VaxArray Seasonal Influenza Assessment of Adjuvant-containing Vaccine formulation

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Introduction & Background

WHO guidelines dictate flu vaccine producers determine vaccine potency and stability prior to and as a function of time of release. Currently, the gold standard for the measurement of hemagglutinin (HA) concentration is the single radial immunodiffusion (SRID) assay. This labor and reagent intensive method is not sufficiently sensitive and is not compatible with all common adjuvants that are known to enhance vaccine performance. Vaccine manufacturers require more rapid and accurate tools to characterize the potency and stability of their products. Therefore, the new alternative potency assay VaxArray Influenza (VXI) seasonal hemagglutinin (sHA) manufactured by InDevR, Inc. was used to quantify potency in quadrivalent adjuvant-containing vaccines. The accuracy of VXI with vaccines that included different concentrations of the nasal adjuvant Endocine™ (Eurocine Vaccines AB) was assessed and compared to SRID values.

Figure 1| The VXI sHA assay is a new alternative potency assay to measure the concentration of functional hemagglutinin (HA). HA has been established to be the key component of whole virus vaccines and the dominant target of protective antibodies following vaccination or infection. Multiple influenza strains can be quantified within 2 hours. The VXI platform is comprised of the Influenza Seasonal Hemagglutinin Potency Assay Kit and the VaxArray Imaging System.

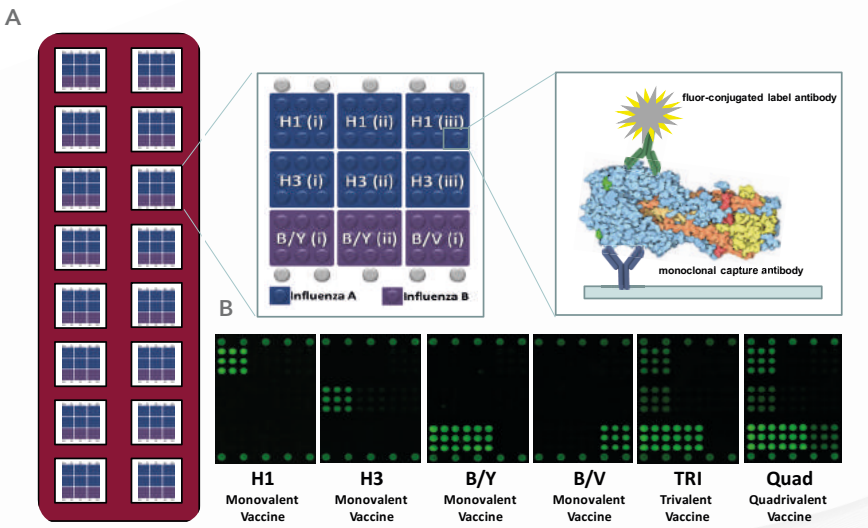
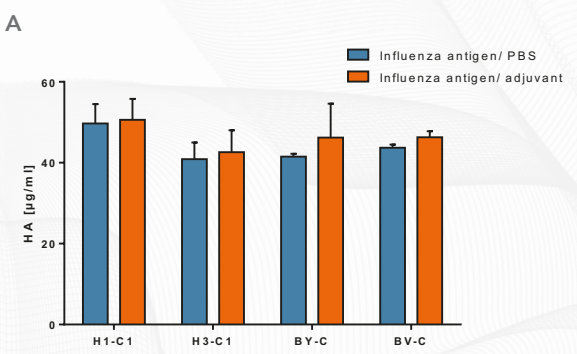


Figure 2| VXI sHA is based on a panel of subtype-specific broadly reactive monoclonal antibodies. A) Subtype specific antibodies to A/H1, A/H3 subtypes as well as B/Yamagata and B/Victoria lineages are printed in an array on a glass substrate. A multiplexed immunoassay is performed with signal readout based on fluorescence from a conjugated "universal" antibody label. **B)** Representative fluorescence images: H1/Christchurch, H3/Switzerland, B/Phuket (Yamagata lineage), B/Brisbane (Victoria lineage). Trivalent vaccine composed of H1, H3, and B/Yam-like, and quadrivalent vaccine composed of H1, H3, B/Yam-like, and B/vic-like. The brightness of the green spots is an indication of signal intensity.

