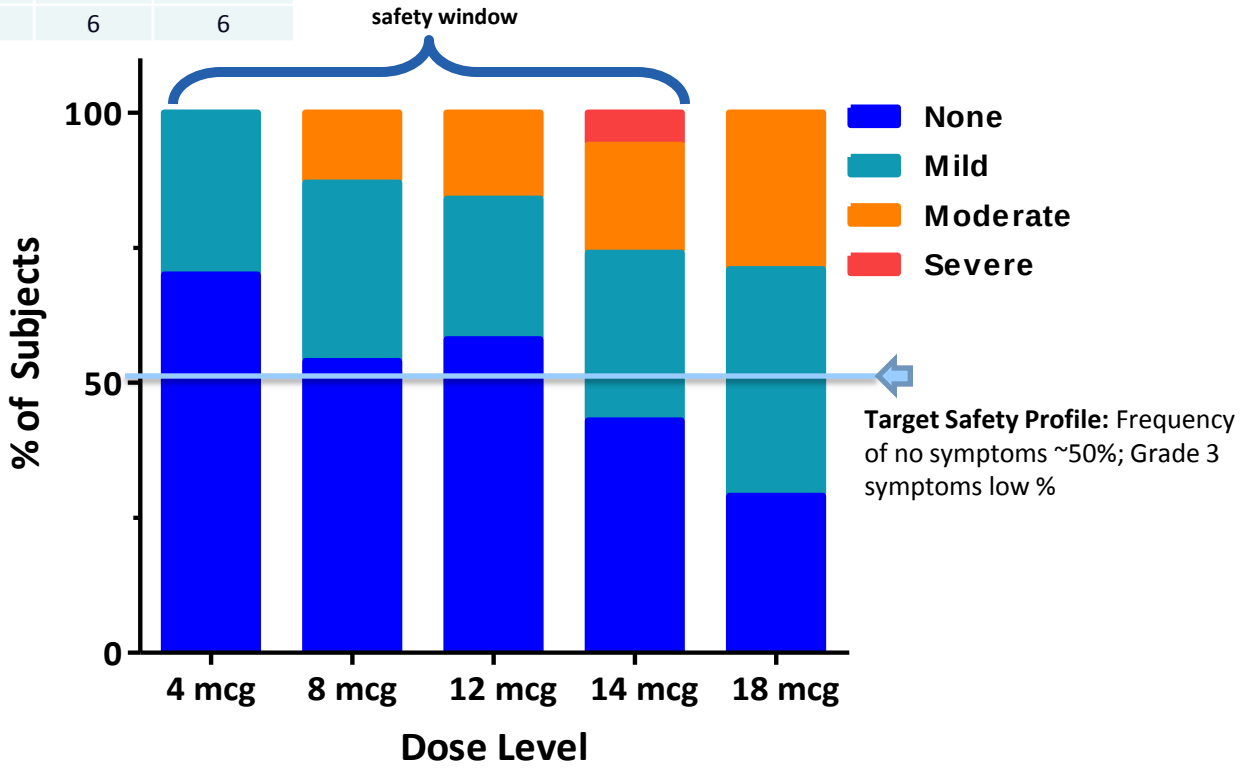
	VAX2012Q					
Dose	No. of	Dose (µg)				Total
Level	Subjects	H1N1	H3N2	B-YAM	B-VIC	Dose
1	20	1	1	1	1	4
2	48	2	2	2	2	8
3	51	2	4	4	4	14
4*	48	2	4	6	6	18
5	49	3	3	3	3	12
6	51	2	2	2	2	8
7	49	3	3	3	3	12
Total	316					

*Paused after this group for interim analysis

VAX2012Q-01

Systemic Safety by Dose Level

Total Dose	N	Dose (µg)			
		H1N1	H3N2	B-YAM	B-VIC
4	20	1	1	1	1
8	99	2	2	2	2
12	98	3	3	3	3
14	51	2	4	4	4
18	48	2	4	6	6

