VaxArray Assessment of Influenza Vaccine Potency and Stability

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Abstract

Background: **There is an on-going effort within the influenza vaccine industry to identify and validate an alternative to the single-radial immunodiffusion assay (SRID) for potency and stability assessment. The VaxArray Seasonal Influenza (VSI) potency assay was developed under the Influenza Vaccine Manufacturing Initiative and performance has been evaluated against key regulatory requirements such as accuracy, precision, and ability to track vaccine stability. This report focuses on a summary of results for a diverse set of flu vaccines tested in collaborative studies.**

Methods: **VSI is based on a panel of monoclonal antibodies printed in a microarray format. A simple sandwich assay is used for simultaneous quantification of all hemagglutinin components within mono- and multi-valent influenza vaccines, including differentiation between the two Influenza B lineages. A standard protocol was used to evaluate the potency of mono-valent and multi-valent influenza vaccines produced in eggs and cell-culture, including recombinant and virus-like particle vaccines. Accuracy was defined with respect to SRID and (or) purity-adjusted total protein content. Potency was also evaluated as a function of time under accelerated stress conditions.**

Conclusions: **VSI was found to exhibit high precision (CV < 15%) and good correlation (R > 0.85) with SRID for most vaccines, and if calibrated appropriately, excellent agreement with SRID values (100 ± 5%). In thermal stability testing all strains in multi-valent vaccines exhibited a decrease in potency over time. While the absolute measured potency for degraded vaccines was not always in agreement with the SRID value, the general trends were consistent with those observed for SRID. These studies indicate that VSI is a reliable and robust alternative potency assay.**

Background

- Potency assays measure the concentration of functional hemagglutinin (HA), which is an influenza virus surface protein. HA has been established to be the key component of whole virus vaccines and the dominant target of protective antibodies following vaccination or infection.
- Currently, the gold standard for influenza vaccine potency is the single radial immunodiffusion (SRID) assay, which has inherent disadvantages including labor-intensive protocols and the requirement for reference reagents that do not necessarily accurately represent the composition of vaccines.
- VaxArray Influenza (VSI) is a rapid alternative potency assay. A comparison between SRID and VSI is demonstrated in Figure 1.

Figure 1: SRID vs VaxArray

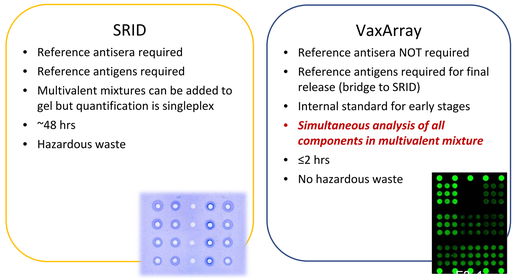
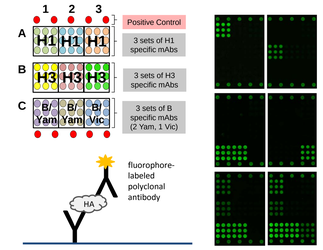


Figure 2: Method Overview – Biochemistry

VSI is a simple multiplexed sandwich immunoassay that utilizes a microarray slide printed with broadly reactive yet subtype specific antibodies for A/H1, A/H3, B/Yamagata-like, and B/Victoria-like strains (Kuck et al., 2014). A "universal" fluorophore-labeled polyclonal antibody mixture is used to quantify all components of monovalent and multivalent HA mixtures through measurement of fluorescent signal intensity.

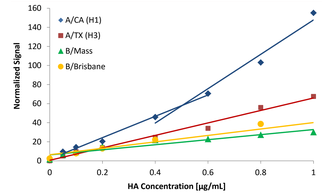


Array Position	Epitope Type	Epitope Location	Neutralizing	Detection Target
A1	Conformational	HA1	YES	A/CA/2009 (pdm-like H1)
A2	Conformational	HA2	YES	Broad H1
A3	Linear	HA1		Broad H1
B1	Conformational	HA1	YES	Broad H3
B2	Conformational	HA2		Broad H3
B3	Linear	HA1		Broad H3
C1	Conformational	HA1	YES	Broad B/Yam
C2	Linear	HA1		Broad B/Yam
C3	Conformational	HA1	YES	Broad B/Vic

Figure 3: Method Overview – Analysis

The automated image analysis associated with the VaxArray platform determines the linear ranges of the standard curve (either internal or CBER/NIBSC reference antigen) for each component. Samples of unknown potency are evaluated and concentration is calculated using the appropriate standard curve.

