Array Layout and Specificity

VaxArray® Influenza Seasonal NA Potency Assay v1.0

Overview

The VaxArray Seasonal Neuraminidase Potency Assay is a new tool for Neuraminidase (NA) protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple capture antibodies specific to seasonal N1, N2, and NB strains are printed in an array format on a functionalized 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 Neuraminidase Potency Assay v1.0 is illustrated in **Figure 1.** The array contains 9 replicate spots of each monoclonal antibody. There are 2 distinct antibodies for N1, N2 and NB subtypes. The presence of two antibodies for each subtype 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 (fluor-conjugated) are available, ranging from a broadly reactive polyclonal antibody to more specific monoclonal antibodies.

Specificity

The specificity of antibodies incorporated in the VaxArray Influenza Neuraminidase Potency Assay v1.0 array toward NA subtypes was evaluated using a range of NA antigens. **Figure 2** shows representative responses for N1, N2, and NB subtypes. Over the range of antigens tested the microarray exhibited excellent specificity and minimal cross-reactivity between subtypes. It is evident in **Figure 2** that individual N1 strains may respond differently to the panel, but in all tested cases, quantification of NA using at least one antibody was possible.

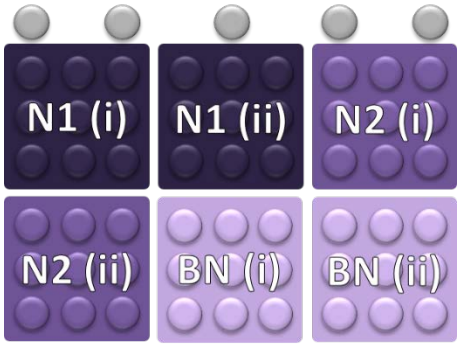

Figure 1 – Array Layout. The array contains 9 replicate spots (200 µm in diameter) of each monoclonal antibody.

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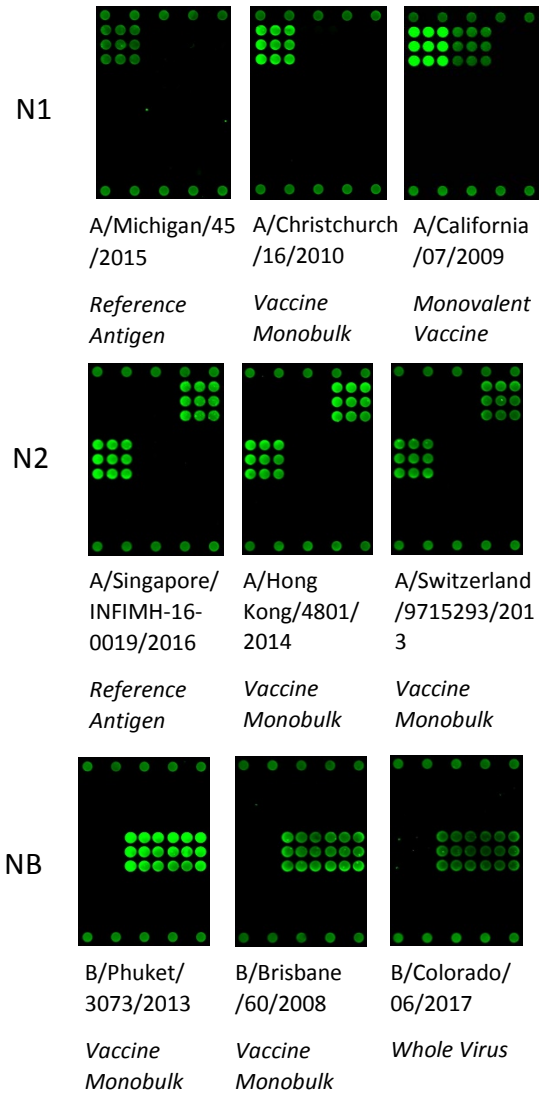

Figure 2 – Representative fluorescence response for seasonal NA subtypes and lineages

Advantages

One advantage of the VaxArray Influenza Neuraminidase Potency Assay is that more than one NA surface epitope is probed for each subtype. **Table 1** details the information known about the binding properties of the relevant capture antibodies.

Table 1: Antibody Information

Array Location	NA Inhibiting	Epitope Location	Relevant Publication
N1(i)	--	--	N/A
N1(ii)	No	NA Head	Wohlbold, 2015
N2(i)	--	--	N/A
N2(ii)	--	--	N/A
BN(i)	Yes	--	Wohlbold, 2017
BN(ii)	Yes	NA Head	Wohlbold, 2017

A similar multiple capture approach for each subtype would be impractical for an ELISA system due to 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 multiplexed 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 Neuraminidase Potency Assay offers subtype specificity for analysis of monovalent or multivalent formulations, as well as robust response despite antigenic drift.

References

Wohlbold TJ, et al. Vaccination with adjuvanted recombinant neuraminidase induces broad heterologous, but not heterosubtypic, cross-protection against influenza virus infection in mice. mBio 2015;6:1-13. Doi:10.1128/mBio.02556-14

Wohlbold TJ, et al. Broadly protective murine antibodies against influenza B virus target highly conserved neuraminidase epitopes. Nat Microbiol. 2017;2:1415-24. Doi:10.1038/s41564-017-0011-8

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