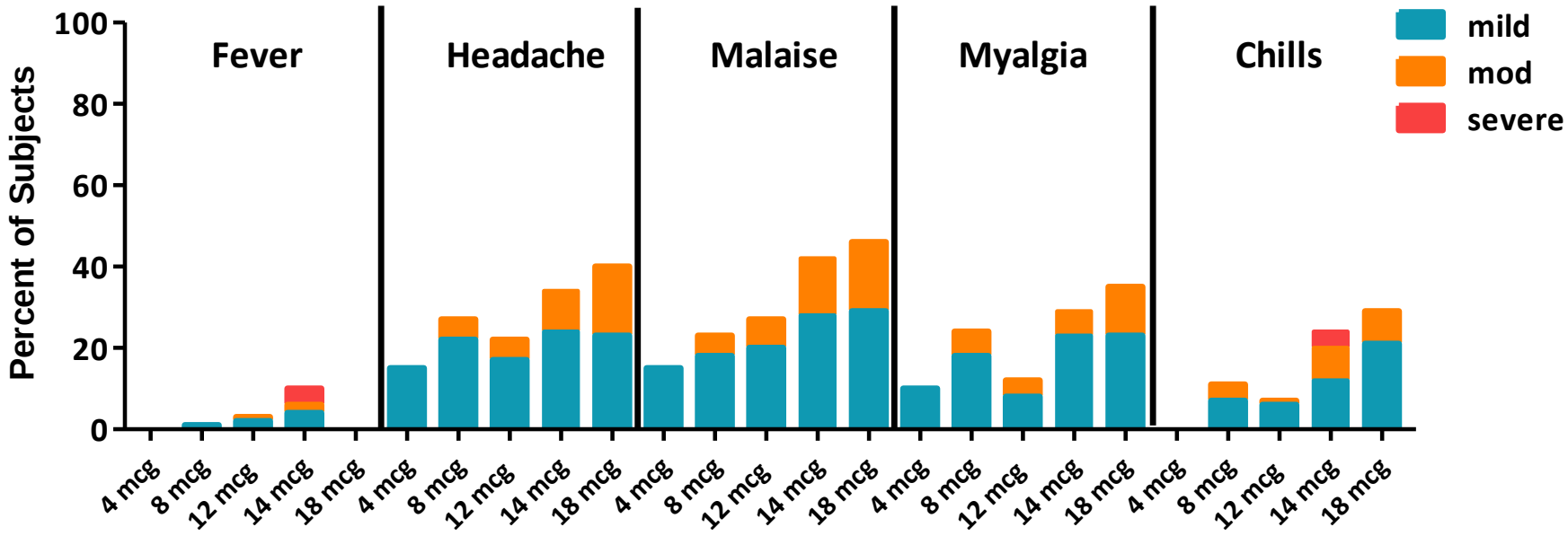


Target Safety Profile: Frequency of no symptoms ~50%; Grade 3 symptoms low %

VAX2012Q-01

VAX2012Q-01: Systemic Safety by Symptom for Dose Levels of 4, 8, 12 or 14 mcg

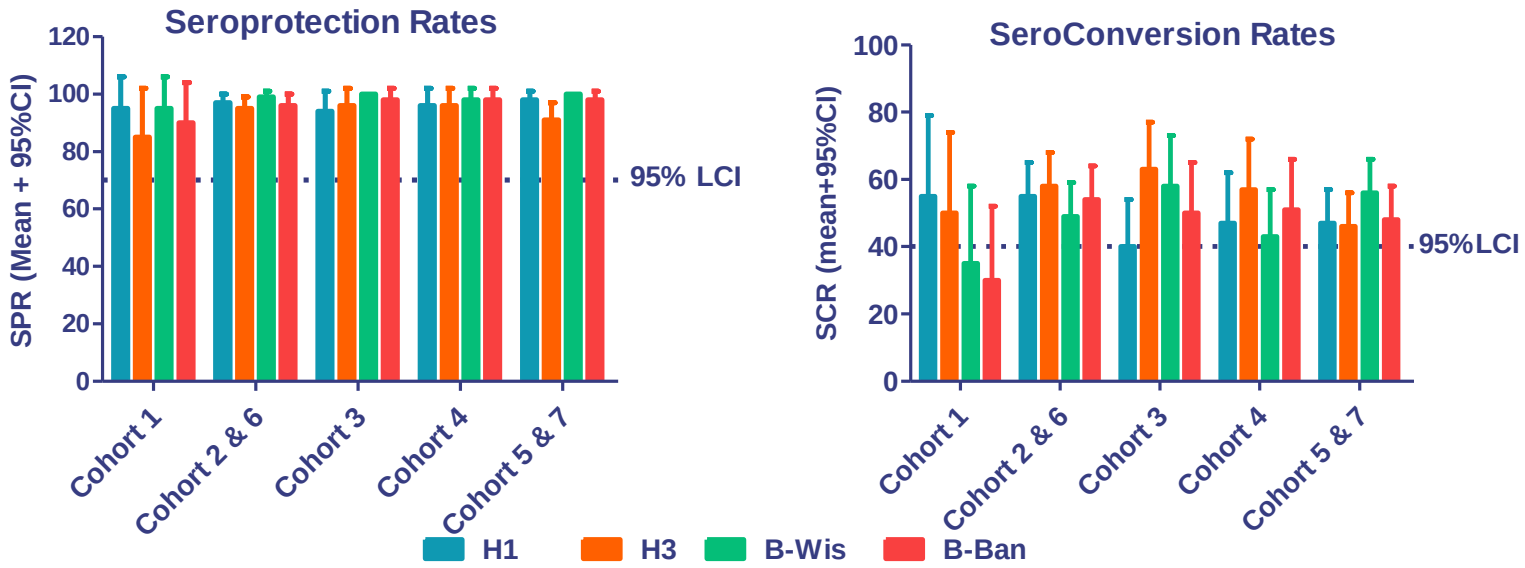
Total Dose	N	Dose (µg)			
		H1N1	H3N2	B-YAM	B-VIC
4	20	1	1	1	1
8	99	2	2	2	2
12	98	3	3	3	3
14	51	2	4	4	4
18	48	2	4	6	6



