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Fast and highly selective determination of hemagglutinin content in quadrivalent influenza vaccine by reversed-phase high-performance liquid chromatography method

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ABSTRACT

Seasonal inactivated quadrivalent influenza vaccines are currently formulated to include antigens from two strains of influenza A and a strain from each of the two circulating influenza B virus lineages. However, the applicability of the potency assay currently required for the release of vaccines has been hindered due to cross-reactivity between the two B strains. In this study, a reversed-phase high-performance liquid chromatography method previously developed for the separation and quantitative determination of the hemagglutinin content in trivalent influenza vaccine preparations was further extended and found to be adaptable for the assessment of all four hemagglutinin antigens present in quadrivalent influenza vaccines. Vaccines prepared from monovalent bulks and commercial quadrivalent products from the past three vaccination seasons in the Northern Hemisphere were tested with the new method. The results showed excellent resolution of all four hemagglutinins from frequently interfering formulation agents such as surfactants. This method provides a simple approach for fast evaluation of quality and hemagglutinin strain identification in influenza vaccines. It is also the only physicochemical method capable of distinguishing the B strains in quadrivalent influenza vaccines.

1. Introduction

Influenza is a major public health threat where each year the human influenza viruses cause seasonal influenza in about 10% of the world population with three to five million severe cases of illness and up to half a million deaths [1–3]. Among the strategies for the prevention of the disease, manufacturing of vaccines ahead of the targeted season using strains recommended by the WHO remains the most effective countermeasure against influenza [4–6]. Influenza viruses found in circulation are divided in three types (A, B and C) where only the A-type is subdivided according to the surface antigen content of the virus (e.g. H1N1) [7]. Moreover, since C-type infections are very mild and uncommon, only the A and B types are deemed responsible for the infection of humans [8,9].

In order to provide immunity against circulating strains, seasonal influenza vaccines are classically composed of two A-subtypes and only one B-type of influenza virus for a trivalent influenza vaccine (TIV) formulation. In such vaccines, each strain is composed of the immunogenic surface protein hemagglutinin (HA) and neuraminidase (NA) responsible for host protection against the Influenza virus [10]. In the case of the B-type, two antigenically distinct lineages of influenza B have been co-circulating over the past 15 years in the North America [11–13]. Unfortunately, the success rate in the prediction of the right B strain to be included in the TIV was less than 50% in the US from 2000 to 2010 [13]. In case of mismatch between the latter and the one selected for formulation, the vaccine provides little-to-no protection against the other B lineage

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and may lead to lower vaccine efficacy. In 2013, the WHO recommended the addition of a second B strain to the TIV leading to the preparation of a quadrivalent influenza vaccine (QIV) that would provide larger coverage [9] and cost effective protection against influenza [10,14–16]. Indeed, some cases of vaccine-mismatch have warranted the advantage of introducing QIV [17].

For the past forty years, the Single Radial Immunodiffusion (SRID) test has been the required method for the determination of influenza vaccine potency [18,19]. Briefly, an agarose gel containing the antibody specific to the strain used in the vaccine is exposed to a serial dilution of the antigen to be assayed. Formation of an antigen-antibody precipitate is then analysed and compared to a reference antigen of known potency [20,21]. Although the SRID was successfully used for decades, one major disadvantage is the requirement for production of time-consuming calibrated reagents. This was evident in the 2009 H1N1 pandemic where fast production and release of vaccines was delayed because of the unavailability of SRID reagents [22,23]. Furthermore, SRID has been found to be inadequate for the identity and potency testing of the B-influenza strains in QIV because of the cross-reactivity between the potency reagents [5,20]. This lead to the conclusion that on top of improving the assessment of vaccine potency by SRID, the development and use of alternative approaches is necessary [22–25].

Among several antibody-independent approaches under development [23], chromatographic approaches to separate and assess the influenza virus HA proteins in TIV have been published by a few groups [26–29]. HPLC is a well-established technique that provides fast and reliable separation and quantification of proteins in solution. As such, approaches using RP-HPLC for the quantification of the HA content through the release of the HA1 subunit have been reported by the groups of Kapteyn [26] and Girard [30]. The sensitivity of these methods was optimized with the use of fluorescence detection over UV detection [31] and also by optimizing other parameters as described in Lorbetskie et al. [28]. In particular, the quantification of HAs by RP-HPLC compared favourably to that obtained by the standard SRID used by regulatory agencies for the analysis of the 2009 pandemic monovalent H1N1 vaccine. Now with the advent of the QIV formulation, the new co-eluent and adjuvants used in the manufacturing of these vaccines, and the limitations of the SRID assay, we have sought to adapt the developed method to these new products.

