Randomized Open-Label Trial to Compare the Immunogenicity of Cell Culture-Based and Recombinant Unadjuvanted Quadrivalent Influenza Vaccines to Conventional Egg-Based Unadjuvanted Quadrivalent Influenza Vaccines among Healthcare Personnel Aged 18-64 Years

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421. Title of Project: Randomized Open-Label Trial to Compare the Immunogenicity of Cell Culture-Based and Recombinant Unadjuvanted Quadrivalent Influenza Vaccines to Conventional Egg-Based Unadjuvanted Quadrivalent Influenza Vaccines among Healthcare Personnel Aged 18-64 Years

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Protocol Version 5, Modified on May 24, 2018, (29Aug2018)

2. List of Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
GMT	Geometric mean titers
HCP	Healthcare Personnel
HA	Hemagglutinin
HI	Hemagglutination Inhibition
ILI	Influenza-like illness
MDCK	Madin-Darby Canine Kidney
MFR	Mean fold rise
MN	Microneutralization
NA	Neuraminidase
NAI	Neuraminidase antibody mediated inhibition
rHA	Recombinant Hemagluttinins
SCR	Seroconversion rate

3. Project Summary

This randomized, open-label trial will assess humoral and cell-mediated immune responses to cell culture-based and recombinant unadjuvanted quadrivalent influenza vaccines compared to conventional egg-based unadjuvanted quadrivalent standard dose (15µg of HA per strain) influenza vaccines among persons aged 18-64 years. The trial will be conducted at two sites in the United States during two influenza seasons (2018-19 and 2019-20). Stratified enrollment procedures will be used to enroll a mix of participants based on age and reported receipt of influenza vaccine during the prior season as a proxy for frequency of influenza vaccination.

Eligible participants at each site will be randomized 2:2:1:1 to receive a single dose of cell culture-based vaccine (Flucelvax™ Quadrivalent by Seqirus, Inc., 15µg of HA per strain) versus recombinant vaccine (Flublok® Quadrivalent by Sanofi Pasteur, 45µg of HA per strain) versus one of two standard dose egg-based vaccines (Fluzone® Quadrivalent by Sanofi Pasteur, 15µg of HA per strain and Fluarix® Quadrivalent by GlaxoSmithKlein, 15µg of HA per strain) during August-September of 2018 and again during August-September of 2019. All study

vaccines are licensed for use in adults aged ≥18 years in the United States. Participants will have blood collected just prior to vaccination and at approximately 28 days and 6 months post-vaccination (or at the end of influenza virus circulation as determined by available surveillance data) to evaluate humoral immune responses to vaccination. Additional blood will be collected from a subset of participants pre-vaccination and at approximately 7 days, 28 days and 6 months post-vaccination (or at the end of influenza virus circulation) to evaluate longitudinal cell-mediated immune responses to vaccination. Active surveillance with mid-turbinate nasal swab collection for influenza-like illness (ILI) defined as new onset of cough or worsening of chronic cough within the preceding 7 days will be conducted during the period of influenza circulation at each study site. Additional blood will also be collected at 6 months post-vaccination (or at the end of influenza virus circulation) from participants with reverse-transcription polymerase chain reaction (RT-PCR)-confirmed ILI during the influenza season to evaluate cell-mediated immune responses to natural influenza virus infection.

Sites will aim to enroll 864 participants (432 per site) at the start of the 2018-19 season, including up to 200 participants (up to 100 per site) who will contribute additional blood at all study visits to evaluate cell-mediated immune responses to vaccination. Efforts will be made to retain participants enrolled in the first year of the study for both years of the study. Sites will also enroll additional participants at the start of the 2019-20 season to make up for participants who withdraw or are lost to follow-up prior to the start of the 2019-20 season. Both participants and study investigators will be aware of study arm assignments with the exception of laboratory investigators who will be blinded to study arm assignment until testing is completed, as appropriate.

Relative efficacy of single doses of study vaccines will be assessed by comparing immunologic responses to vaccination among participants between study arms using Fluzone® Quadrivalent and Fluarix® Quadrivalent as the comparator groups for participants in the Flucelvax™ Quadrivalent or Flublok® Quadrivalent arms. In addition, the effect of frequency of prior vaccination during the preceding five years on immunologic responses to vaccine will be evaluated in subgroup analysis. Both humoral (influenza antibody) and cell-mediated (influenza-specific CD4 and CD8 T cell) immune responses will be evaluated.