VAX2012Q-01

VAX2012Q-01: Immune Responses by Dose Group

Cohort	Total Dose	N	Dose (µg)			
			H1N1	H3N2	B-YAM	B-VIC
1	4	20	1	1	1	1
2&6	8	99	2	2	2	2
5&7	12	98	3	3	3	3
3	14	51	2	4	4	4
4	18	48	2	4	6	6

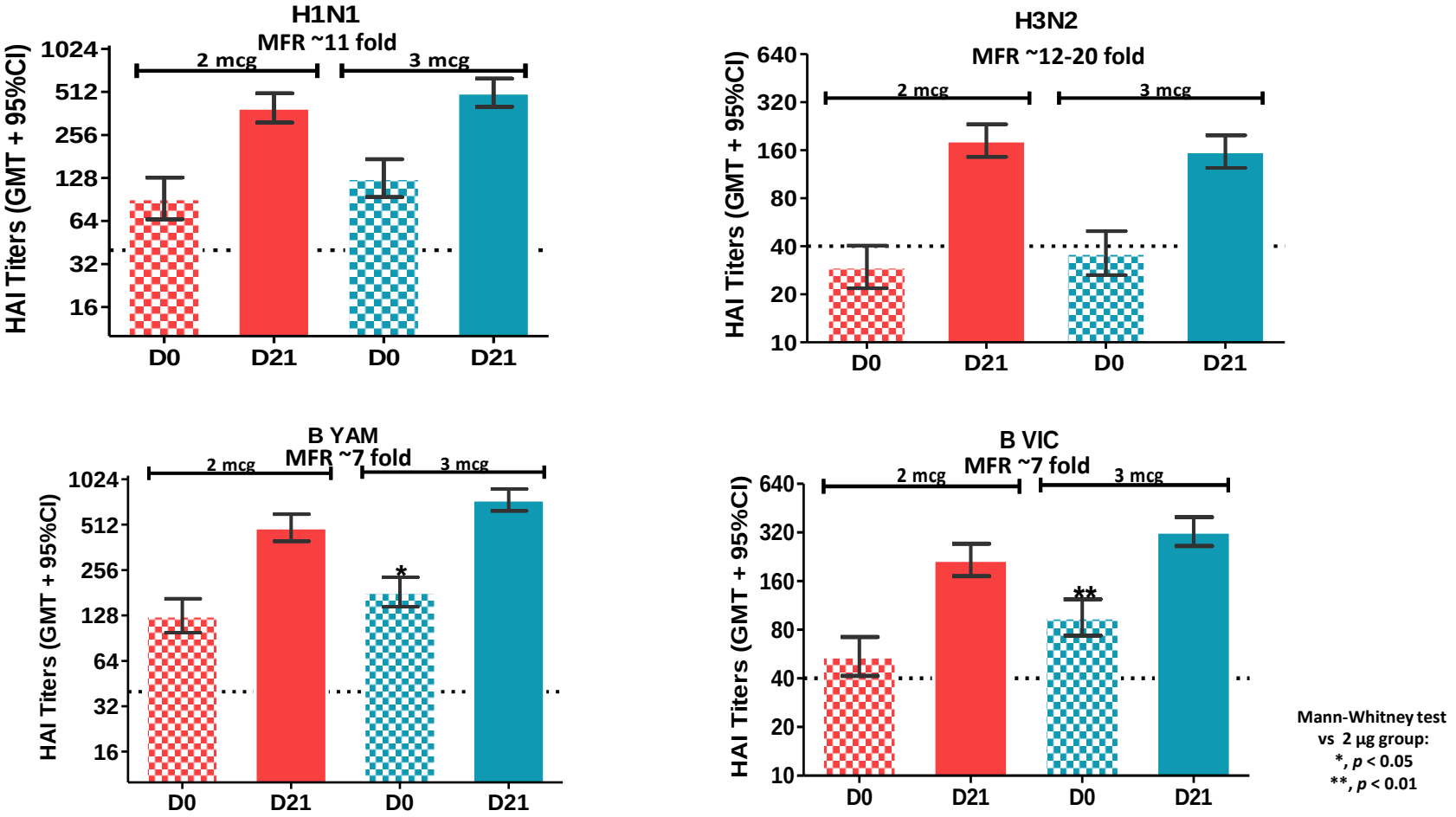