In this study we have modified and optimized method parameters to ensure good separation of the four different HA1 species present in QIV and also separate these from interfering components such as the widely used detergents, Triton X-100 and polysorbate. We further prepared different mixtures from monovalent bulk material to show how this method can be adapted to elucidate different combinations of HA that may occur in future formulations. In an effort to develop alternative methods to the SRID, we find that this approach could be quite beneficial in pandemic situations where rapid approval and distribution of an effective vaccine is critical.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical reagent grade and obtained from several sources. 1,4-Dithiothreitol (DTT), trifluoroacetic acid (TFA), Triton X-100, Tween 80 and β-propriolactone were from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and 2-propanol were purchased from Merck KGaA (Darmstadt, Germany). Distilled water (dH_2O) was deionized on a Nanopure Diamond™ system (Barnstead International, Dubuque, IA, USA).

2.2. Samples

a) Vaccines from manufacturer A

1) 1) 2014-15 Influenza Virus Vaccine Quadrivalent Types A and B (Split Virion)
2) 2) 2015-16 Influenza Virus Vaccine Quadrivalent Types A and B (Split Virion)
3) 3) 2016-17 Influenza Virus Vaccine Quadrivalent Types A and B (Split Virion)

b) Vaccines made from monovalent bulks:

1) 2015-16 Influenza Virus Vaccine Quadrivalent Types A and B (Split Virion) from manufacturer B
2) 2015-16 Influenza Virus Vaccine Quadrivalent Types A and B (Split Virion) from manufacturer C

c) NIBSC Influenza Antigen Reagents:

1) A/California/7/2009 (H1N1) (NYMC-X181), NIBSC code: 16/106, lyophilized reference, 45 μg HA/mL
2) A/Switzerland/9715293/2013 (H3N2) (NIB88), NIBSC code: 14/254, lyophilized reference, 55 ug HA/mL
3) A/Texas/50/2012 (H3N2) (NYMCX-223), NIBSC code: 13/112, lyophilized reference, 74 ug HA/mL
4) A/Hong Kong/4801/2014 (H3N2) (NYMCX-263B), NIBSC code: 15/230, lyophilized reference, 67 ug HA/mL
5) A/turkey/Turkey/1/2005 (H5N1) (NIBRG-23), NIBSC code: 07/112, lyophilized reference, 80 ug HA/mL
6) A/Anhui/1/2013 (H7N9) (NIBRG-268), NIBSC code: 14/250, lyophilized reference, 37 ug HA/mL
7) B/Brisbane/60/08, NIBSC code: 08/352, lyophilized reference, 27 μg HA/mL
8) B/Massachusetts/02/2012, NIBSC code: 13/134, lyophilized reference, 35 ug HA/mL
9) B/Phuket/3073/2013, NIBSC code: 14/252, lyophilized reference, 32 ug HA/mL
10) B/Brisbane/60/08, NIBSC code: 13/234, lyophilized reference, 42 μg HA/mL

2.3. Instrumentation

The analytical HPLC system consisted of a Waters Alliance 2695 chromatograph equipped with a column heater and an autosampler with a sample cooling device coupled to a Waters 2475 Multichannel Fluorescence Detector with a 8 μL flow cell working at λ_{ex} 280 nm and λ_{em} 335 nm and a Waters 2996 UV–vis photodiode array detector (Waters, QC, Canada). Data acquisition and integration were performed with Empower 3 Chromatography Data Software from Waters.

2.4. Conditioning and separation conditions

Separation of HA in QIV formulations was obtained by optimization of a previously reported method by Lorbetskie et al. [28]. Non-porous silica-based columns were MICRA® HPLC NPS-ODSI, 33 mm × 4.6 mm, 1.5 μm particles (Eprogen, Darien, IL, USA). Chromatographic separations were carried out at 55 °C with an AB gradient elution of 19 min at a flow rate of 1.0 mL/min as shown in Table 1. Eluent A was 0.04% (v/v) aqueous TFA and eluent B was 0.03% (v/v) TFA in 25% ACN and 75% 2-propanol.

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