agents and in the absence of HA protein standards as provided by WHO collaborating centers. Clearly, a strong need in the art exists for robust, accurate and fast methods for reliable isolation and quantification of antigen, including viral antigens such as HA, in vaccine manufacturing before antigen standards are available from WHO collaborating centers. The following disclosure describes the specifics of such methods.

SUMMARY

[0009] The methods described herein were developed to provide a means of measuring antigen content in a vaccine in the absence of available antigen standards. Therefore, the disclosure~~invention~~ addresses one or more needs in the art relating to fast and accurate quantification of antigen concentration in a vaccine during the vaccine development and manufacturing process without the need of international standards. Thus, the methods provided herein allow vaccine manufacturers to more quickly produce a vaccine which can be delivered to the public without waiting for WHO to develop and provide a standard.

[0010] More specifically, the disclosure~~invention~~ provides fast and robust methods of isolating and accurately quantifying vaccine antigens, which are accurate and reproducible, in the absence of the use of standards. The disclosure is applicable for use with a variety of antigens, thereby providing an improved method in the art of vaccine manufacturing to date.

[0010a] In one aspect, there is provided a method for isolating an antigen from a vaccine composition, the method comprising the steps of: (a) solubilizing the antigen in the vaccine composition by reduction without a detergent and without an alkylating agent, wherein the reduction comprises an incubation temperature of about 70°C, about 75°C, about 80°C, about 85°C, or about 90°C; and (b) isolating the antigen or an antigen subtype by fractionation.

[0011] The disclosure~~invention~~ provides methods for isolating an antigen from a vaccine composition, the method comprising the steps of: (a) solubilizing the antigen in the vaccine composition without a detergent and without an alkylating agent; and (b) isolating the antigen or an antigen subtype by fractionation.

[0012] In some embodiment~~aspect~~s, the solubilizing step is carried out by reduction. In some embodiment~~aspect~~s, the reduction comprises treating the vaccine composition with dithiothreitol. In some embodiment~~aspect~~s, the reduction comprises an incubation time from about 5 minutes to about 20 hours. In other embodiment~~aspect~~s, the reduction comprises an incubation time from about 30 minutes to about 2 hours. In more particular embodiment~~aspect~~s, the reduction comprises an incubation time of about 1 hour. In further embodiment~~aspect~~s, the reduction comprises an incubation temperature from about 20°C to about 100°C. In various

embodimentaspects, the reduction comprises an incubation temperature from about 50°C to about 90°C. In certain embodimentaspects, the reduction comprises an incubation temperature at about 85°C. In further embodimentaspects, the reduction step is pH-controlled. In various embodimentaspects, the reduction is carried out at a pH from about pH 6 to about pH 11. In particular embodimentaspects, the reduction is carried out at a pH from about pH 7 to about pH 10. In additional embodimentaspects, the solubilizing step further comprises denaturing with a chaotropic agent. In various embodimentaspects, the chaotropic agent is guanidine hydrochloride, urea, thiourea, lithium, perchlorate, or thiocyanate. In further embodimentaspects, the reduction is further carried out by acidification of the antigen or antigen subtype to prevent disulfide bond formation between separated antigen subtypes. In various embodimentaspects, the acidification is carried out with phosphoric acid, hydrochloric acid, sulfuric acid, trifluoroacetic acid, pentafluoropropionic acid, heptafluorobutyric acid, or formic acid.

[0013] In some embodimentaspects, fractionation is carried out by chromatography. In various embodimentaspects, the chromatography is high performance liquid chromatography (HPLC), reversed-phase HPLC (RP-HPLC), ion exchange-HPLC (IEX-HPLC), affinity chromatography, hydrophobic interaction chromatography (HIC), or size exclusion chromatography (SEC). In an exemplary aspect, the chromatography is reversed-phase (RP)-HPLC.

[0013a] In one aspect, there is provided a method for quantifying antigen or antigen subtype in a vaccine composition, the method comprising the method as defined herein with a further step of quantifying the antigen or antigen subtype.

[0014] The disclosureinvention further provides methods for quantifying antigen or antigen subtype content in a vaccine composition. Such methods include all of the methods described herein above for isolating an antigen from a vaccine composition, with a further step of quantifying the antigen or antigen subtype. In exemplary embodimentaspects, the quantifying step is carried out without using an antigen standard. In various embodimentaspects, the quantifying step comprises quantifying antigen by amino acid analysis. In some embodimentaspects, the antigen is viral or

bacterial. In certain embodimentaspects, the antigen is hemagglutinin (HA). In particular embodimentaspects, the HA is from an Influenza virus vaccine composition, a measles virus vaccine composition, a parainfluenza virus vaccine composition, or a mumps virus vaccine composition. In some embodimentaspects, the vaccine composition is an Influenza virus vaccine composition. In further embodimentaspects, the Influenza virus vaccine composition provides protection from an Influenza virus selected from the group consisting of Influenza A and Influenza B. In various embodimentaspects, the antigen subtype is any one of Influenza A HA1, HA2, HA3, HA4, HA5,

HA6, HA7, HA8, HA9, HA10, HA11, HA12, HA13, HA14, HA15, and HA16 and Influenza B HA. In certain embodimentaspects, the antigen subtype is Influenza A HA subtype HA1, HA2, HA3, HA5, HA7, HA9, HA10, or influenza B HA. In an exemplary embodimentaspect, the Influenza A HA subtype is HA1. In further embodimentaspects, the Influenza vaccine composition provides protection from an Influenza type selected from the group consisting of Influenza A H1N1, H1N2, H2N2, H3N2, H5N1, H7N1, H7N2, H7N3, H7N7, H9N2, H10N7, and Influenza B. In various embodimentaspects, the method quantifies antigen content with a relative standard deviation (RSD) of less than about 2.5%. In more particular embodimentaspects, the RSD is about 2.2%. In even more particular embodimentaspects, the RSD is about 1.3%.

[0015] The foregoing summary is not intended to define every embodimentaspect of the disclosure, and additional embodimentaspects are described in other sections, such as the following detailed description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document. Other features and advantages of the disclosureinvention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the disclosure, are given by way of illustration only, because various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION

[0016] The disclosure provides a novel method for isolating and preparing antigen and determining antigen concentration in vaccine development and manufacturing in the absence of international standards, for example, World Health Organization (WHO) International Standards, which are biological reference preparations with defined biological activity. The method includes improvements over the prior art by solubilizing antigen from virus in a vaccine composition without the use of detergent and without the use of alkylation. Solubilized antigen is then separated by fractionation and quantified by quantitative amino acid analysis.