Impact of adjuvant on VXI analysis



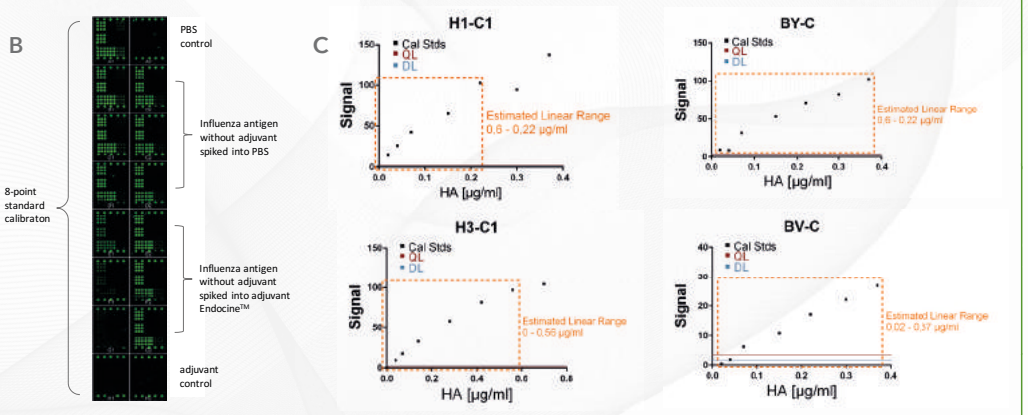
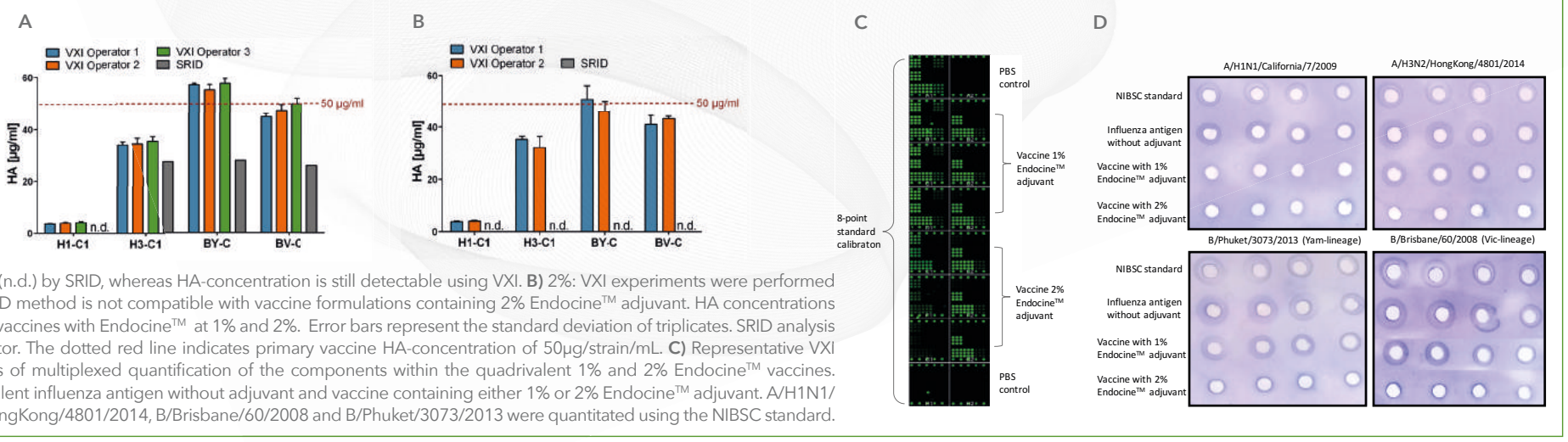


Figure 3| VXI analysis of quadrivalent influenza antigen without Endocine™ adjuvant spiked into PBS or Endocine™ (final PBS or adjuvant % = 90). **A)** Adjuvant Endocine™ does not affect HA quantification measured by VXI. Both samples (control influenza antigen without adjuvant in PBS and influenza antigen spiked into adjuvant) show similiar HA concentrations. Error bars represent the standard deviation of triplicates.

B) Representative VXI fluorescence montage images of multiplexed quantification of the components within the quadrivalent influenza antigen formulation. **C)** Representative calibration curves for H1, H3, Yamagata and Victoria-lineage components within the quadrivalent influenza antigen formulation. The dotted orange box indicates the estimated linear range.

Quantification of 1% and 2% adjuvant-containing Vaccine using VXI and SRID

Figure 4| VXI analysis of quadrivalent vaccine containing either 1% or 2% Endocine™ adjuvant. **A)** 1%: VXI experiments were performed by three different operators. VXI results are in good agreement between operators and RSD values for triplicate measurements are low. VXI analysis is more sensitive compared to SRID since H1-C1 is not detectable (n.d.) by SRID, whereas HA-concentration is still detectable using VXI. **B)** 2%: VXI experiments were performed by two different operators. SRID method is not compatible with vaccine formulations containing 2% Endocine™ adjuvant. HA concentrations obtained by VXI are similiar in vaccines with Endocine™ at 1% and 2%. Error bars represent the standard deviation of triplicates. SRID analysis was performed by one operator. The dotted red line indicates primary vaccine HA-concentration of 50µg/strain/mL. **C)** Representative VXI fluorescence montage images of multiplexed quantification of the components within the quadrivalent 1% and 2% Endocine™ vaccines. **D)** SRID evaluation of quadrivalent influenza antigen without adjuvant and vaccine containing either 1% or 2% Endocine™ adjuvant. A/H1N1/California/7/2009, A/H3N2/HongKong/4801/2014, B/Brisbane/60/2008 and B/Phuket/3073/2013 were quantitated using the NIBSC standard.



Conclusions

We established that the VaxArray Seasonal Influenza potency assay performs well with quadrivalent vaccine formulations containing either 1% or 2% Endocine™ adjuvant, while SRID analysis failed to analyze vaccine formulation containing 2% Endocine™. This experiment demonstrated that VXI exhibited an accurate quantification of HA with quadrivalent vaccines in only one VaxArray run, good overall producibility between different users and higher assay sensitivity when compared to SRID. This work establishes VXI as a promising alternative to SRID.

VaxArray (VXI)
- Works with adjuvanted vaccines and in crude matrix
- Reference antisera not required
- Simultaneous analysis of all components in multivalent mixture
- Analysis standardized with 21 CFR Part 11
- Works with vaccines below LOD for SRID
- Sample to result time ≤2 hrs
- No hazardous waste

SRID
- Not compatible with all adjuvanted vaccines
- 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
- Assay takes ~48 hrs
- Produces hazardous waste

VXI related literature

Kuck LR, Sorensen M, Matthews E, Srivastava I, Cox MMJ, et al. (2014) **Titer on Chip: New Analytical Tool for Influenza Vaccine Potency Determination.** PLoS ONE 9(10)

Kuck LR, Saye S, Loob S, Roth-Eichhorn S, Byrne-Nash R, Rowlen KL, et al. (2017) **VaxArray assessment of influenza split vaccine potency and stability.** Vaccine2017.02.028

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