VaxArray® Influenza Seasonal Hemagglutinin Potency Assay: Protein Quantification in Crude Samples

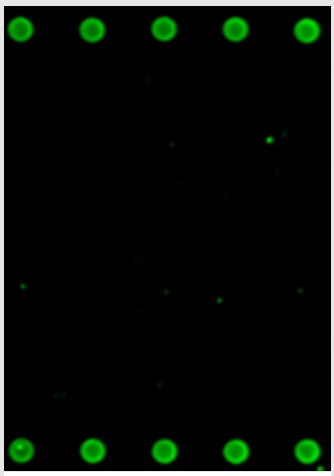
Background: The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin (HA) protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple antibodies against seasonal A/H1, A/H3, B/Yamagata-like and B/Victoria-like strains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

During vaccine development and production it is important to track both virus and protein yields at each step in the process. In this work, we demonstrated that influenza HA can be detected and quantified even in the crudest samples, such as allantoic fluid.

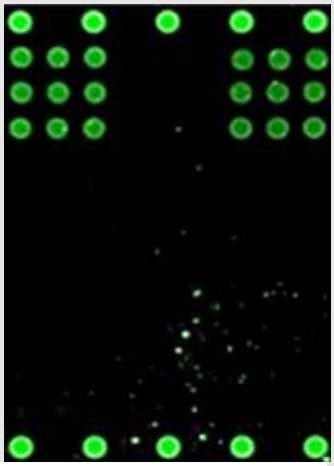
Objective: The objective of this Application Note is to demonstrate the use of VaxArray Influenza for quantifying influenza HA protein in crude samples, such as extract from cell or egg culture.

Cell Culture: Preliminary studies were conducted in order to determine whether VaxArray Influenza can successfully be employed at all stages of the manufacturing process. Potentially the toughest samples would include crude extracts from cell culture where the antigen concentration is low and "contaminant" levels are high. With samples provided by a collaborator, VaxArray Influenza was used to quantify HA in samples of varying purity. **Figure 1** demonstrates specific detection of A/CA/07/2009 in crude cell culture supernatant. As shown, the results were clearly positive on two of the capture antibodies (positions A and C) and

Figure 1 – Representative Images for Detection of HA in Crude Cell Culture Supernatant



Cell Culture Supernatant Negative Control



Cell Culture Supernatant with A/CA/07/2009

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

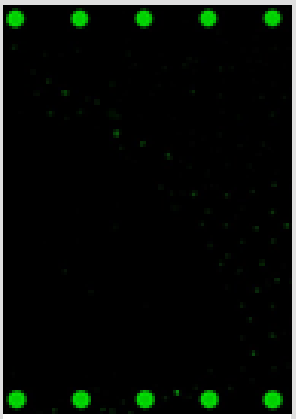
there was no significant background or cross-reactivity evident. For ELISA, the limit of quantification may be elevated when using such crude extract due to non-specific binding on the reference anti-sera used as a capture agent on the assay. Total protein methods such as BCA are not particularly useful for this type of sample due to the high levels of non-target proteins.

Allantoic Fluid: VaxArray Influenza was also tested with allantoic fluid as another challenging crude matrix. Qualitative results are shown in **Figure 2** for allantoic samples including natively produced H1, purified H1 spiked into a negative control (mock infected) and a mock infected negative control. All samples were tested with Zwittergent 3-14 present at 1%. In all cases, VaxArray Influenza resulted in sensitive detection of the protein above a negligible background. Quantification yielded the expected protein content.

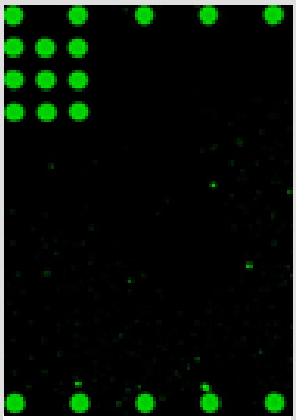
Summary: Both allantoic fluid and cell culture supernatants present analytical challenges for specific protein quantification due to the abundance of other proteins that are present in addition to the hemagglutinin protein of interest.

The representative studies described briefly here indicate that VaxArray Influenza is an exceedingly good choice for analyzing HA content even in the most challenging matrices. Thus, VaxArray Influenza could open up new opportunities for tracking protein content throughout the manufacturing process.

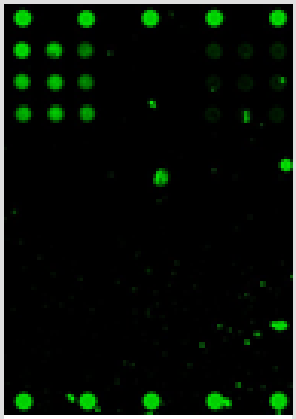
Figure 2 – Representative Images for Detection of HA in Allantoic Fluid



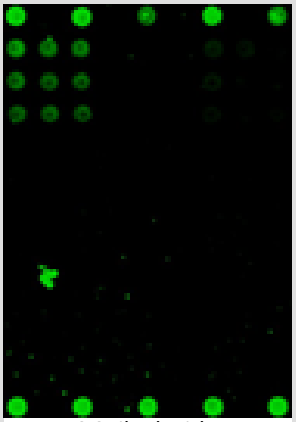
Mock Infected Allantoic Fluid (Negative Control)



Allantoic Fluid infected with H1 Subtype



Mock Infected Allantoic Fluid Spiked with H1 Standard



PBS Spiked with H1 Standard