VaxArray® Influenza Seasonal Hemagglutinin Potency Assay: Accuracy and Precision

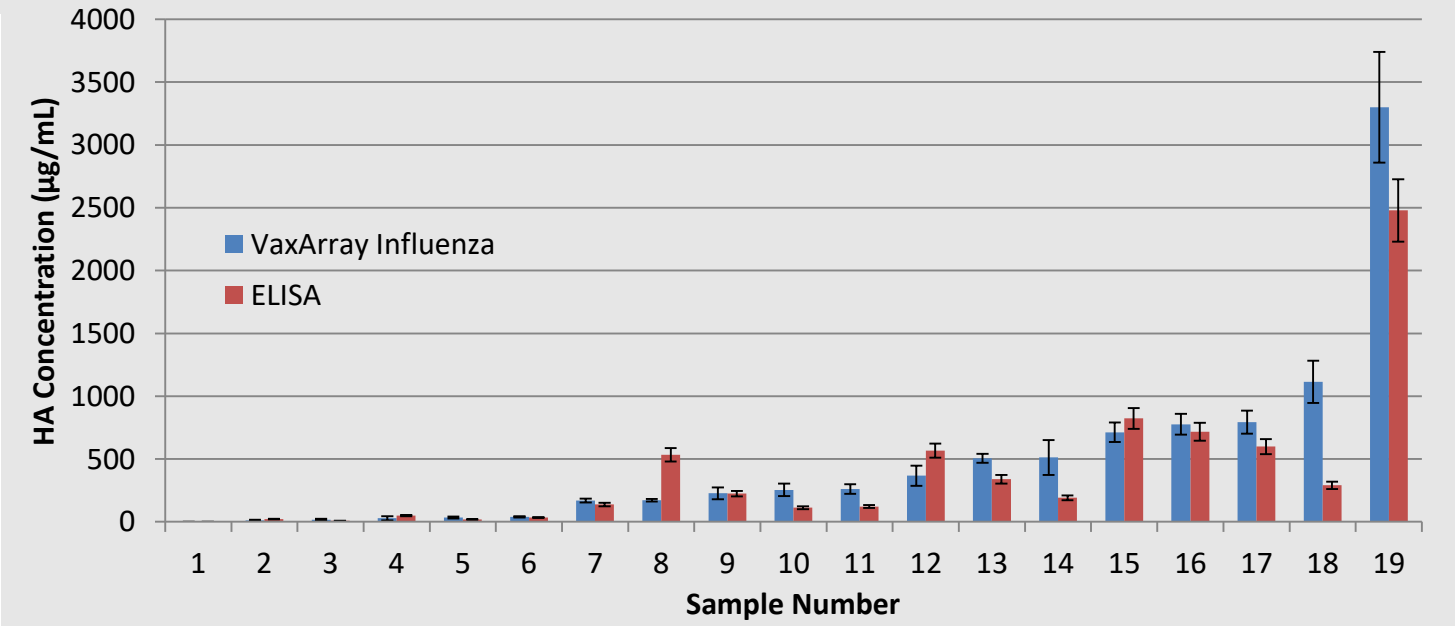
Background: The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin (HA) protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple antibodies against seasonal A/H1, A/H3, B/Yamagata-like and B/Victoria-like strains are printed in an array format on a glass substrate. As a multiplexed immunoassay, signal readout is based on fluorescence from a conjugated "universal" primary antibody label.

ELISA is currently one of the most widely used methods for estimating influenza HA content during early stages in vaccine manufacturing. However, ELISA has significant limitations, including delays due to reliance on reference antisera from CBER (or development of seasonal antibodies), and the requirement for in-house preparation of plates.

Objective: The objective of this Technical Note is to describe the accuracy and precision of the assay compared to ELISA-based quantification of HA.

Comparison of VaxArray Seasonal Hemagglutinin Potency Assay and ELISA: Blinded VaxArray Influenza studies were conducted on 19 recombinant HA (rHA) samples that had previously been quantified by ELISA (CBER reference antisera were used as the capture agents for ELISA). **Figure 1** shows plots of HA content as determined by VaxArray Influenza vs. ELISA for a wide range of sample types from crude extract to bulk drug substance. The measurement error for VaxArray

Figure 1 - HA Concentration as Determined by ELISA and VaxArray Influenza



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

Influenza is included and ± 10% relative error is assumed for ELISA. Nineteen samples were tested and are represented in the plot. Sample #1 was below the quantification limit for ELISA (2.5 µg/mL) and thus measured only by VaxArray Influenza.

A linear plot of VaxArray Influenza versus ELISA results in a slope of 1.2, with a Person's correlation coefficient (R) of 0.94. In this case, the slope implies that VaxArray Influenza typically yields a ~20% higher value for HA content.

As a more rigorous test to determine whether or not a linear relationship exists between the two techniques, the log of each value in both sets of data was taken and the results plotted against each other. In log space, a linear relationship (neglecting the intercept) should yield a slope of 1. That is indeed the case for log (VaxArray Influenza) values versus log (ELISA), where a linear regression yields a slope of 1.03 and an R value of 0.91. Thus, one may conclude that there is a linear relationship between HA content determined by VaxArray Influenza and ELISA.

Reproducibility: In order to evaluate reproducibility, replicate VaxArray Influenza measurements were made on recombinant B rHA. Studies were conducted on two separate days, with two different lots of arrays used on the second day. As shown in **Figure 2**, replicate serial dilutions of antigen quantified using capture antibody I (B/Victoria-like) yield a mean slope of 33 ± 3. Thus, the relative error in replicate serial dilution sets is ~10%, which is excellent.

Summary: VaxArray Influenza provides equivalent or improved results relative to ELISA and offers significant advantages. Specifically, VaxArray Influenza is a "turn-key" kit which eliminates the need to wait on development of seasonal reagents. In addition, VaxArray Influenza eliminates the need for in-house preparation of plates or gels.

Figure 2 - Assay Reproducibility and Precision with Capture mAb I