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

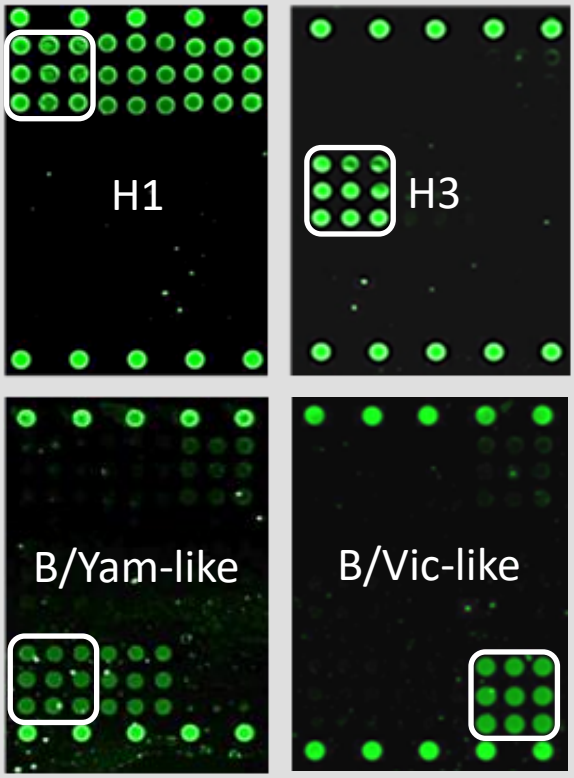
Background: The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin (HA) protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple antibodies against seasonal A/H1, A/H3, B/Yamagata-like and B/Victoria-like strains 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

Figure 1 - Representative Fluorescence Images for Monovalent Bulk Drug Substances



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

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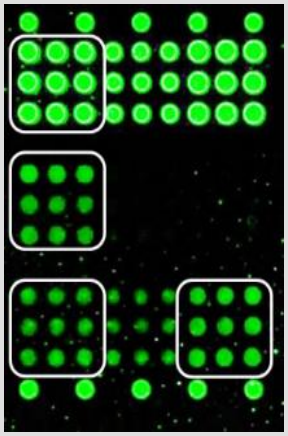
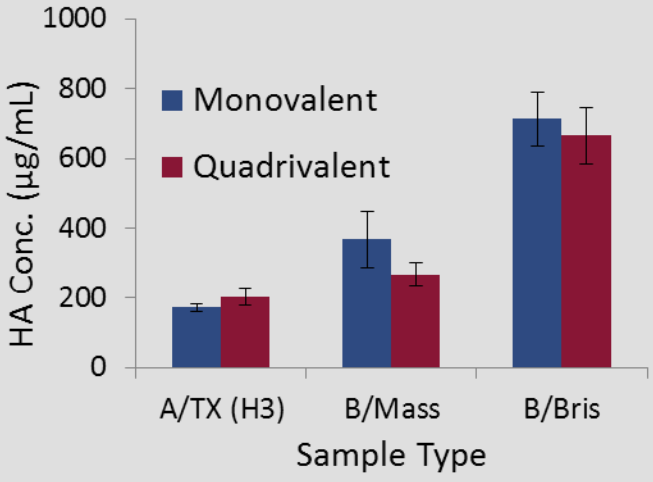
multiplexed quantification was conducted three months after the initial BDS measurements, within error the results were equivalent to those reported for the monovalent BDS (with the exception of A/CA HA for which a new label antibody was tested).

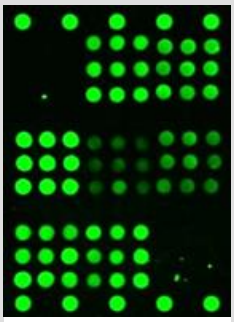
Figure 2 - Representative Image and Results Summary for Multiplexed Quantification of HA in Quadrivalent Formulation



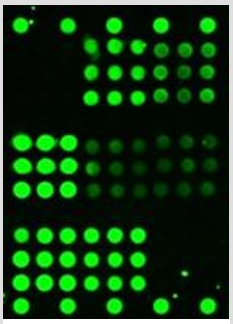


A variety of trivalent vaccines have also been evaluated. Representative fluorescence images are shown in **Figure 3** for vaccines produced by the four major manufacturers over a range of years.

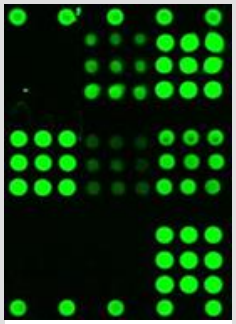
Figure 3 - VaxArray Images for Multiplexed Analysis of Trivalent Vaccines



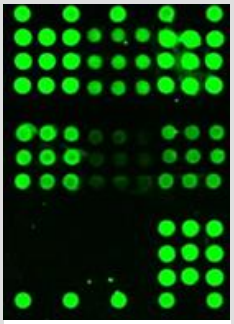
A/Brisbane/59/2007
A/Urug/716/2007
B/Florida/04/2006
2008-2009
Sanofi Fluzone



A/Brisbane/59/2007
A/Urug/716/2007
B/Brisbane/3/2007
2008-2009
GSK Fluarix



A/Solom. Is./3/2006
A/Wisc/67/2005
B/Malaysia/2506/04
2007-2008
Novartis Fluvirin



A/CA/7/2009
A/Vic/210/2009
B/Brisbane/60/2008
2010-2011
CSL Afluria

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.