Virion Splitting Studies

VaxArray® Influenza Potency Assays

Overview

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 conjugated polyclonal or monoclonal antibody labels.

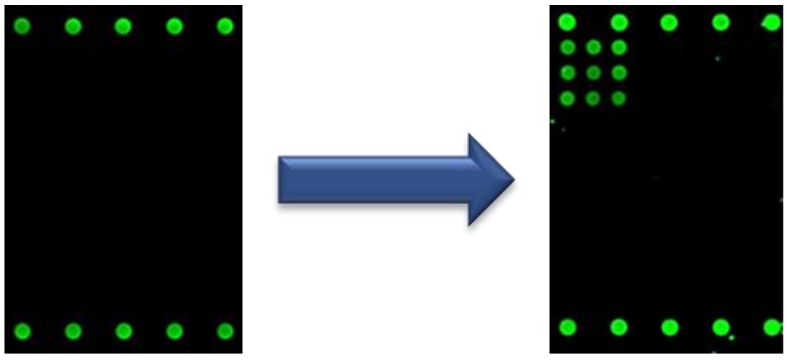
Virion (or virus) "splitting" refers to the process wherein detergent is used to dissociate virus structure into individual proteins. Understanding the efficiency and kinetics of that process is of vital importance to vaccine producers that work with whole viruses.

Virion Splitting

To demonstrate the utility of the VaxArray Influenza Assay for studying virion splitting, whole viruses (inactivated A/CA/07/2009 or B/Brisbane CBER reference antigens) were split (lysed) using 1% Zwittergent 3-14 at room temperature. Aliquots of each sample were exposed for various lengths of time to the detergent and subsequently analyzed by VaxArray.

A qualitative array response is illustrated in **Figure 1**, with representative images showing the A/CA/07/2007 (H1N1) antigen at t = 0 and t = 30 minutes exposure to Zwittergent. At t = 0 minutes there is no measurable fluorescence

Figure 1 – Representative Fluorescence Images for Virus Lysis



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detected on the array, indicating that the assay is not able to detect intact, whole influenza virus (likely due to steric issues. In contrast, the 30 minute time point shows bright and specific fluorescence signal from the pandemic A/CA-like capture antibody in the upper left block of nine spots on the array.

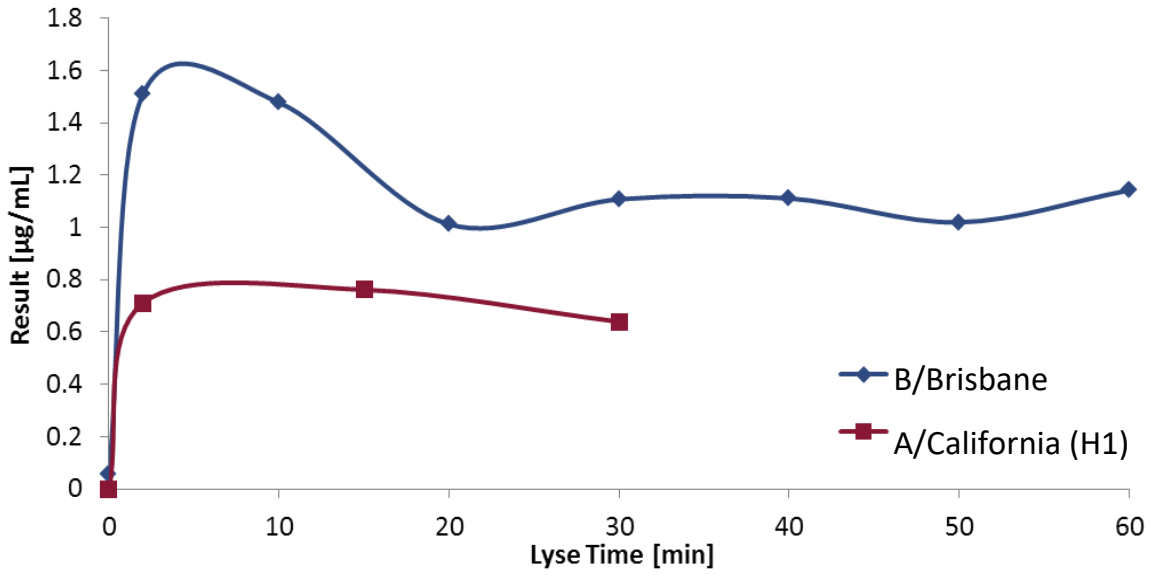
VaxArray results for the relevant capture antibodies for Influenza A and B viruses over a 30 minute time course are shown in **Figure 2**. While the whole virus was not quantifiable on the array, the assay was able to reliably measure HA after as little as 2 minutes of exposure to detergent, which releases individual component proteins from the virus.

It is interesting to note that for B/Brisbane virus, there is a rapid rise in measured protein concentration followed by a slight decrease to an apparent steady state after 20 minutes. This effect is reproducible and may be associated with equilibration between various oligomeric states of HA (e.g., trimer versus aggregates of trimers).

Summary

Data presented here demonstrates the potential application of the VaxArray Influenza Assay for investigating the kinetics of virion splitting by detergents.

Figure 2 - HA Quantification as a Function of Lysis Time



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