Study Sponsor

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4. INTRODUCTION

4.1 Background Information:

Influenza is estimated to result in 9.2-35.6 million illnesses, 140,000-710,000 hospitalizations and 12,000-56,000 deaths in the United States.[1] Influenza vaccination is the most effective method of preventing influenza virus infection, and annual influenza vaccination is recommended for all persons aged ≥6 months in the United States. However, the efficacy of licensed influenza vaccines varies annually depending on the match between vaccine strains and circulating strains and by influenza virus type and subtype. In a meta-analysis of published influenza vaccine effectiveness studies conducted during 2007-2015, pooled influenza vaccine effectiveness was estimated as 61% (95%CI 57-65) against A/H1N1 viruses circulating after the 2009 A/H1N1 pandemic and 54% (95%CI 46-61) against influenza B viruses, but only 33% (95%CI 26-39) against influenza A/H3N2 viruses.[2] In addition, observed influenza vaccine effectiveness has been lower against A/H3N2 viruses than A/H1N1 viruses for the past several influenza seasons in the United States.[3, 4] The relatively low effectiveness of influenza vaccines against influenza A/H3N2 viruses is particularly concerning given that influenza A/H3N2 viruses have been associated with higher influenza-associated hospitalization and mortality rates among adults.[5, 6]

Questions about the preventive benefit of influenza vaccines are especially relevant to healthcare personnel (HCP). Although HCP are believed to be at increased risk of influenza virus infection[7], there is limited information on the effectiveness of influenza vaccines among HCP.[8] Reports of reduced influenza vaccine effectiveness among persons with a history of frequent influenza vaccination in some studies[9, 10] and seasons[11] also make it especially important to examine influenza vaccine effects among HCP who are among the most vaccinated target populations. Recent studies of influenza vaccine immunogenicity among HCP have demonstrated that repeated vaccination can blunt the antibody response to hemagglutinin[12] and neuraminidase.[13]

The conventional method of inactivated influenza vaccine (IIV) production relies on propagation in embryonated chicken eggs of an egg-adapted vaccine seed strain derived from a circulating influenza virus. Both the adaptation process and serial passage of influenza viruses in chicken eggs can result in the development of mutations that may introduce important antigenic differences between the vaccine strain and circulating wild-type strains.[14-17] It has been

hypothesized that this phenomenon may be partially responsible for the lower vaccine effectiveness observed against influenza A/H3N2 viruses in some studies despite a reportedly good match between vaccine seed strains and circulating strains.[16, 18, 19] Historically, the immunogenicity of influenza vaccines has been assessed by measuring antibody responses to egg-grown influenza viruses which may be a suboptimal measure of efficacy if egg-grown viruses differ antigenically from circulating wild-type viruses because of mutations acquired during egg passage.

During the past decade, inactivated influenza vaccines manufactured using alternative vaccine virus production technologies have been licensed in the United States, including a cell culture-based influenza vaccine (Flucelvax™ by Seqirus, Inc.) licensed for persons aged ≥ 4 years and a recombinant influenza vaccine (Flublok® by Sanofi Pasteur) licensed for persons age ≥ 18 years. Studies evaluating these vaccines have measured antibody responses to cell culture-grown viruses in some cases which may be more representative of immune responses to circulating viruses if cell culture-grown viruses possess fewer mutations acquired during the adaptation and viral passage process.

Flucelvax™ is a subunit vaccine formulated from influenza virus cultivated in Madin-Darby Canine Kidney (MDCK) cells. A version known as Optaflu is also licensed in Europe. Although cultivation of vaccine strains is done using MDCK cell lines, seed viruses for the vaccine may be either egg-derived or cell culture-derived. In a phase III, randomized, placebo-controlled, observer-blinded trial comparing either cell culture-based trivalent IIV (Optaflu) or egg-based trivalent IIV (Aggripal) to placebo among 11,404 adults aged 18-49 years, the cell culture-based vaccine was 70% (lower bound of the 95% CI=55%) efficacious against all culture-confirmed influenza-like illness (ILI), 84% (lower bound of the CI=61%) against ILI associated with vaccine-like viruses, and 59% (lower bound of the CI=34%) against ILI associated with drifted viruses.[20] Efficacy was similar for these outcomes for the cell culture- and egg-based vaccines, but relative efficacy was not assessed. In other studies evaluating the immunogenicity of Flucelvax™ or Optaflu compared to standard dose egg-based vaccines, immune responses to the cell culture-based vaccines were generally similar or non-inferior to egg-based vaccines when evaluating responses against homologous egg-grown virus antigens.[21, 22] However, some studies demonstrated a better immune response to cell culture-based vaccine compared to egg-based vaccines when immune responses were evaluated against homologous

