Comparison with IDMS and Activity

VaxArray® Influenza Seasonal NA Potency Assay

Overview

The VaxArray Influenza Seasonal Neuraminidase Potency Assay ("VaxArray NA") is a new tool for confirming the presence of subtype-specific neuraminidase (NA) in flu vaccines.

VaxArray NA is a rapid and quantitative alternative to enzymatic assays. The assay is highly correlated with enzymatic activity, is stability indicating, is compatible with multivalent vaccines, and has been shown to serve as a proxy for immunogenicity in mice [Kuck et al., 2018]. Other advantages include excellent precision and reproducibility, large linear dynamic range for quantification, less sensitivity to environmental and matrix effects than enzymatic assays, compatibility with low-dose and adjuvanted vaccines, influenza NA specific reagents, high quality complete kits manufactured under a certified ISO 13485 quality management system, and 21CFR Part 11 compatible software for enhanced data integrity.

This technical note summarizes a few comparative studies that highlight the performance of the VaxArray NA assay for A/Singapore/GP1908/2015 (H1N1). Please refer to the paper by Kuck et al. [2018] for the results obtained for A/Hong Kong/4801/2014 (H3N2).

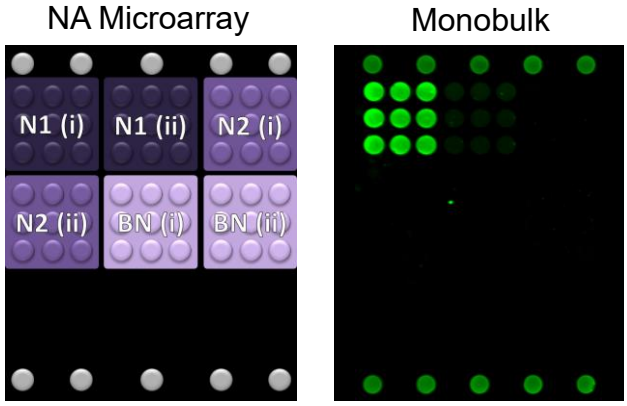

Figure 1 – Left panel is a schematic of the microarray layout. Right panel shows a representative fluorescence image of an A/H1N1 monobulk intermediate vaccine.

VaxArray NA Assay

The VaxArray NA immunoassay is based on capture of antigen by subtype-specific monoclonal antibodies printed in a microarray format. **Figure 1** includes a schematic of the antibody layout for the microarray. A fluor-conjugated antibody (polyclonal or monoclonal) is used to label the captured antigen for fluorescence detection. The assay can be completed in two hours. Figure 1 also includes a representative fluorescence image for an H1N1 A/Singapore/GP1908/2015 (IVR-180) monobulk vaccine intermediate.

InDevR, Inc.
2100 Central Ave, Suite 106, Boulder, CO 80301
www.indevr.com | 303.402.9100 | sales@indevr.com

Selective, Quantitative, and Stability Indicating: Comparison to IDMS

It is well-established in the literature that the total protein concentration for specific NA subtypes can be determined by isotopic dilution mass spectrometry (IDMS) [Williams et al., 2012]. As a means to demonstrate the subtype-specific quantitative and stability indication properties of VaxArray NA, a comparison to IDMS was conducted.

The total NA content for each of five dilutions of A/Singapore/GP1908/2015 (IVR-180) monobulk was determined by IDMS and by VaxArray following standard protocols [Williams et al., 2012].

The five samples ranged in expected concentration (defined with respect to HA content) from 80 µg/mL HA to 5 µg/mL of HA. To evaluate stability indication capabilities, the 80 µg/mL HA sample was also stressed at 56° C for 20 hours.

Figure 2 is a plot of the measured NA content by both methods. The data represented by blue circles are triplicate IDMS measured values. The purple circles represent quadruplicate VaxArray values from the N1(i) capture mAb. The green circles represent quadruplicate VaxArray values from the N1(ii) capture mAb. In all cases, the solid bars represent the average value.

Note that both methods yield similar values within error for all but the thermally stressed sample (designated H1 FD). As expected, the VaxArray measured NA content after thermal stress is significantly lower than the IDMS measured level. This observation is consistent