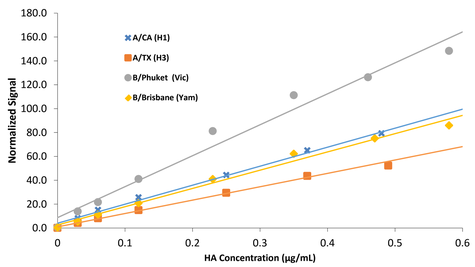
1. Calibration with reference antigens (internal or CBER/NIBSC)
2. Automated image analysis and determination of linear ranges
3. Analysis of unknown, automated calculation of concentration using standard curve metrics



Results

Figure 4: Linear Range Determination

- Due to the multiplexed nature of VSI, quadrivalent samples can be analyzed.
- The analysis software determines linear response ranges for each Influenza type and subtype.
- Quantification is determined via linear regression analysis as described previously (Kuck et al, 2014).
- From calibration curves, HA concentrations can be determined for samples of monovalent or multivalent nature without requiring multiple tests (as with SRID).



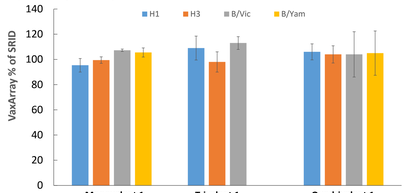
Limit of Detection

A significant advantage of the VaxArray platform is the low Limit of Detection (LOD). Whereas the SRID LOD of HA is 6 µg/mL, VSI can detect and quantify samples of less than 0.02 µg/mL concentration of HA precisely and accurately.

Figure 5: Precision and Accuracy as Compared to SRID

- The data below is a summary of potency measured by VSI and SRID (expressed as %VSI:SRID) for monovalent, trivalent, and quadrivalent split virion vaccines from the 2014-2015 season.
- When calibrated appropriately, VSI measured potency was within 20% of SRID values for each antigen component.
- Potency measurements are repeatable with an average relative error of 10% for VSI replicate measurements of the same sample.
- Thus, VaxArray is both precise and accurate.

A



B

SAMPLE	H1	H3	B/Vic	B/Yam
Monovalent 1	95 ± 5	99 ± 3	107 ± 1	106 ± 4
Monovalent 2	65 ± 3	55 ± 5	108 ± 7	75 ± 1
TIV 1	109 ± 9	98 ± 8	113 ± 5	--
TIV 2	97 ± 4	100 ± 6	107 ± 4	--
QIV 1	106 ± 6	104 ± 7	104 ± 18	105 ± 18
QIV 2	103 ± 3	81 ± 1	111 ± 9	105 ± 14
AVERAGE:	96 ± 2	90 ± 2	108 ± 4	98 ± 6

Figure 6: Stability Indication

- Potency of samples was measured before and after a 20 hour incubation at 56 ˚C, and the ratio of %(T20/T0) was used to evaluate stability.
- In general, VSI exhibited similar qualitative trends to SRID for loss of potency under these conditions for multivalent vaccines.
- The agreement was somewhat poorer for monovalent bulk drug substance relative to both HPLC and SRID.
- From a qualitative standpoint, VSI is stability indicating – as expected for an immunoassay.

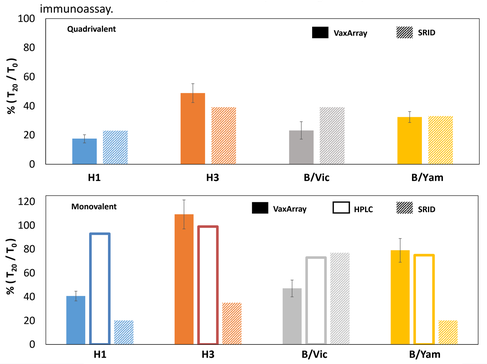
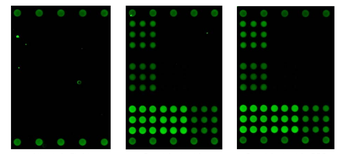


Figure 7: In-Process Analysis

- Quadrivalent vaccine samples were spiked into allantoic fluid and PBS (control) and analyzed with VSI.
- Allantoic fluid is not cross-reactive with the assay and does not inhibit HA binding (**A**).
- Potency was determined by VSI (**B**) and in both allantoic fluid and PBS, potency values were similar to the predetermined potency from SRID (dotted line).
- VSI can be used throughout the vaccine production process to monitor HA production and potency.

