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VaxArray assessment of influenza split vaccine potency and stability

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ABSTRACT

Vaccine manufacturers require more rapid and accurate tools to characterize the potency and stability of their products. Currently, the gold standard for influenza vaccine potency is the single radial immunodiffusion (SRD) assay, which has inherent disadvantages. The primary objective of this study was to investigate the ability of the VaxArray Influenza (VXI) seasonal hemagglutinin (sHA) potency assay to accurately quantify potency and stability in finished vaccines as well as to quantify hemagglutinin protein (HA) within crude in-process samples. Monobulk intermediates and mono- and multivalent vaccines were tested using VXI. Quantification of HA in crude samples was evaluated by spiking known concentrations of HA into allantoic fluid. VXI generated SRD equivalent potency measurements with high accuracy (within ±10%) and precision (CV 10 ± 4%) for antigen components of monobulk intermediates and multivalent split vaccines. For these vaccines and vaccine intermediates, the VXI linear dynamic range was ~0.01–0.6 μg/mL, which is 12× greater than the linear range of SRD. The measured sample limit of detection (LOD) for VXI varied from 0.005 to 0.01 μg/mL for the different subtypes, which in general is ≥600× lower than the LOD for SRD. VXI was able to quantify HA in crude samples where HA only accounts for 0.02% of the total protein content. Stability indication was investigated by tracking measured potency as a function of time at elevated temperature by both SRD and VXI. After 20 h at 56 °C, the ratio of VXI to SRD measured potency in a quadrivalent vaccine was 76%, 125%, 60%, and 98% for H1/California, H3/Switzerland, B/Phuket and B/Brisbane, respectively. Based on the study results, it is concluded that VXI is a rapid, multiplexed immunoassay that can be used to accurately determine flu vaccine potency and stability in finished product and in crude samples from upstream processes.

1. Introduction

Seasonal and pandemic influenza infections pose significant public health threats. The rapid development of vaccines serves as a foundation for the prevention of seasonal and pandemic influenza outbreaks. The trivalent and quadrivalent split influenza vaccines tested in this study were developed by GlaxoSmithKline (GSK) for active immunization for the prevention of disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. According to guidelines provided by the World Health Organization, vaccine producers must determine the potency at the time of release and throughout the approved shelf life of the product [1]. Potency assays measure the concentration of the influenza surface protein hemagglutinin, which has been established to be the dominant target of protective antibodies following vaccination or infection [2,3]. The single radial immunodiffusion assay (SRD aka SRID) measures the immunological reaction between antisera and test antigen and is the only current internationally recognized method for establishing and tracking influenza vaccine potency and stability [4]. Developed in 1978, SRD is a labor intensive assay that relies on seasonal reference reagents that result from a complex interaction between surveillance laboratories, vaccine producers, and regulatory agencies. New reference reagents must be developed when a strain change is required for the seasonal vaccine and this process can take up to four months, thereby complicating the vaccine development. Furthermore, it has been acknowledged that potency determined by SRD does not provide "*an exact correlate between vaccine potency and clinical outcome*" [5,6]. For these reasons and others [7,8], there is an extensive effort to develop and test alternative influenza vaccine potency assays.

The technologies currently being examined as replacements for SRD include HPLC [9–11], surface plasmon resonance (SPR) [12,13], mass spectrometry [14–17], and several different immunoassays [18–22]. There are limitations associated with each technology. For example, immunoassays require well-characterized antibody reagents. HPLC [23] and mass spectrometry methods [15] require

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complex sample preparation and non-biologically relevant sample conditions. While offering improved sensitivity to SRD, adsorption methods based on SPR detection (e.g., Biacore) [12,13], are generally considered too expensive [6]. Additionally most of the published alternative potency assays, like SRD, are limited to analysis of a single antigen component during measurement, which can be tedious, time-consuming, and materials intensive.

Despite reliance on specific antibodies, immunoassays offer distinct advantages for quantifying structurally intact proteins and therefore tracking protein stability in addition to providing a direct measure of HA concentration [12,14,18–20]. The stability indication capability of a potency assay is crucial because licensure of influenza vaccines requires potency determination as well as evaluation of stability in both accelerated and real-time testing [24]. Due to the time-sensitive nature of producing seasonal vaccines on a yearly basis, forced degradation (accelerated) studies are considered to be critical tools for quickly assessing stability [25].

The goal of this work was to evaluate the VaxArray platform as a rapid influenza vaccine potency assay for use with seasonal vaccines. VXI is a simple multiplexed sandwich immunoassay that utilizes a glass substrate printed with broadly reactive yet subtype specific antibodies for A/H1, A/H3, B/Yamagata-like, and B/Victoria-like strains in a microarray format [20]. A "universal" polyclonal label antibody is used to quantify all components of monovalent and multivalent HA mixtures. In a previous study, VaxArray (formerly known as Titer on a Chip) generated nearly equivalent potency determination relative to SRD for *recombinant HA* produced in a baculovirus expression system [20]. This study expands on the previous work to include the suitability of VXI for potency determination and stability indication of mono- and multivalent *split virus vaccine*s produced in eggs. The vaccines used in this study were developed by vaccine manufacturer GSK for the 2015–2016 flu season.

