Array Layout and Specificity

VaxArray® Influenza Hemagglutinin Potency Assay v1.2

Overview

The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin 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 conjugated polyclonal or monoclonal antibody labels.

Array Layout

The microarray layout for the VaxArray Influenza Hemagglutinin Potency Assay v1.2 is illustrated in **Figure 1.** The array contains 9 replicate spots of each monoclonal antibody. There are 3 distinct antibodies for A/H1 and A/H3, 2 antibodies for B/Yamagata-like, and a single antibody for B/Victoria-like strains. The presence of multiple antibodies against the same strain provides breadth of coverage across variations in the virus, as well as complementary insight into protein structure.

Analysis is conducted in a simple sandwich assay format. Multiple detection labels are possible ranging from a broadly reactive polyclonal antibody (fluor-conjugated) to more specific monoclonal antibodies.

Specificity

The specificity of antibodies incorporated in the VaxArray Influenza Hemagglutinin Potency Assay v1.2 array toward HA subtypes was evaluated using a range of recombinant HA antigens. **Figure 2** shows representative responses for A/H1, A/H3, B/Yamagata-like, and B/Victoria-like subtypes. Over the range of antigens tested, including recombinant HA from 1999-2011 origin strains, the microarray exhibited excellent specificity and no cross-reactivity between subtypes. It is evident in **Figure 2** that individual H1 strains may respond differently to the panel, but in all tested cases, quantification of HA using at least one antibody was possible.

Figure 1 – Array Layout

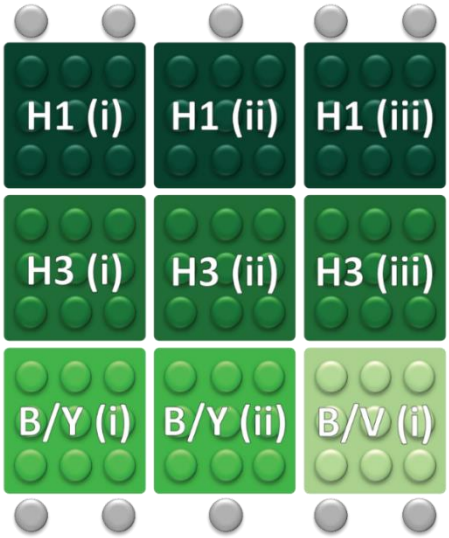

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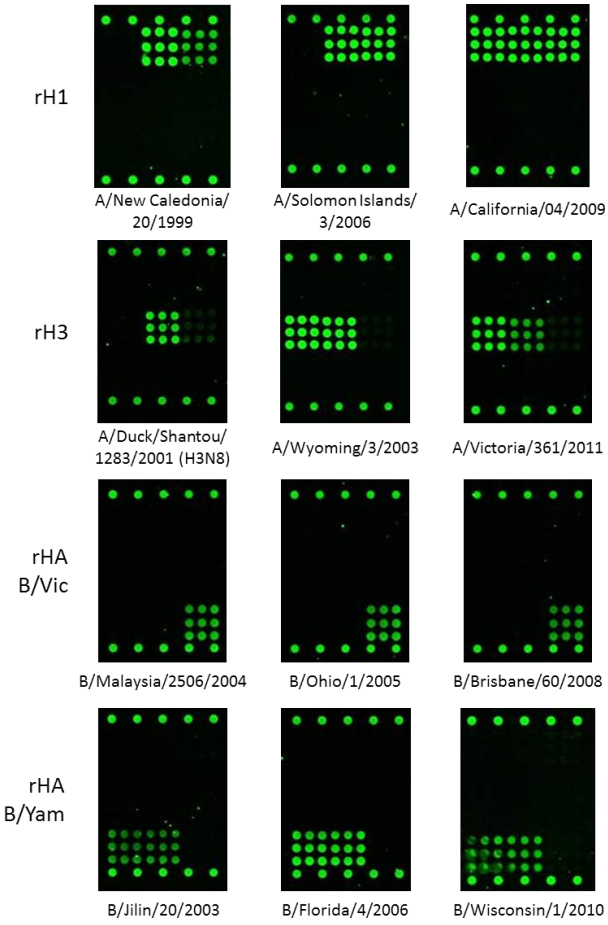
Figure 2 – Representative Fluorescence Images for Seasonal HA Subtypes and Lineages



Advantages

The advantage of including multiple distinct monoclonal antibodies binding to either variable HA1 or conserved HA2 domains can be seen in **Figure 2**. The capture antibody in position H3 (ii) reliably responded to all of the H3 subtypes originating from viruses first isolated over the time period of 2001 to 2011, despite antigenic drift. Antibody H3 (ii) is known to bind to the conserved HA2 stem region of the protein. The same observation is made for recombinant H1's from viruses isolated over the time period of 1999 through 2009. The mAb that responded to all H1's (array position H1 (ii)) is known to recognize a conserved region of HA2.

A similar multiple capture approach for each subtype would be impractical for an ELISA system due to reagent cost and low sample throughput per plate (4-6 samples per plate). In contrast, microarrays are ideally suited for the task due to sparing use of reagents (picograms per spot) and multiplex design capability. For example, the amount of antibody needed to coat *a single* ELISA plate can be used to produce over *400* microarrays.

Summary

The VaxArray Influenza Assay displays strain specificity, which enables analysis of monovalent or multivalent formulations, as well as robust response despite antigenic drift.

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