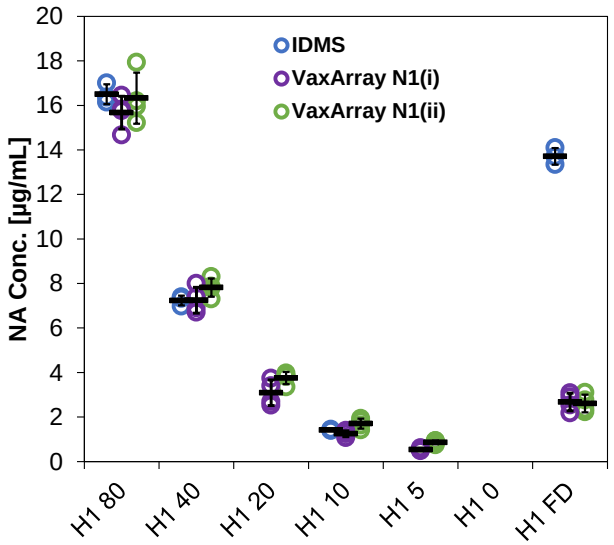

Figure 2 – Plot of NA N1 concentration as measured by VaxArray (two antibodies) and IDMS. The sample designations on the x-axis correspond to expect HA concentration in µg/mL. H1 0 corresponds to a negative control and H1 FD corresponds to the force degraded sample. There was no IDMS measurement for the H1 20 sample.

with VaxArray being stability indicting and IDMS being a total protein measurement.

It is also worth noting that the measured NA content is approximately a factor of 5 less than the expected HA concentration, consistent with reported ratios of HA to NA [Williams et al., 2012].

The final point to consider is that the limit of quantification of VaxArray is lower than it is for IDMS. For example, the lowest concentration sample contained ~1 µg/mL N1 NA, which was quantified by VaxArray and was below the quantification limit for IDMS.

InDevR, Inc.
2100 Central Ave, Suite 106, Boulder, CO 80301
www.indevr.com | 303.402.9100 | sales@indevr.com

Correlation with Enzymatic Activity

The same five samples were also analyzed by a MUNANA-like enzyme activity assay. **Figure 3** shows the correlation between VaxArray measured concentration and NA activity. The two measurements are highly correlated, with a Pearson's correlation coefficient of 1. The enzymatic activity of the thermally stressed sample was below the limit of detection for the activity assay; however VaxArray indicated the presence of ~2-3 µg/mL of intact NA, compared to the non-stressed NA concentration of ~16 µg/mL.

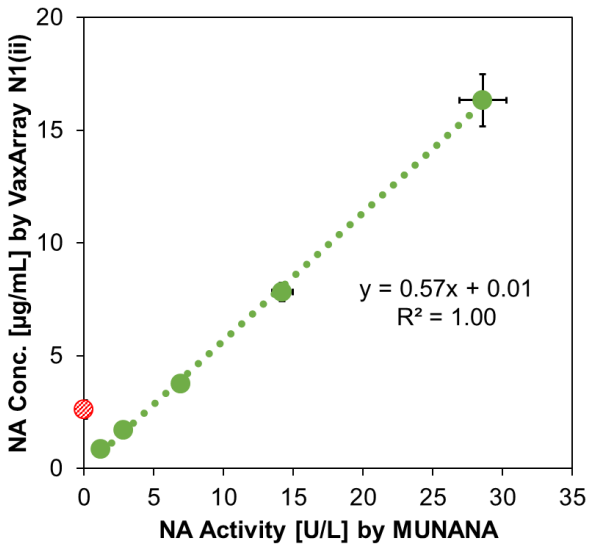

Figure 3 – Correlation plot of VaxArray N1 NA relative to NA activity. The error bars are ± one standard deviation from four replicate measurements. The data point represented by the red circle is the thermally stressed sample (designated H1 FD in Figure 2).

VaxArray NA as a Proxy for Immunogenicity

Kuck et al. [2018] recently demonstrated a clear and predictive relationship between the NA levels in an A/H3N2 vaccine (A/Hong Kong/4801/2014 (X-263B)) and neuraminidase inhibiting antibody levels induced in mice.

Summary

The VaxArray Influenza Seasonal NA potency assay exhibits excellent correlation with enzymatic activity and immunogenicity, good agreement with subtype-specific IDMS, and is stability indicating. The assay is also equally applicable to multivalent vaccines. With a number of advantages over enzymatic activity assays, VaxArray NA should serve as a more efficient means to confirm the presence of subtype-specific NA levels in both vaccine intermediates and in final multivalent formulations.

Acknowledgements

We thank Dr. Maryna Eichelberger and her team at CBER/FDA for the IDMS measurements.

The A/Singapore/GP1908/2015 (IVR-180) samples were graciously supplied by a vaccine producer (unnamed but much appreciated).

References

Kuck, LR, Byrne-Nash, RT, Gillis J, Bueter, K, Couzens, LK, Eichelberger, MC, Rowlen, KR. VaxArray for hemagglutinin and neuraminidase potency testing of influenza vaccines, Vaccine, Volume 36, Issue 21, 2018, Pages 2937-2945

Williams TL, Pirkle JL, Barr JR. Simultaneous quantification of hemagglutinin and neuraminidase of influenza virus using isotope dilution mass spectrometry. Vaccine, Volume 30, 2012, Pages 2475–2482

InDevR, Inc.
2100 Central Ave, Suite 106, Boulder, CO 80301
www.indevr.com | 303.402.9100 | sales@indevr.com