1:1 ratios of either 2 or 3 mcg per component elicit the most balanced immune response across all strains

VAX2012Q-01

HAI Titers Prior to and After Vaccination

2 mcg/component, N=98; 3 mcg/component, N=96



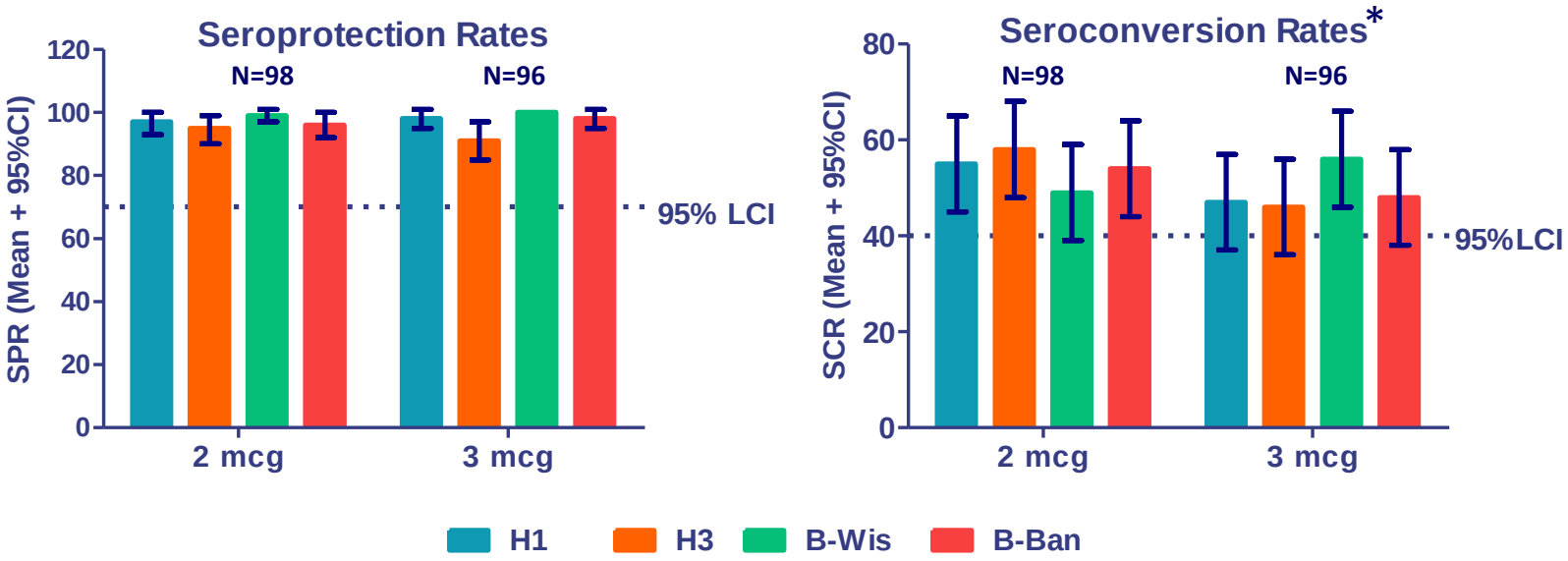
Mann-Whitney test
vs 2 µg group:
*, $p < 0.05$
**, $p < 0.01$