cell culture-grown virus antigens.[21, 23, 24] Early trials of Flucelvax™ used vaccine produced in cell culture from egg-derived seed viruses (likely resulting in vaccine viruses that were more similar to those used in egg-based vaccines), whereas more recent formulations of Flucelvax™ (e.g. the 2017-2018 formulation) included an H3N2 vaccine strain grown from a cell culture-derived seed virus. The 2018-2019 formulation of Flucelvax™ will be made from cell-culture derived seed strains for both the H3N2 and H1N1 vaccine strains.

Flublok® is a recombinant vaccine formulated with purified HA protein from cell-culture isolates of the vaccine reference virus. HA is grown using baculovirus vector technology in an insect cell line from the fall armyworm. Since production of Flublok® does not use egg-adapted seed strains, the recombinant HA (rHA) antigens contained in the vaccine are thought to completely match those in the vaccine reference virus.[25] In contrast to most other vaccines licensed for use in adults aged 18-64 years in the United States, Flublok® contains 45 µg of HA per strain. In a phase III randomized placebo-controlled trial of trivalent Flublok® among healthy adults aged 18-49 years, Flublok® was 45% (95%CI 19-63%) efficacious against culture-confirmed influenza during the 2007-2008 influenza season when the majority of circulating influenza A/H3N2 and B viruses were drifted from the vaccine strains. Flublok® was also found to be highly immunogenic for all three vaccine strains with 99% of participants having an HI titer of $\geq$1:40 against the H1N1 vaccine strain at 28 days post-vaccination, 97% against the H3N2 vaccine strain, and 96% against the B vaccine strain; HI was measured against baculovirus derived rHAs.[26] In a subsequent phase III randomized trial comparing quadrivalent Flublok® to quadrivalent Fluarix, an egg-based vaccine, among adults aged 18-49 years, Flublok® and Fluarix recipients had similar post-vaccination HI titers against all four vaccine components using egg-grown antigens.[27] Randomized trials in adults aged $\geq$50 years also demonstrated similar immune responses at 28 days post-vaccination as measured by HI against baculovirus derived rHAs between trivalent Flublok® and Fluzone®[28] and showed that Flublok® was more efficacious than Fluarix against RT-PCR-confirmed ILI (relative vaccine efficacy, 30%, 95% 10-47%) during the 2014-2015 influenza season when drifted A/H3N2 viruses predominated.[29]

Both Flucelvax™ and FluBlok® were licensed in the United States based on demonstrations of clinical efficacy and non-inferiority using immunogenicity outcomes according to the criteria of the Centers for Biologic Evaluation and Research. Studies assessing

the immunogenicity of Flucelvax largely assessed immune response against homologous egg-based antigens, although some studies also assessed responses against homologous cell-grown antigens. In contrast, studies assessing Flublok assessed immune responses against baculovirus-derived rHAs. To date, there are few data directly comparing the immunogenicity of cell culture-based and recombinant influenza vaccines to egg-based vaccines using the same immunogenicity outcome measures against the same antigenic targets for all three vaccine types, including evaluation of response to cell-grown viruses that may be more representative of circulating strains. It also remains unclear whether cell culture-based or recombinant influenza vaccines that are free from mutations in the viral HA introduced during the egg adaptation and passage process of egg-based vaccine production are more effective against circulating influenza viruses. This randomized, open-label trial will assess humoral and cell-mediated immune responses to cell culture-based and recombinant quadrivalent influenza vaccines compared to two conventional egg-based quadrivalent standard dose (15µg of HA per strain) influenza vaccines among persons aged 18-64 years using both cell-grown and egg-grown vaccine reference viruses as antigenic targets. Two egg-based influenza vaccines will be included as comparators in this trial since other vaccine components that vary by vaccine regardless of method of vaccine virus growth might influence immune response to vaccination including neuraminidase (NA) content, preservatives, and other substances retained as part of the inactivation or purification processes. The primary study hypothesis is that a single dose of cell culture-based or recombinant influenza vaccine may be more immunogenic than a single dose of split-virus/subvirion egg-based influenza vaccine to cell-grown viruses that are more representative of circulating strains, regardless of the vaccine product among persons aged 18-64 years.

