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VaxArray for hemagglutinin and neuraminidase potency testing of influenza vaccines

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ABSTRACT

Practical methods to measure the potency of influenza vaccines are needed as alternatives for the standard single radial immunodiffusion (SRID) assay. VaxArray assays for influenza hemagglutinin (HA) and neuraminidase (NA) have been developed to address this need. In this report, we evaluate the use of these assays to assess the potency of HA and NA of an A/H3N2 subunit vaccine by determining the correlation between the amounts measured by VaxArray and the immunogenicity in mice. The antibody response after one and two doses of five formulations of the vaccine ranging from 5 μg/mL to 80 μg/mL of HA, was measured by hemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) assays. For hemagglutinin, vaccine potency determined by VaxArray was equivalent to potency measured SRID and these amounts were predictive of immunogenicity, with excellent correlation between potency measured by VaxArray and the HAI geometric mean titers (GMT). Likewise, the amount of NA measured by VaxArray was predictive of the NAI GMT. The VaxArray NA assay reported non-detectable levels of intact NA for a sample that had been heat degraded at 56 °C for 20 h, demonstrating that the assay measures the native, active form of NA. Similarly, the HA potency measured by VaxArray in this heat-treated sample was very low when a monoclonal antibody was used to detect the amount of antigen bound. Importantly, the force degraded sample induced low HAI titers and the NAI titers were not measurable, supporting the conclusion that the VaxArray HA and NA assays measure the immunogenic forms of these A/H3N2 antigens. This study indicates that VaxArray assays can be used to assess the potency of HA and NA components in influenza vaccines as a proxy for immunogenicity.

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1. Introduction

As highlighted in recent publications, the single radial immunodiffusion (SRID) assay has served the influenza vaccine industry well for forty years [1–3]. However, these sources also point out a number of limitations for SRID including the lengthy time required to develop reference reagents, inadequate sensitivity for dose-sparing vaccines, and its unsuitability for new influenza vaccines based on emerging platform technologies such as cell culture generated recombinant proteins or virus like particles produced in cell culture or plants [1–7]. Furthermore, there is a strong motivation in both public health and the flu vaccine industry to improve the efficacy of flu vaccines. One aspect of that drive is an effort to deepen our understanding of the role played by other viral proteins in the vaccine, such as neuraminidase (NA), nucleoprotein (NP), and the matrix protein [8–12]. For example, recently a new focus group (NAction!) was created to promote research on the role of NA plays in flu vaccine efficacy [13]. Current regulations for lot release testing of influenza vaccines specify that the presence of NA must be confirmed, but there is no requirement for quantification and the levels of NA can vary significantly from one season to another [13–15].

To address the limitations of SRID and to enable rapid, quantitative assessment of neuraminidase in flu vaccines, we developed and previously reported on the VaxArray vaccine potency testing platform [16,17]. The system is based on a multiplexed immunoassay printed in a microarray format. For the "seasonal hemagglutinin" microarray, each array contains subtype specific monoclonal antibodies against all of the hemagglutinin (HA) antigens within quadrivalent seasonal vaccines [16]. Previous studies demonstrated good accuracy, precision, applicability to dose-sparing vaccines due to excellent sensitivity, and applicability to

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Table 1
Standards and samples included in the study.

Reference antigen	Lot # or ID	Source	[HA] by SRID (μg/mL)	[HA] by paBCA (μg/mL)	[NA] by IDMS (μg/mL)
Standards					
A/Hong Kong/4801/2014 (X-263B)	2016/109B	TGA	112	–	10 ± 1
A/Hong Kong/4801/2014 (X-263B)	Monovalent Bulk	Mfg.	341 ± 26	355 ± 35	37 ± 1

Sample ID	Type	Virus strain	Expected HA (μg/mL)
Samples analyzed			
H3 80	Monovalent Vaccine	A/Hong Kong/4801/2014 (X-263B)	80
H3 40	Monovalent Vaccine	A/Hong Kong/4801/2014 (X-263B)	40
H3 20	Monovalent Vaccine	A/Hong Kong/4801/2014 (X-263B)	20
H3 10	Monovalent Vaccine	A/Hong Kong/4801/2014 (X-263B)	10
H3 5	Monovalent Vaccine	A/Hong Kong/4801/2014 (X-263B)	5
H3 0	Negative Control		0
H3 FD	Degraded 80 μg/mL	A/Hong Kong/4801/2014 (X-263B)	17 ± 2 (by SRID)

SRID (single radial immunodiffusion assay); paBCA (purity adjusted bicinchoninic acid); IDMS (isotopic dilution mass spectrometry).

in-process samples as well as adjuvanted vaccines [17]. Although the results have not been published, the VaxArray platform performed well, in the large comparative studies sponsored by the U.S. Department of Health and Human Services (HHS), the National Institute for Biological Standards and Controls (NIBSC), and the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) [1].

