Quantification of Neuraminidase in Vaccines

Overview

The VaxArray Influenza Seasonal Neuraminidase Potency Assay is a new tool for confirming the presence of subtype-specific neuraminidase (NA) in flu vaccines derived from whole viruses. While the exact levels of NA in flu vaccines are not currently regulated, the presence of immunogenic forms of NA must be confirmed during vaccine production using a “suitable enzymatic or immunological” method [Ph. Eur. Monograph 0869; WHO 927, 2005]. For flu vaccines made from inactivated whole virus it is also necessary to verify that the inactivation method does not significantly alter the immunogenic properties of NA.

Currently, the confirmation of NA presence in flu vaccines is most often accomplished by measuring the enzymatic activity of NA. One of the most popular NA activity assays is based on the generation of a fluorometric product from sodium 4-methylumbelliferyl-α-D-N-acetylneuraminic acid (4-MUNANA). However, enzymatic assays are not ideal for a number of reasons, including the lack of harmonized influenza neuraminidase standards by which to compare relative activity, the effects of buffer composition and temperature on activity, and the inability to distinguish between different subtypes in multivalent vaccines.

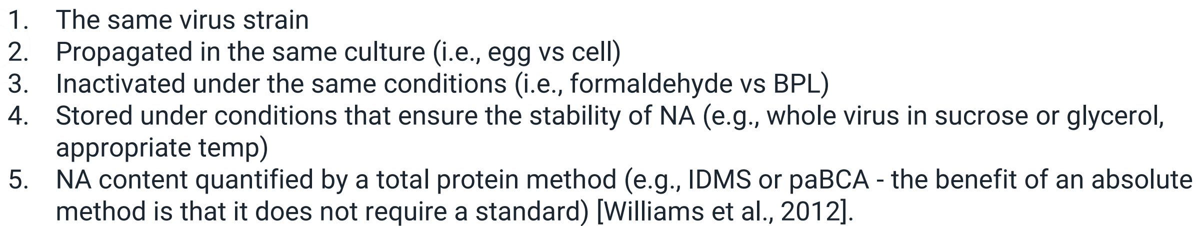
The VaxArray NA assay is a rapid and quantitative alternative to enzymatic assays. The assay is highly correlated with enzymatic activity, is stability indicating, and has been shown to serve as a proxy for immunogenicity in mice [Kuck et al., 2018]. Other advantages include:

1. influenza NA specific reagents
2. subtype specific reagents
3. excellent precision and reproducibility
4. large linear dynamic range for quantification
5. less sensitivity to environmental and matrix effects than enzymatic assays
6. compatibility with low-dose and adjuvanted vaccines
7. complete kits manufactured under a certified ISO 13485 quality management system
8. 21CFR Part 11 compatible software for enhanced data integrity.

Quantification

The characteristics of a “suitable enzymatic or immunological” method to confirm the presence of NA must include specificity for NA, a quantitative relationship between NA concentration and signal response, and a sensitivity to conformationally intact NA. Ideally, the method would be equally applicable to both monovalent and multivalent vaccines.

In order to quantify NA in terms of protein concentration (i.e., µg/mL) it is essential to have a matched standard with known NA concentration. The characteristics of an ideal matched standard for NA in a vaccine derived from a whole virus are:



1. The same virus strain
2. Propagated in the same culture (i.e., egg vs cell)
3. Inactivated under the same conditions (i.e., formaldehyde vs BPL)
4. Stored under conditions that ensure the stability of NA (e.g., whole virus in sucrose or glycerol, appropriate temp)
5. NA content quantified by a total protein method (e.g., IDMS or paBCA - the benefit of an absolute method is that it does not require a standard) [Williams et al., 2012].

In other words, the ideal calibration reagent for NA would likely be an internal standard generated under the same conditions as a prospective “unknown” but characterized by an absolute method early in the vaccine production process. Additional potential calibrants, including recombinant proteins and ERL-released reference antigens, are discussed below.

Calibration with Recombinant Proteins

Recombinant proteins work well in the VaxArray Seasonal NA assay and are attractive as potential standards because they can be produced and purified at relatively high concentrations and do not require chemical inactivation. Because the purity can be >95%, quantification by absorbance (an absolute method) is straightforward.

Unfortunately, for many immunoassays including VaxArray, recombinant proteins generally do not serve well as standards for HA and NA derived from whole virus. This is likely due to binding constant differences that arise from chemical modification during inactivation as well as differences in glycosylation patterns. The structural form of the NA (monomer versus tetramer) may also be different for split whole viruses and recombinant proteins.

