VaxArray Seasonal Influenza Assessment of Cell-Derived Influenza Vaccine Potency

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Abstract

Background and novelty: WHO guidelines dictate flu vaccine producers determine potency & stability prior to and as a function of time after release. The current accepted potency determination assay is single radial immunodiffusion (SRID) to measure hemagglutinin (HA) concentration. SRID is labor and reagent intensive, established for egg-based vaccines and is not always optimal, or even possible, for novel vaccine produced outside of eggs, such as VLPs. The VaxArray seasonal HA assay (VXI sHA) is a new alternative potency assay compatible with cell-based vaccines during all manufacturing stages, ranging from crude samples in tissue culture media to monobulk drug substances & multivalent formulations. VXI sHA is based on a panel of subtype-specific yet broadly reactive monoclonal antibodies. Multiple antibodies against seasonal subtypes are printed in an array on a glass substrate, & a multiplexed immunoassay is performed with signal readout based on fluorescence from a conjugated "universal" antibody label.

Experimental approach: To demonstrate utility of VXI sHA in characterizing cell-derived vaccines, a panel of 20 cell-derived HA antigens were analyzed for assay detection and coverage. To demonstrate assay sensitivity and binding, a serial dilution of a cell-derived reference antigen was analyzed via VXI sHA and a binding curve was analyzed. Spiking study were performed to demonstrate the use of VXI sHA in the upstream vaccine manufacturing of cell-derived vaccines and in the presence of alum-based and squalene-based adjuvants.

Results and discussion: The VXI sHA assay has been previously demonstrated to work well with egg-based vaccines (Kuck et al., 2017). This study expanded the compatibility of the VXI sHA assay to include cell-derived vaccines. The assay detected all 20 cell-derived HA antigens tested, demonstrating 100% coverage. The assay also demonstrated a quantitative response to increasing concentrations of cell-derived HA antigen with an estimated linear range of 0.02-1.75 µg/mL. Vaccine potency quantitation was not affected by the presence of crude matrixes common to upstream vaccine manufacturing (allantoic fluid, tissue culture media, 20% sucrose), nor by the presence of common adjuvants at vaccine-relevant concentrations.

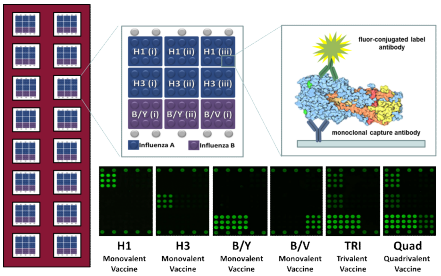
Background

- 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 incompatibility with novel vaccines such as VLPS, labor-intensive protocols, and the requirement for reference reagents that do not necessarily accurately represent the composition of vaccines.
- The VaxArray Influenza (VXI) alternative potency assay allows for potency determination in 2 hours.

The VaxArray Platform

Three components: 1) Reagent kit, 2) Fluorescence imaging system, 3) 21CFR Part 11/Annex 11 compatible software.

VaxArray Influenza (VXI) Technology

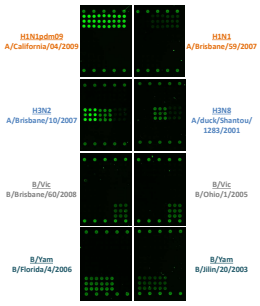


VXI sHA is a simple multiplexed sandwich immunoassay that utilizes a microarray slide printed with broadly reactive yet subtype specific antibodies for seasonal influenza strains (Kuck et al., 2014). A "universal" fluorophore-labeled antibody is used to quantify all components of monovalent and multivalent HA mixtures.

Results

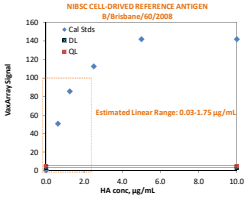
Antigen Screening & Coverage

- To assess the utility of VXI sHA in assessing potency of tissue culture-derived influenza antigens, we screened a panel of 20 publically available (BEI/IRR), tissue culture-derived antigens consisting of recombinant HA produced in Sf9 insect cells, whole virus antigen from MDCK and Per.C6 cells, and cell-derived reference antigens (CBER and NIBSC) on the VaxArray assay.
- **VSI sHA demonstrated 100% coverage of the cell-derived antigens, detecting 20/20 antigens. Representative images are presented to the right.**



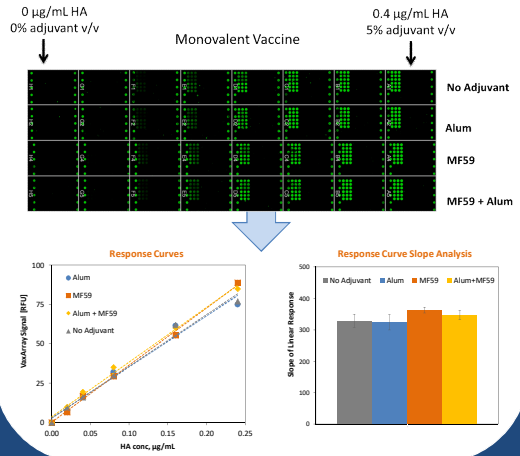
Binding Curve Assessment

- To assess the linear response of the VXI sHA assay to increasing HA concentrations, a Per.C6 cell-derived B/Brisbane/60/2008 (NYMCBX-35) NIBSC reference antigen was serially diluted and analyzed via VXI sHA.
- **VXI sHA demonstrated a quantitative response to increasing cell-derived HA antigen.**
- **A linear range of 0.02-1.75 µg/mL was estimated from this study.**

