VaxArray Assessment of Influenza Vaccine Potency and Stability

Rose Byrne-Nash, Ph.D. | Laura Kuck, Ph.D. | Kathy Rowlen, Ph.D.
InDevR, Inc. | 2100 Central Avenue, Suite 106 | Boulder, CO 80301 | Ph: 303.402.9100 | www.indevr.com | E-mail: info@indevr.com

Abstract

Background: There is an on-going effort within the influenza vaccine industry to identify and validate an alternative to the single-radial immunodiffusion assay (SRID) for potency and stability assessment. The VaxArray Seasonal Influenza (VSI) potency assay was developed under the Influenza Vaccine Manufacturing Initiative and performance has been evaluated against key regulatory requirements such as accuracy, precision, and ability to track vaccine stability. This report focuses on a summary of results for a diverse set of flu vaccines tested in collaborative studies.

Methods: VSI is based on a panel of monoclonal antibodies printed in a microarray format. A simple sandwich assay is used for simultaneous quantification of all hemagglutinin components within mono- and multi-valent influenza vaccines, including differentiation between the two influenza B lineages. A standard protocol was used to evaluate the potency of mono-valent and multi-valent influenza vaccines produced in eggs and cell-culture, including recombinant and virus-like particle vaccines. Accuracy was defined with respect to SRID and (or) purity-reflected total protein content. Precision was also evaluated as a function of time under accelerated stress conditions.

Conclusions: VSI was found to exhibit high precision (CV < 15%) and good correlation (R > 0.85) with SRID for most vaccines, and if calibrated appropriately, excellent agreement with SRID values (100 ± 5%). In thermal stability testing all strains in multi-valent vaccines exhibited a decrease in potency over time. While the absolute measured potency for degraded vaccines was not always in agreement with the SRID value, the general trends were consistent with those observed for SRID. These studies indicate that VSI is a reliable and robust alternative potency assay.

Background

- Potency assays measure the concentration of functional hemagglutinin (HA), which is an influenza virus surface protein. HA has been established as the key component of whole virus vaccines and the dominant target of protective antibodies following vaccination or infection.
- Currently, the gold standard for influenza vaccine potency is the single-radial immunodiffusion assay (SRID) which has inherent disadvantages including labor-intensive protocols and the requirement for reference reagents that do not necessarily accurately represent the composition of vaccines.
- VaxArray Influenza (VSI) is a rapid alternative potency assay. A comparison between SRID and VSI is demonstrated in Figure 1.

Results

- The automated image analysis associated with the VaxArray platform determines the linear range of the standard curve (Figure 2). Samples of unknown potency are evaluated and concentration is calculated using the appropriate standard curve.
- Calibration with reference antigens (monovalent or B/Victoria) for each component allows for easy integration into existing protocols.
- Assay robustness to older strains is demonstrated in Figure 3.

The Future of the VaxArray Platform

- Rapid Quantification of FluNArray
  - The VaxArray platform is highly versatile and can be utilized to screen for a number of different influenza antigens.
  - The array analysis software determines linear ranges for each influenza type and subtype.

Application to Pandemic Influenza Vaccines

- VSI is in concert with the WHO and US government, a number of vaccine producers prepare pandemic vaccines against globally circulating virus strains.
- VSI can be used throughout the vaccine production process to monitor HA production and potency.
- VSI can be used in bridging to single radial immunodiffusion (SRID) assay, making it a valuable tool for vaccine producers, especially during a pandemic.
- With constant monitoring of potential pandemic influenza, VSI assays will be adapted to be ready for the next pandemic influenza outbreak.

Other Advances

- VSI is being validated as a multiplexed sandwich immunoassay that utilizes a microarray slide printed with broadly reactive yet subtype-specific antibodies.
- Neuraminidase (NA), the second most abundant influenza surface glycoprotein, is gaining support for inclusion in pandemic influenza vaccines.
- VSI can be used in existing protocols, as a pre-screening tool.
- VSI can be used in a real-time setting to monitor vaccine potency.

Characteristics for an Improved Potency Assay for Inactivated Influenza Vaccines

<table>
<thead>
<tr>
<th>WHO Expert Committee on Biological Standardization</th>
<th>Geneva Oct 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biologically relevant potency assay</td>
<td>Applicability to existing cell lines</td>
</tr>
<tr>
<td>Correlation with clinical efficacy</td>
<td>Applicability to different strains</td>
</tr>
<tr>
<td>Stability indicating assay</td>
<td>Measure of exposure</td>
</tr>
<tr>
<td>Independent of strain-specific factors</td>
<td>Reduced amount of reagents required for assay</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Equivalence of vaccine potency</td>
</tr>
<tr>
<td>Efficacy and safety profile</td>
<td>Efficient regulatory review</td>
</tr>
<tr>
<td>Applications worldwide</td>
<td>Accomplishing high dose release</td>
</tr>
</tbody>
</table>

Acknowledgements

We gratefully acknowledge funding from the National Institute for Allergy and Infectious Disease (NIAID R44 AI023181).

FIGURES

- Figure 1: VSI vs SRID
- Figure 2: Method Overview – Biochemistry
- Figure 3: Method Overview – Analysis
- Figure 4: Linear Range Determination
- Figure 5: Precision and Accuracy as Compared to SRID
- Figure 6: Stability Indication
- Figure 7: In-Process Analysis
- Figure 8: Robust Response to Older Strains