VaxArray® Influenza Seasonal Hemagglutinin Potency Assay: Correlation with SRID

Background: The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple antibodies against seasonal A/H1, A/H3, B/Yamagata-like and B/Victoria-like strains are printed in an array format on a glass substrate. Signal readout for this multiplexed immunoassay is based on fluorescence from a conjugated "universal" primary antibody label.

The single radial immunodiffusion assay (SRID) has been used to quantify influenza vaccine potency since 1978 and it remains the FDA- and WHO-approved gold standard method. SRID is an antigen-antibody based assay that relies on reference antigens and reference antisera generated and distributed by Essential Reference Laboratories around the world. While reference antigens can be produced with reasonable expediency, vaccine producers often experience significant delays in materials characterization due to the complexities associated with generating and validating reference antisera. This limitation, as well as the tedious nature of the assay, has resulted in a push for alternatives to SRID and an overt effort to identify a replacement. However, it is understood that any potential alternative must be correlated with SRID.
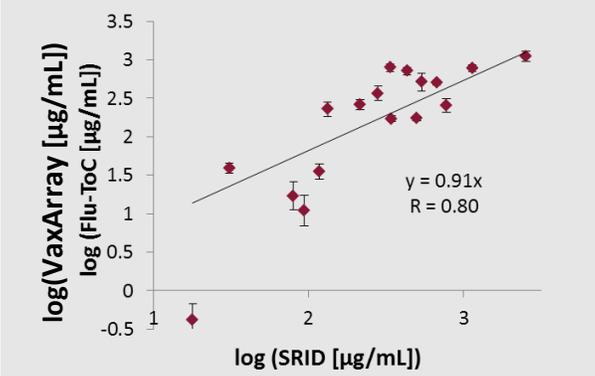
Objective: The objective of this Technical Note is to demonstrate that the HA protein content determined by VaxArray Influenza is linearly correlated with the HA content measured by SRID.

Recombinant HA: A blind study was conducted in conjunction with scientists at Protein Science Corporation (PSC) on a range of samples containing varying amounts of recombinant influenza HA from A/CA, A/Victoria, B/Brisbane and B/Massachusetts strains. The HA content was determined by SRID at PSC and by VaxArray Influenza at InDevR. Zwittergent 3-14 was not used in the VaxArray Influenza analysis.

A log-log plot of the data from 17 samples is shown in **Figure 1**. In log space, a linear relationship (neglecting the intercept) should yield a slope of 1. As evident in **Figure 1**, the log-log relationship between SRID and VaxArray Influenza exhibits a slope of 0.91 with a Pearson's correlation coefficient (R) of 0.80. Based on a statistical analysis of these results, we conclude that a linear correlation exists between the HA content measured by VaxArray Influenza and by SRID.

Figure 1 - Log-Log Plot of Recombinant HA Concentration as Determined by SRID vs. VaxArray Influenza

$$y = 0.91x$$
$$R = 0.80$$

Table 1 – Vaccine Potency by VaxArray Influenza

BEI Cat. #	Supplier	Year	Formula	VaxArray Result [µg/mL]	% SRID (original)
NR-20347	SANOFI	2009	Monovalent	26	87
NR-20083	NOVARTIS	2009	Monovalent	26	87
NR-31797	NOVARTIS	2010/2011	Trivalent	33	110
NR-31044	SANOFI	2010/2011	Trivalent	38	127
NR-31799	GSK	2010/2011	Trivalent	33	110
NR-31798	CSL	2010/2011	Trivalent	38	127
NR-36747	SANOFI	2011/2012	Trivalent	40	133
			Average:	33 ± 6	112 ± 19

Commercial Vaccines: In order to evaluate the reliability of the VaxArray Influenza as a potency assay for vaccines, seven archived vaccines spanning the time period of 2009 to 2012 were analyzed for A/CA/2009 H1 HA concentration. CBER reference antigen A/CA/07/2009 (lot number H1-Ag-1107) was used as the calibration standard for all seven vaccines. The CBER reference antigen was lysed with Zwittergent 3-14 and each dilution in the calibration set was maintained at 1% Zwittergent. Likewise, 1% Zwittergent was added to the vaccines and maintained at that level for each dilution. Each vaccine was analyzed in duplicate. The results are summarized in **Table 1**.

It is presumed that each of the vaccines had SRID HA concentrations of 30 µg/mL at release date, with 20% relative error. Therefore the expected HA content range was 30 ± 6 µg/mL. As evident in **Table 1**, four of the values measured by VaxArray Influenza were within the expected range. Three of the vaccines exhibited VaxArray Influenza values outside the range. A degree of deviation was anticipated due to the age of the vaccines but in general lower values were expected. On average, VaxArray Influenza yielded a result of 112% of the original SRID value, which is reasonable given the limitations of the study.

Virus-Like Particles: A similar study was conducted on virus-like particle Bulk Drug Substance in order to evaluate the utility of VaxArray Influenza for these novel vaccines. The samples were split with detergent and analyzed by both VaxArray Influenza and SRID. The results are summarized in **Table 2.** Although only 3 samples were analyzed, the results support the conclusion that VaxArray Influenza provides a measurement of functional forms of HA and is correlated with SRID.

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Table 2 - Quantification of HA in VLP Bulk Drug Substance

Vaccine (BDS)	SRID Result (µg/mL)	VaxArray Result (µg/mL)	Accuracy (% of SRID)	CBER Ref. Standard
A/H3	189.7	170 ± 13	90	A/Texas/50/2012 X-223A Lot 75
A/H1	212.2	231 ± 15	109	A/CA/07/2009 X-181 Lot H1-Ag-1107
B/Brisbane	217.1	216 ± 7	100	B/Bris/60/2008 Lot 68

Summary: VaxArray Influenza is correlated with SRID and, in many cases, yields equivalent results.

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