The objectives of the study described herein were twofold: (i) to establish the relationship between HA measured by VaxArray and its immunogenicity, and (ii) to evaluate the performance of a new VaxArray assay for NA as a predictor of immunogenicity. The immunogenicity induced by HA was determined via the hemagglutination inhibition (HAI) assay and the immunogenicity induced by NA was determined by a neuraminidase inhibition (NAI) assay [18]. Neuraminidase inhibition titers have been shown to be well correlated with vaccine protection [10].

2. Materials and methods

2.1. Standards and samples

The standards and samples included in this study are listed in Table 1. Given the initiative to enable potency testing prior to the availability of reference reagents, a monovalent bulk (MB) intermediate was evaluated as a possible internal standard by comparing its response to that of a matched reference antigen distributed by the Therapeutics Goods Administration (TGA) in Australia. To characterize the MB, the matched TGA reference antigen was used in conjunction with the appropriate reference antisera to determine the potency of the MB by SRID. The MB was also characterized by a physiochemical method, as described below. Note that for simplicity the term "vaccine" is used to describe each of the concentrations of the monovalent A/H3N2 preparations that were made by sterile serial dilution of a known concentration of the MB. The force degraded sample was prepared from the 80 μg/mL vaccine. Specifically, samples were heated in a water bath for 20 h (T20) while a control was retained at 4 °C (T0). The water temperature was continuously monitored and was 55–56 °C during the entire degradation time period. After degradation, the vials were briefly cooled on ice and then stored at 4 °C until analysis later that day. Each vial was re-weighed before analysis to check for possible evaporation during degradation. All weights showed <0.07% difference after degradation.

2.2. Potency by SRID

The SRID assay was performed as described previously [19] with minor modifications. Specifically, each vaccine preparation was analyzed in 6 replicates randomly dispersed across 3 gels (two replicates of each vaccine preparation per gel). Two replicates of the standard curve were also analyzed on each gel, for a total of 6 replicates of each standard. Fig. 1 shows an example SRID gel after processing and staining.

2.3. Purity adjusted total protein (paBCA)

For the vaccine monobulk, 20 μL were denatured at 95° with 0.35% SDS and 55 mM DTT before being deglycosylated in the presence of PNGase F (V4831, Promega) overnight at 37 °C. The XCell *SureLock* gel box and NuPAGE™ pre-cast 4–12% bis-tris gradient gels (ThermoFisher NP0322) were used to evaluate the deglycosy-

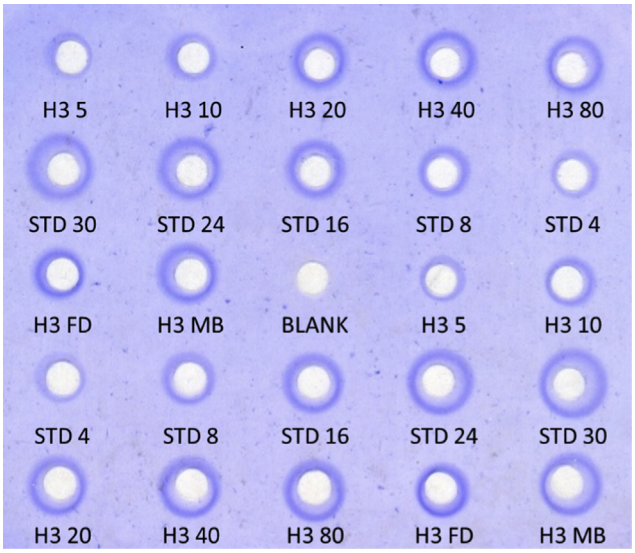

Fig. 1. A representative image of a SRID gel. A 5-point standard curve of reference antigen was analyzed in duplicate on each gel. Each sample, including the monovalent bulk (H3 MB), was lysed with 1% Zwittergent and diluted such that the expected HA content fell within the standard curve and was analyzed in duplicate. The values at the end of each label describe the expected HA concentration of the sample/standard. H3 FD is the forced degraded vaccine sample.

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