

VaxArray® Influenza Seasonal Hemagglutinin Potency Assay: Stability Indicating

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

Essential for any vaccine potency assay is that it be stability indicating. As vaccines degrade over time, the HA concentration as measured by SRID decreases. Since SRID is believed to be directly correlated with immunogenicity, the decrease in measured concentration corresponds to lower potency. It was recently demonstrated that immunoassays can also be used to track stability, as determined by forced degradation studies [Bodle et al. (2013) and Hashem et al. (2013)]. Since VaxArray Influenza is a high-information-content immunoassay, it is similarly expected to exhibit stability indicating behavior.

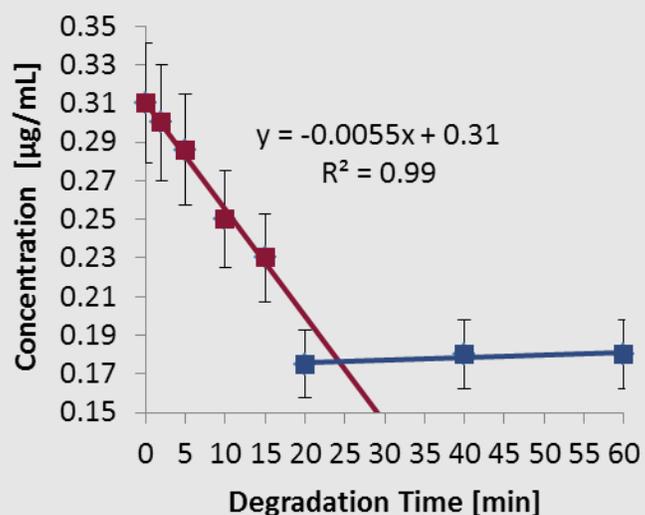
Objective: The objective of this Application Note is to demonstrate the use of VaxArray Influenza to monitor influenza hemagglutinin protein stability.

To explore the use of VaxArray Influenza as a stability indicating method, preliminary studies were conducted using elevated temperature and low pH forced degradation protocols.

Thermal Degradation: CBER reference antigen (A/CA/07/2009) was lysed with 1% Zwittergent 3-14, aliquoted, and each aliquot subjected to heat exposure at 50°C for times ranging from 0 to 60 minutes. The A/CA H1-specific capture mAb (conformational and neutralizing) was used for quantification.

Protein concentration as a function of time exposed to elevated temperature is shown in **Figure 1**. The concentration decreased linearly (zero order) by ~45% within the first 25 minutes to reach a steady state measured concentration. This trend is qualitatively similar to the SRID and ELISA results published by Hashem et al. [2013] for A/CA/07/2009 in which a ~20% decrease in SRID-measured concentration and a ~90% decrease in ELISA-measured concentration was observed after exposure to 50°C for 1 hour.

Figure 1 - Time Dependent Thermal Degradation

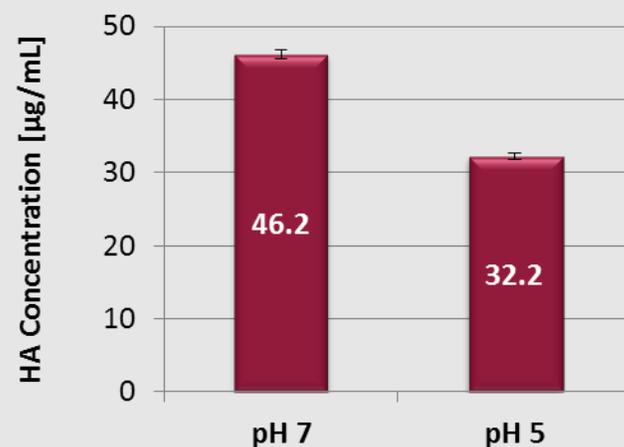


Stability is also commonly tested at low pH [Hasija et al., 2013] where HA is known to undergo significant conformational changes [Fontana et al., 2012]. With respect to SRID measurements at low pH, the result is a decrease in measured concentration of the trimeric form [Hashem et al., 2013].

To evaluate the pH response of VaxArray Influenza, lysed A/CA/07/2009 was exposed to pH 5.0 for 30 minutes and neutralized back to pH 7.2. Zwittergent 3-14 was added to obtain a final concentration of 1% prior to analysis. The treated sample was compared to an equivalent concentration aliquot that had not been exposed to low pH.

As shown in in **Figure 2**, a decrease of ~30% in measured concentration was observed after exposure to low pH, which is qualitatively similar to the SRID and ELISA results published by Hashem et al. [2013]. In their work, the SRID value decreased ~80% and the ELISA value decreased ~100% after 1 hour exposure to pH 5.

Figure 2 - pH Dependent Degradation



Summary: These preliminary studies provide strong evidence that VaxArray Influenza, as expected, is indeed a stability indicating immunoassay.

Cited References

Bodle J, Verity EE, Ong C, Vandenberg K, Shaw R, Barr IG, Rockman S. "Development of an Enzyme-Linked Immunoassay for the Quantitation of Influenza Haemagglutinin: an Alternative Method to Single Radial Immunodiffusion." (2013) *Influenza Other Respir Viruses*. 7(2), 191-200.

Fontana, J., Cardone, G., Heymann, J. B., Winkler, D.C., Steven, A.C. "Structural Changes in Influenza Virus at Low pH Characterized by Cryo-Electron Tomography" (2012) *J. Virology*, 86(6), 2919.

Gupta, R. "Form of HA Measured by SRID for Potency of Inactivated Influenza Vaccines and Alternate Methods to Measure this Form of HA"



(2012) CBER/FDA Presentation at PhRMA Annual Flu Meeting (available on-line).

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Hasija, M., Li, L., Rahman, N., Ausar, S.F. “Forced Degradation Studies: An Essential Tool for the Formulation Development of Vaccines” (2013) Vaccine: Development and Therapy 3, 11-3

