

Accuracy and Precision

VaxArray® Imaging and Analysis System

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. As a multiplexed immunoassay, signal readout is based on fluorescence from conjugated polyclonal or monoclonal antibody labels.

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.

Comparison to ELISA

Blinded VaxArray Influenza Assay 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 the VaxArray Influenza Assay vs. ELISA for a wide range of sample types from crude extract to bulk drug substance. The measurement error for the VaxArray Influenza Assay is included and $\pm 10\%$ relative error is assumed for ELISA. Nineteen

Figure 1 - HA Concentration by ELISA and VaxArray

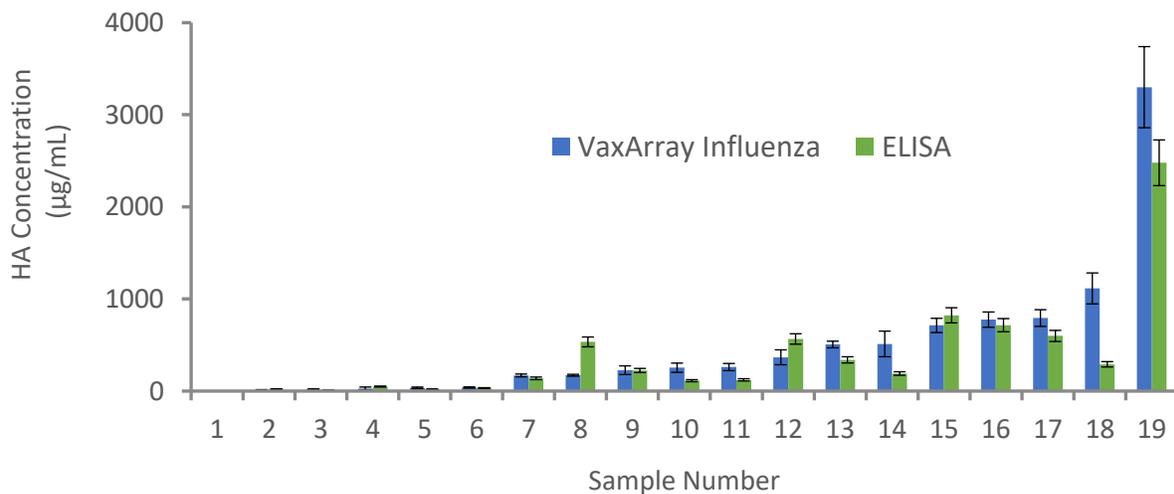
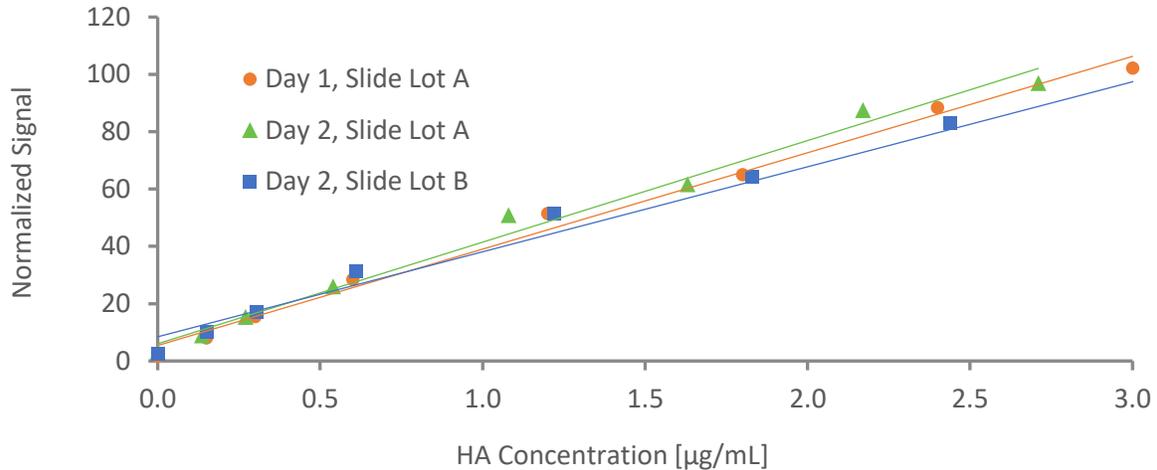


Figure 2 - Assay Reproducibility and Precision



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.

A linear plot of VaxArray results versus ELISA results in a slope of 1.2, with a Pearson's correlation coefficient (R) of 0.94. In this case, the slope implies that the VaxArray Influenza Assay 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 results) versus log (ELISA results), 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 and ELISA.

Reproducibility

In order to evaluate reproducibility, replicate VaxArray 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%.

Summary

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