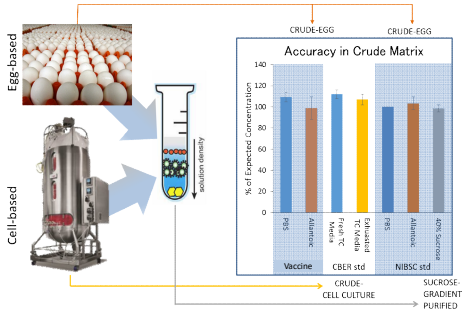


VaxArray Analysis in the Presence of Adjuvants

- A monovalent vaccine was diluted to 0.4 µg/mL HA in the presence or absence of 5% Alum (15x vaccine relevant , MF59, and a combination of the two adjuvants.
- Serial dilutions were performed of each sample and each analyzed on a column of a VaxArray slide.
- Response curves to decreasing HA and adjuvant concentrations were compared to the non-adjuvanted sample (bottom left) and the resulting slopes of each response curves were plotted (bottom right).
- **Even at the highest adjuvant concentrations, background and array image quality was not affected.**
- **From 0-3% adjuvant by volume, the assay was unaffected by the presence of adjuvants. Due to the highly sensitive nature of the assay, adjuvants can easily be diluted to less than 3% by volume, which is 15x the vaccine relevant concentration of alum-based vaccines and 4x the vaccine relavant concentration of squalene-based vaccines liked MF59.**



Application in Bioprocess Improvement



- To illustrate the capability of the VXI sHA assay to quantify immunogenic HA in crude matrixes common to upstream vaccine bioprocessing, a spiking experiment was performed. Three samples were spiked into PBS, allantoic fluid, fresh and exhausted tissue culture media, and 40% sucrose to mimic samples at harvest from egg- and cell-based manufacturing and after a sucrose gradient purification. Each sample was analyzed via VaxArray and compared to the predicated HA concentration.
- **VXI sHA is unaffected by complex matrixes and can be utilized to 1) monitor HA yield during viral propagation, 2) determine a starting HA concentration at virus harvest, 3) monitor/optimize yield of sucrose gradient purification, 4) track yield through any additional downstream purification efforts, 5) assess HA concentration/potency of bulk drug substance, and 6) assess potency of final multivalent formulations.**

Conclusions

The VaxArray Seasonal Influenza alternative potency assay (VXI-sHA) can quantify potency of cell-based vaccines. The VXI sHA assay has been previously demonstrated to work well with egg-based vaccines (Kuck et al., 2017). This study expanded the compatibility of the VXI sHA assay to include cell-derived vaccines. The assay demonstrated high coverage, detecting all cell-derived antigen tested in this study. The assay also demonstrated a quantitative response to increasing concentrations of cell-derived HA antigen with an estimated linear range of 0.02-1.75 µg/mL. Vaccine potency quantitation was not affected by the presence of crude matrixes common to upstream vaccine manufacturing, nor by the presence of common adjuvants at vaccine-relevant concentrations.

SRID

- **NOT COMPATIBLE WITH NOVEL VACCINES SUCH AS VLPs**
- Reliant on seasonal reference antisera & antigens
- Requires in-house prep of gels or plates
- Limited to singleplex quantification
- Analysis & interpretation not standardized
- Limited dynamic range
- Does not work with crude samples
- Does not work with adjuvanted vaccines
- Slow. Assay takes ~48 hrs
- Produces hazardous waste

VaxArray (VXI)

- Compatible with novel vaccines
- Reference antisera NOT required; Reference antigens required for final release (bridge to SRID)
- ***Simultaneous analysis of all components in multivalent mixture***
- Analysis standardized with 21 CFR Part 11 compatible software
- Works with samples in crude matrix
- Works with dose-sparing vaccines below LOD for SRID
- Works with adjuvanted vaccines
- Sample to result time ≤2 hrs
- No hazardous waste

Recommended Reading:
Kuck, L.R., Saye, S., Loob, S., Roth-Eichhorn, S., Byrne-Nash, R.T., Rowlen, K. "VaxArray Assessment of Influenza Split Vaccine Potency and Stability." Vaccine. (2017)
Kuck LR, Sorensen M, Matthews E, Srivastava I, Cox MMJ, Rowlen KL. "Titer on chip: New analytical tool for influenza vaccine potency determination." PLoS One (2014)

Acknowledgements:
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