A



B

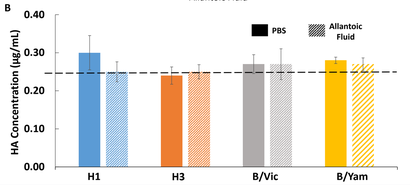
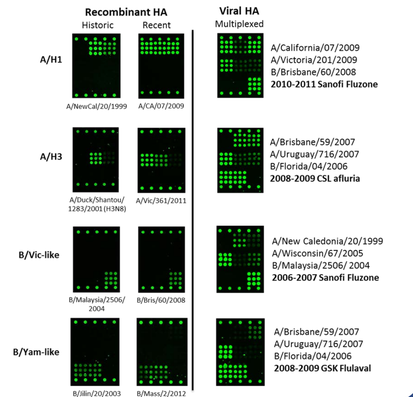


Figure 8: Robust Response to Older Strains

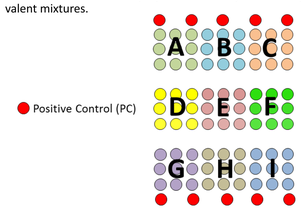
- To test the response of VSI to antigenic drift, archived vaccines and recombinant HA from older strains were analyzed by VSI.
- Recombinant proteins and archived vaccines representing a 10 year time period were readily detected on VSI.



The Future of the VaxArray Platform

Rapid Quantification of Flu Neuraminidase

- The VaxArray platform is highly versatile and can be outfitted to screen for a number of different influenza proteins.
- Neuraminidase (NA), the second most abundant influenza surface glycoprotein, is gaining support for inclusion in seasonal vaccinations (Eichelberger et al, 2015).
- The NA chip is currently under development and will be printed with broadly reactive, subtype specific anti-neuraminidase antibodies (similar to VSI) to **A/N1, A/N2**, and **B-Influenza neuraminidase**.
- As with VSI, a "universal" polyclonal antibody is used to quantify all neuraminidase in mono-valent and multi-valent mixtures.



Array Position	Detection Target
A	Broad N1
B	Broad N1
C	Broad N1
D	Broad N2
E	Broad N2
F	Broad N2
G	Broad N2
H	Broad B NA
I	Broad B NA

Unlike HA, neuraminidase (NA) does not have a potency gold standard (like SRID for HA), although MUNANA is widely used. We anticipate the this new microarray assay will be able to quantify not only total NA protein content, but also NA enzymatic activity, both important outputs for vaccine production and regulation.

Application to Pandemic Influenza Vaccines

- In concert with the WHO and US government, a number of vaccine producers prepare monovalent vaccines against potentially pandemic flu viruses.
- We are developing the VaxArray Pandemic Influenza (VPI) potency assay for **A/H5, A/H7**, and **A/H9** vaccines.
- Current studies are ongoing to select for antibodies (shown below) that will ensure that precise, rapid quantification is attainable for high risk pandemic influenza strains.

H5N1 Antigens (27)																											
Antibody ID	Sub-type	1	35	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	H5	75% Coverage, 100% Specificity																											

H7N9 Antigens (11)												
Antibody ID	Sub-type	28	29	30	31	32	33	34	35	36	37	38
2	H7	64% Coverage, 100% Specificity										
3	H7	64% Coverage, 100% Specificity										

H9N2 Antigens (11)												
Antibody ID	Subtype	39	40	41	42	43	44	45	46	47	48	49
4	H9	70% Coverage, 100% Specificity										

VPI will allow rapid, efficient screening of pandemic influenzas as necessary. VPI can function on the leading edge of pandemic vaccine production without requiring reference antisera (similar to VSI), making it a valuable tool for vaccine production, especially during a pandemic. With constant monitoring of potential pandemic influenzas, VPI assays will be adapted to be ready for the next pandemic influenza outbreak.

Other Advances

- 21 CFR Part 11 capable software is in development to enable easy integration into regulated environments.
- The Vidia Microarray Imaging System is CE certified.
- Compatible with automated and high throughput processing options.
- Please contact us if you are interested in collaborating or in beta testing new products

Acknowledgements

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Characteristics for an Improved Potency Assay for Inactivated Influenza Vaccines
WHO Expert Committee on Biological Standardization; Geneva Oct 2011

Biologically relevant potency measure ✓	Applicability to existing and novel vaccines ✓
Correlation with clinical efficacy (?)	Unaffected by adjuvants (?)
Stability indicating ✓	Measure low doses ✓
Bridging to single radial immunodiffusion (SRD) test ✓	Independent of strain specific reagents X
Precision and accuracy ✓	Reduced amount of reagents (required for assay) ✓
Reproducibility ✓	Robustness of reagent supply, speed of supply, volume/quantity of supply ✓
(Sub)type specificity ✓	Usable in process control (i.e. in presence of other proteins, contaminants) ✓
Flexibility and maximum practicability ✓	Efficient regulatory review ✓
Applicable world-wide ✓	Accelerating lot release ✓
Quick availability/usability following a strain change ✓	