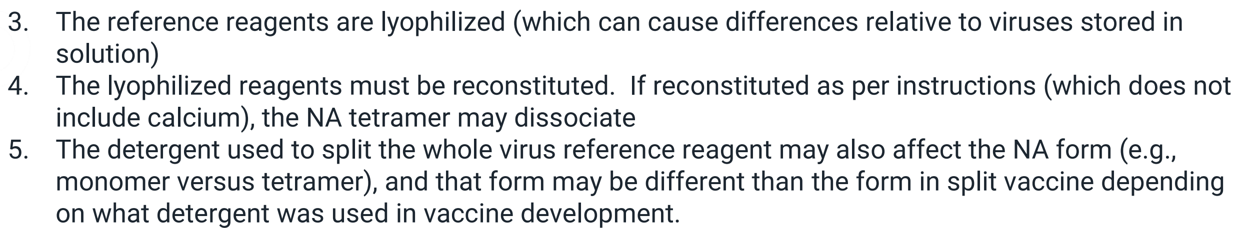
Calibration with ERL-Released Reference Antigens

Since the perfect calibration standard is difficult to obtain for vaccines derived from whole virus, we investigated the use of the reference antigens released by the Essential Regulatory Laboratories (ERLs). These antigens are developed and calibrated specifically for analysis of HA in the single radial immunodiffusion assay. We postulated that they may also be useful for NA.

To test this approach, we have collaborated with scientists at the CDC and FDA for determination of the total NA content of those reference reagents by Isotopic Dilution Mass Spectrometry (IDMS). We have also used an absorbance-based purity adjusted total protein assay to determine the total NA content.

Based on testing conducted to date, we have observed some cases where an ERL reference antigen serves as a reasonable standard for split vaccine in the VaxArray assay. However, we have also observed cases where the reference antigen does not serve well. At this point, there is no clear explanation for the observed differences, but we note the following non-idealities:

1. The ERL reference reagents may be inactivated differently than the whole viruses that will be split prior to vaccine formulation
2. The reference reagents are not purified and stabilized in the same way as vaccine formulations



3. The reference reagents are lyophilized (which can cause differences relative to viruses stored in solution)
4. The lyophilized reagents must be reconstituted. If reconstituted as per instructions (which does not include calcium), the NA tetramer may dissociate
5. The detergent used to split the whole virus reference reagent may also affect the NA form (e.g., monomer versus tetramer), and that form may be different than the form in split vaccine depending on what detergent was used in vaccine development.

In addition to the limitations outlined above, Webby and co-workers have reported that a single amino acid change in the stalk domain (at 66Y for N1 in H1N1 viruses) could dramatically affect tetramer stability and enzymatic activity [Zanin et al., 2016] – so defining an absolute standard is clearly going to be a challenge.

Calibration Based on Enzymatic Activity

One approach for immediate implementation of the VaxArray NA assay for confirming the presence and stability of NA relies on calibration of an internal standard by enzymatic activity. The concept is outlined as follows:

1. Early in vaccine production an appropriate standard is selected (e.g., seed virus or monobulk intermediate) for each NA subtype
2. The standard is calibrated by an enzymatic assay (i.e., is assigned a units/mL activity value)
3. That standard is then used as a calibrant for all VaxArray NA measurements throughout the remainder of the manufacturing campaign (the activity of the standard can be checked from time to time)

Disadvantages of this approach include a relative concentration expressed as VaxArray-equivalent activity units rather than in µg/mL and the fact that the buffer of the standard must be matched to the buffer of the sample to be quantified. However, one of the distinct advantages of this approach is that the calibrant directly bridges a long-established method to this new more robust method, which may facilitate regulatory acceptance.

Relative Potency

An additional approach for immediate implementation of the VaxArray NA assay is to track relative NA levels throughout the vaccine manufacturing process. This process could be performed by:

1. Determining the VaxArray signal of an early vaccine production intermediate
2. Using this early intermediate as a ‘standard’ in the VaxArray NA assay and comparing all downstream vaccine intermediates in terms of percentage of the early intermediate

This method would allow the manufacturer to track enrichment and/or loss of NA throughout the purification process. The disadvantage of this approach is that the result is a relative potency value. Advantages of this approach include the highest flexibility and ease of use for tracking NA throughout the production campaign. Since the VaxArray NA assay is relatively unaffected by buffer/matrix composition, users would not have to be concerned about matching the standard matrix to the sample matrix.

Summary

The ultimate solution for appropriate NA calibration standards remains to be defined. Addressing the issue will likely require a group of scientists working across a broad spectrum of agencies – groups such as NAction! [Krammer et al., 2018]. In the meantime, relative measurements for NA may represent a first step toward tracking NA potency throughout the manufacturing process.

References

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