

Crude (In-Process) Samples

VaxArray® Influenza Potency Assays



Overview

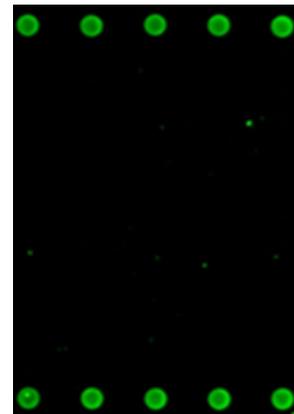
The VaxArray Seasonal Hemagglutinin Potency Assay is a new tool for hemagglutinin (HA) protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple antibodies against seasonal A/H1, A/H3, B/Yamagata-like and B/Victoria-like strains are printed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

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.

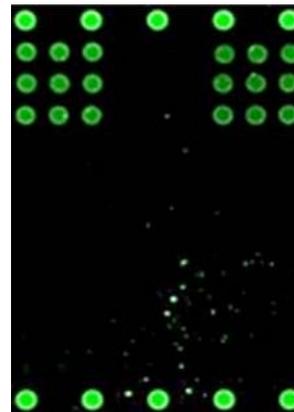
Cell Culture

Preliminary studies were conducted in order to determine whether the VaxArray Influenza Assay can successfully be employed at all stages of the manufacturing process. Potentially challenging 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, the VaxArray Influenza Assay 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

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

positive on two of the capture antibodies (positions A and C) and there was no significant background or cross-reactivity present. Using an ELISA, the limit of quantification may be elevated when using 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

The VaxArray Influenza Assay 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, the VaxArray Influenza Assay resulted in sensitive detection of the protein above a negligible background. Quantification yielded results consistent with 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, however, the representative studies described here indicate the VaxArray Influenza Assay is a good choice for analyzing HA content even in the most challenging matrices. The VaxArray Influenza Assay could open up new opportunities for tracking protein content throughout vaccine manufacturing processes.

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