2. Materials and methods

2.1. Sample receipt and handling

Eight monovalent intermediate bulks, two monovalent vaccines, and four multivalent vaccines were provided by GSK, along with GSK in-house standards and NIBSC reference standards (see Tables 1 and 2). The strain-specific GSK-in-house standards were obtained from purified split virus fractions originating from commercial vaccine manufacturing (proprietary technology) and were calibrated for their HA content using SRD, SDS-PAGE and HA-HPLC (see below). Upon receipt, all samples and GSK standards were stored at 4 °C. Lyophilized standards from NIBSC and CBER were stored at −20 °C and then at 4 °C once reconstituted with water as per use specifications.

2.2. HA quantification using VaxArray seasonal influenza potency assay

The assay and array layout for VXI are illustrated in Fig. 1A-B. Briefly, HA proteins are captured by sub-type specific monoclonal "capture" antibodies and detected by a "universal" polyclonal antibody conjugated with a "Cy3" equivalent fluorophore (excitation at 532 nm and emission at 570 nm). The array can be used for simultaneous analysis of HA proteins from A/H1, A/H3, B/Yamagata-like and B/Victoria-like influenza viruses.

VXI reagents kits (#6500, InDevR) contain two microarray slides (75 × 25 mm), each printed with 16 arrays per slide, Positive Control Label, Protein Blocking Buffer (PBB), and two Wash Buffers. Prior to use, VXI slides were removed from the refrigerator and equilibrated at room temperature for 30 min in their foil pouch. For quantification in this study, eight arrays were used for an 8-point calibration curve and up to 24 arrays were used for

Table 1
Standards used in this study.

Reference antigen	Lot # or ID	Source	Concentration by SRD (μg/mL)	Concentration by SDS-Page (μg/mL)
A/Christchurch/15/2010 (H1N1) (NIB-74)	10/258 (29 μg/mL)	NIBSC	NA	NA
A/Switzerland/9715293/2013 (NIB-88)	14/254 (55 μg/mL)	NIBSC	NA	NA
B/Phuket/3073/2013	14/252 (32 μg/mL)	NIBSC	NA	NA
B/Brisbane/60/08	13/234 (42 μg/mL)	NIBSC	NA	NA
A/Shanghai/02/2013	78 (60 μg/mL)	CBER	NA	NA
H1N1 Standard A/Christ/16/10 NIB-74XP	3-A/Christ (Standard)	GSK In-house split virus (MB)	180	164
H3N2 Standard A/Switz/9715293/2013 NIB-88	4-A/Switz (Standard)	GSK In-house split virus (MB)	217	183
B/Yamagata-like Standard B/Phuket/3073/2013	10-B/Phuk (Standard)	GSK In-house split virus (MB)	250	341
B/Victoria-like Standard B/Brisbane/60/2008	9-B/Bris (Standard)	GSK In-house split virus (MB)	176	347
H7N9 Standard A/Shanghai/02/2013	14-H7N9 (Standard)	GSK In-house split virus (MB)	121 (10/2013); 88 (01/2014)	120

Table 2
Samples analyzed in this study.

Samples received for analysis by VaxArray		
Sample ID	Type	Virus Strain
1-A/Christ	Monovalent Intermediate Bulk	H1N1 A/Christ/16/10 NIB-74xP
2-A/Christ	Monovalent Intermediate Bulk	H1N1 A/Christ/16/10 NIB-74xP
5-A/Switz	Monovalent Intermediate Bulk	H3N2 SWITZ/9715293/2013 NIB-88
6-A/Switz	Monovalent Intermediate Bulk	H3N2 SWITZ/9715293/2013 NIB-88
7-B/Bris	Monovalent Intermediate Bulk	B/vic-like Brisbane/60/2008
8-B/Bris	Monovalent Intermediate Bulk	B/vic-like Brisbane/60/2008
11-B/Phuk	Monovalent Intermediate Bulk	B/Yam-like Phuket/3073/2013
12-B/Phuk	Monovalent Intermediate Bulk	B/Yam-like Phuket/3073/2013
13-H7N9	Monovalent Vaccine	A/Shanghai/02/2013
15-TIV	Trivalent Vaccine	H1N1, H3N2 and B/Yam-like Phuket/3073/2013
17-TIV	Trivalent Vaccine	H1N1, H3N2 and B/Yam-like Phuket/3073/2013
16-QIV	Quadrivalent Vaccine	H1N1, H3N2, B/Yam-like Phuket/3073/2013 and B/vic-like Brisbane/60/2008
18-QIV	Quadrivalent Vaccine	H1N1, H3N2, B/Yam-like Phuket/3073/2013 and B/vic-like Brisbane/60/2008
19-Pan	Monovalent Vaccine	A/Shanghai/02/2013