

VaxArray® Influenza Seasonal Hemagglutinin Potency Assay: Appropriate Calibration Standards

Background: The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin (HA) 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. As a multiplexed immunoassay, signal readout is based on fluorescence from a conjugated “universal” primary antibody label.

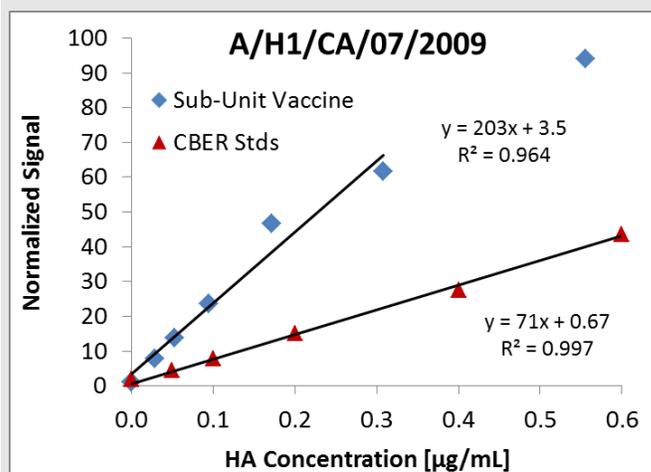
Based on a range of studies we know that determining an appropriate calibration standard for vaccine potency assays can be challenging. For the VaxArray Influenza potency assay, whole virus reference standards such as those provided by CBER or NIBSC may not be appropriate for split vaccines.

Objective: The objective of this Technical Note is to establish the need for use of a vaccine-specific secondary standard in the VaxArray Influenza assay.

Current Use of Secondary Standards: The use of secondary standards in the quantification of influenza HA has been established for vaccines composed of recombinant proteins or virus-like particles (VLPs). In addition, an alternative potency assay based on ELISA exhibited good performance for sub-unit vaccines when bulk vaccine was used as a secondary standard [see Bodle et al., 2013].

Comparing Primary and Secondary Standards: Since sub-unit vaccines are composed of relatively pure proteins, whole virus reference antigens may not be appropriate standards for sub-unit or split vaccines. Even after splitting the whole virus with detergent, in the absence of purification steps, there are significant differences in the matrix and possible differences in the protein structure.

Figure 1 – A/H1 Calibration Curves for CBER Reference Antigens versus Sub-Unit Vaccine.



In order to compare the relative response of split whole virus standards and purified sub-unit vaccines, VaxArray Influenza was performed on each sample type using the v1.0 protocol (InDevR, 2014). Normalized signal for each of the samples analyzed over a range of concentrations was evaluated. The resulting “calibration curves” are shown in **Figure 1** for A/CA/07/2009. Note that for the linear ranges, there is approximately a factor of

3 difference in the slope, with the assay exhibiting greater sensitivity to the purified sub-unit vaccine relative to the split, whole virus reference standards. In this case, if the reference standards were used for calibration, the measured concentration of the vaccine would be 3 times higher than it would be if an equivalent sub-unit standard were used.

The same effect is observed for other strains within current trivalent and quadrivalent vaccines, as well as for VLP vaccines.

Recommendations: When absolute quantitative values are required, we recommend that a secondary standard be used with the VaxArray Influenza potency assay. The secondary standard should be created from the specific vaccine (e.g., split vaccine or recombinant or VLP) which itself has been characterized by SRID using the appropriate reference antigens. This approach is consistent with that used for ELISA [Bodle et al.,

2013]. If reference reagents are not yet available, the vaccine standard can be established using purity adjusted SDS-PAGE results. If needed, the appropriate correction factor can be applied to the results once reference reagents become available.

Cited References:

Kuck LR, Sorensen M, Matthews E, Srivastava I, Cox MMJ, et al. "Titer on Chip: New Analytical Tool for Influenza Vaccine Potency Determination." *PLoS ONE* 9.10 (2014): e109616.

Bodle J, Verity EE, Ong C, Vandenberg K, Shaw R, Barr IG, Rockman S. "Development of an Enzyme-Linked Immunoassay for the Quantitation of Influenza Haemagglutinin: an Alternative Method to Single Radial Immunodiffusion." *Influenza Other Respir. Viruses.* (2013) 7(2):191-200.

InDevR. (2014) *VaxArray Influenza Operation Manual*. Boulder, CO.