Robust HAI titers were measured in the 2 and 3 mcg/component dose groups. Note the high starting titers, which were significantly higher for the B strains in the 3 mcg dose group

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VAX2012Q-01

2 vs 3 mcg per component



*. **Logistic regression models using baseline titers as a covariate, indicate that seroconversion rates are comparable between the two dose levels for H1, H3 and B Bangladesh and are <u>positively</u> impacted by dose for the B Wisconsin strain**

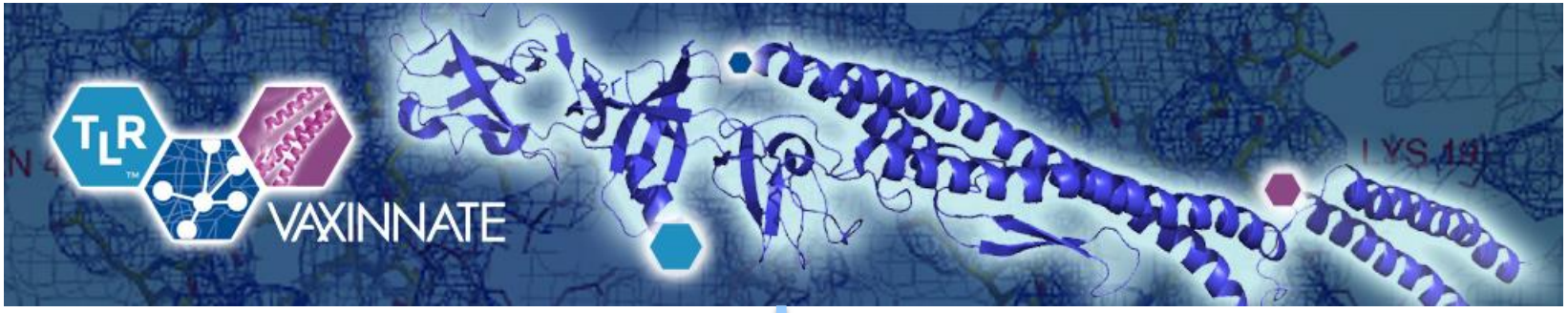
The immune responses were largely overlapping for the 2 and 3 mcg per component dose levels. A positive effect of dose was observed for one of the Bs.

VAX2012Q-01

VAX2012Q-01: Conclusions & Next Steps

- **The VAX2012Q-01 results provide the clinical proof of concept for VaxInnate's recombinant approach to a quadrivalent seasonal product**
- Doses of 2 or 3 mcg per component of VAX2012Q have comparable safety and immunogenicity profiles
  - SP and SC rates are consistent with serological criteria established by CBER for accelerated licensure
- Three mcg per component, and above, has been selected for evaluation in a Phase 1b dose ranging study in the elderly
- Two dose levels (2 and 3 mcg per component) will be evaluated in a Phase 2 dose confirmation study (VAX2012Q-03) in healthy adults 18-64 years old in 1H 2015
  - A US licensed quadrivalent vaccine will be included as a comparator (matched for 2 strains)
- The End of Phase 2 meeting is projected to take place in 1H 2016
  - Suitability of the process and analytical validation package, the overall Phase 3 study design and the size of the safety database will be determined

Our Platform Supports a Range of Production Systems and Vaccine Targets



Prokaryotic Production

Established and Emerging Targets

Vector-Borne Disease Portfolio

Eukaryotic Production

Adjuvants

Discovery

Chikungunya

Yellow Fever

WNV/JE

C.difficile

Preclinical

Dengue

Pandemic Flu

Seasonal Flu

Phase 1

Acknowledgements

- Lynda Tussey, Ph.D—CSO
- Bruce Weaver—VP Manufacturing
  - Yan Chen—Director Process Development
    - Youngsun Kim
- Devin Wigington—Director QC
  - Kelly Russell
  - Vanessa Diaz
- Ge Liu—Senior Director Research
  - Haijun Tian
  - Fu Hou
- Immunology Group
  - Rodney Bell
  - John Gathuru, Ph.D.
  - Kalyani Ginjupali

Funded by BARDA Contract HHSO100201100011C