Stability Indication

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. Signal readout for this multiplexed immunoassay is based on fluorescence from conjugated polyclonal or monoclonal antibody labels.

Stability Indication

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 the VaxArray Influenza Assay is a high-information-content immunoassay, it is similarly expected to exhibit stability indicating behavior.

To explore the use of VaxArray 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.

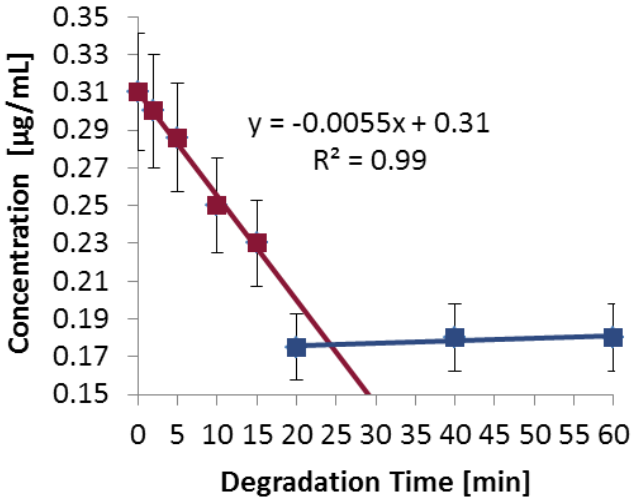

Figure 1 - Time Dependent Thermal Degradation

Protein concentration determined by VaxArray 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.

pH 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 the VaxArray Influenza Assay, 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.

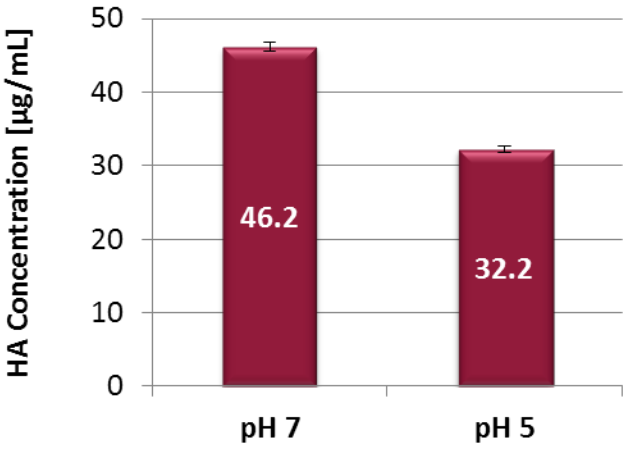

Figure 2 - pH Dependent Degradation

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

Summary

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

References

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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).

Hashem, A.M. and 12 others "A Novel Synthetic Receptor-Based Immunoassay for Influenza Vaccine Quantification" (2013) PLOS One, 8(2) e55428

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