**VaxArray® Influenza Seasonal Hemagglutinin Potency Assay: Monovalent and Multivalent Vaccines**

**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. Signal readout for this multiplexed immunoassay is based on fluorescence from a conjugated “universal” primary antibody label.

**Objective:** The objective of this Application Note is to present VaxArray Influenza as a tool for accurate quantification of HA in both monovalent bulk drug substance and multivalent formulations including finished quadrivalent vaccines.

**Monovalent:** Figure 1 shows representative fluorescence images for monovalent Bulk Drug Substance (BDS) samples analyzed on a VaxArray Influenza Seasonal Hemagglutinin Potency Assay Slide. The antibodies used to quantify the protein are highlighted in a white box. The samples were quantified against reference antigens (rHA) used as calibration standards. Quantitative results are summarized in Figure 2, with standard error from replicate measurements.

**Multivalent:** A significant advantage of VaxArray Influenza over ELISA and SRID is the ability to work with a variety of sample types on a single analytical platform. In addition to the specific measurement of monovalent BDS samples, VaxArray Influenza allows users to simultaneously analyze all components in trivalent or quadrivalent vaccine products. For example, Figure 2 shows representative fluorescence image of multiplexed analysis of all components within a quadrivalent formulation (constructed from a mixture of monovalent BDS), where the white boxes outline the mAbs used to quantify each component. The error for quadrivalent formulation is 12% RSD, based on the average relative precision determined in separate studies. Despite the fact that
A variety of trivalent vaccines have also been evaluated. Representative fluorescence images are shown in Figure 3 for vaccines produced by the four major manufacturers over a range of years.

**Summary:** The ability of VaxArray Influenza to accurately quantify both monovalent and multiplex samples in a single assay provides a dramatic advantage in comparison to other platforms. This level of multiplexing is not possible with ELISA or SRID.
In addition, a single VaxArray Influenza experiment generally allows for 8-24 individual samples to be analyzed simultaneously.