VaxArray Influenza Seasonal NA Potency Assay Crude and In-Process Samples

Overview

The VaxArray Influenza Seasonal Neuraminidase Potency Assay is a new tool for Neuraminidase (NA) protein quantification based on a panel of subtype-specific but broadly reactive monoclonal antibodies (mAbs). Multiple antibodies against seasonal A/N1, A/N2, and B-NA are printed in an array format on a glass substrate. Signal for this multiplexed immunoassay is fluorescence from conjugated 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 NA 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 Seasonal Neuraminidase Potency 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 CBER, the VaxArray Influenza Seasonal Neuraminidase Assay was used to quantify NA in samples of varying purity. Figure 1 demonstrates specific quantitative analysis of a trivalent sample spiked into crude cell culture supernatant. As shown, the presence of cell culture supernatant did not affect quantification of any sample in the mixture as all samples quantified within 20% of the expected NA concentration. In contrast, when 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.

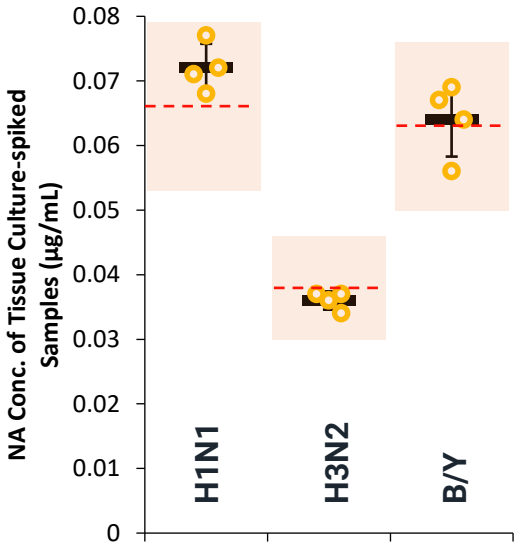

Figure 1 – Quantitative analysis of samples spiked into cell culture supernatant. Orange circles are individual replicates (n=4). Black line is average result. Red dashed line is the expected NA concentration. Orange box is the 20%-of-expected interval.

Allantoic Fluid

The VaxArray Influenza Seasonal Neuraminidase Assay was also tested with allantoic fluid as another challenging crude matrix. Quantitative results are shown in Figure 2 for a trivalent sample spiked into mock-infected allantoic fluid. For all NA proteins in the mixture, the VaxArray Influenza Seasonal Neuraminidase Assay resulted in sensitive detection of the protein above a negligible background. Quantification yielded results consistent with the expected protein content.

Sucrose

A trivalent sample spiked into 40% sucrose was evaluated with the VaxArray Influenza Seasonal Neurminidase Potency Assay to evaluate the potential for use after sucrose-gradient centrifugation. Figure 3 demonstrates that 40% sucrose does not affect quantification of NA with the VaxArray assay as all components were consistent with expected protein content.

Summary

Allantoic fluid, cell culture supernatants, and sucrose present analytical challenges for specific protein quantification due to the abundance of other proteins and interfering substances that are present in addition to the protein of interest. However, the representative studies described here indicate the VaxArray Influenza Seasonal Neuraminidase Assay is a good choice for analyzing NA content even in the most challenging matrices. The VaxArray Influenza Seasonal Neuraminidase Assay could open up new opportunities for tracking protein content throughout vaccine manufacturing processes.

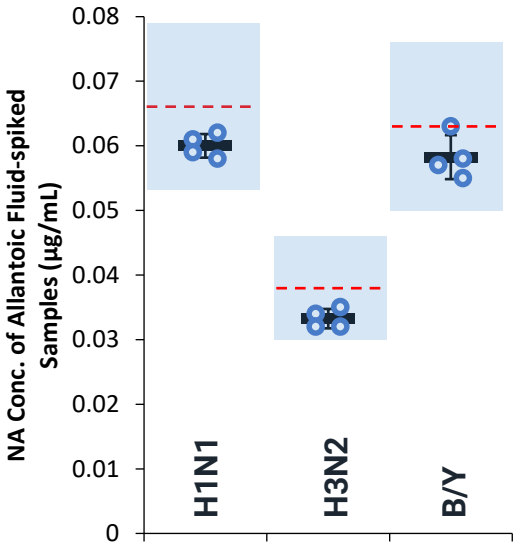

Figure 2 – Quantitative analysis of samples spiked into allantoic fluid. Blue box is the 20%-of-expected interval.

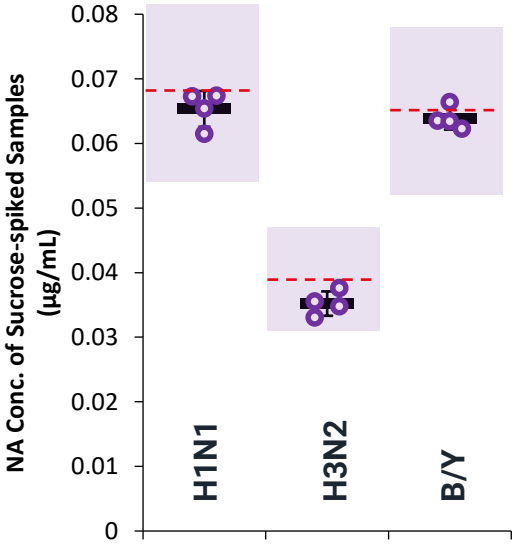

Figure 3 – Quantitative analysis of samples spiked into 40% sucrose. Purple box is the 20%-of-expected interval.

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