4.2 Justification:

Influenza vaccines are the most effective method of influenza prevention and are recommended for all persons aged ≥6 months in the United States. However, the efficacy of licensed influenza vaccines varies annually depending on the match between vaccine strains and circulating strains and varies by influenza virus type and subtype. Data from the last several influenza seasons showing lower vaccine effectiveness against influenza A/H3N2 viruses combined with studies documenting challenges with growing recently circulating influenza A/H3N2 viruses in embryonated chicken eggs for vaccine production have raised questions

about the impact of egg adaptation and propagation of vaccine viruses on the effectiveness of egg-based influenza vaccines. At the same time, influenza vaccines made by alternative methods of production that avoid the egg adaptation and/or egg propagation steps are now licensed in the United States, including cell culture-based vaccines and recombinant influenza vaccines. At present, the Advisory Committee on Immunization practices does not preferentially recommend any specific influenza vaccine for use in adults in the United States. Studies are needed to compare the immunogenicity of conventional egg-based vaccines versus vaccines manufactured using alternative methods of production using standard assays and endpoints. Data from such studies will inform whether a preferential recommendation for vaccines based on a specific method of production are warranted.

4.3 Expected benefits from the proposed study

Influenza results in substantial morbidity and mortality, and annual vaccination remains the most effective method to prevent influenza and its complications. Through this study, we will gain a better understanding of whether the cell culture-based vaccine Flucelvax™ Quadrivalent or the recombinant vaccine Flublok® Quadrivalent offer greater protection to HCP aged 18-64 years than the conventional egg-based vaccines Fluzone® Quadrivalent and Fluarix® Quadrivalent as measured primarily by humoral immune responses to MDCK cell-grown vaccine strains. Because we will assess immune responses at 6 months after influenza vaccination (or the end of influenza virus circulation) in all participants, this study will also provide information about how long protective immune responses to each influenza vaccine last.

In the United States, all four study vaccines are licensed for use in adults aged ≥18 years based on phase III trials demonstrating efficacy in comparison to placebo[26] or non-inferiority to conventional egg-based trivalent influenza vaccines. Therefore, study participants may directly benefit from receipt of study vaccine by receiving protection against circulating influenza viruses during the 2018-19 and 2019-20 influenza seasons.

5a. STUDY OBJECTIVES

- **Primary Objective**
    - Compare immune responses among persons aged 18-64 years vaccinated with either a single dose of the cell culture-based vaccine Flucelvax™ Quadrivalent or

the recombinant vaccine Flublok® Quadrivalent versus a single dose of the conventional egg-based vaccines Fluzone® Quadrivalent or Fluarix® Quadrivalent at approximately 28 days after receipt of the 2018-19 and 2019-20 vaccines as measured by

- hemagglutination inhibition (HI) titers for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria strains, and
- microneutralization (MN) titers for influenza A/H3N2 viruses

- **Secondary Objectives**
  - Compare immune responses at 28 days post-vaccination as stated in the primary objective but with secondary indicators of immune response, including by:
    - HI titers for influenza A/H3N2 viruses, as appropriate
    - MN titers for other virus subtypes/lineages, as appropriate
  - Evaluate the effect of frequency of prior influenza vaccination during the preceding five years on immune responses after a single dose of Flucelvax™ Quadrivalent, Flublok® Quadrivalent, Fluzone® Quadrivalent or Fluarix® Quadrivalent at approximately 28 days after receipt of the 2018-19 and 2019-20 vaccines as measured by
    - HI titers for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria strains and
    - MN titers for influenza A/H3N2 viruses
- **Exploratory Objectives**
  - Make the same comparisons as stated in the primary objective but at approximately 6 months after vaccine receipt to assess duration of immune responses as measured by
    - HI titers for all virus subtypes/lineages
    - MN for virus subtypes/lineages, as appropriate
  - Evaluate the relative efficacy of a single dose of the cell culture-based vaccine Flucelvax™ Quadrivalent or the recombinant vaccine Flublok® Quadrivalent versus a single dose of the conventional egg-based vaccines Fluzone®

Quadrivalent or Fluarix® Quadrivalent to prevent reverse-transcription polymerase chain reaction (RT-PCR)-confirmed influenza-like illness (ILI).

- Characterize humoral immune responses as measured by HI, MN titers, neuraminidase antibody mediated inhibition (NAI) titers and other appropriate humoral immunity assays, and characterize cell-mediated immune responses of B, CD4 and CD8 T-cells among persons who experience 'vaccine failure' defined as the development of RT-PCR-confirmed ILI more than 14 days after receipt of vaccine.
- Characterize cell-mediated immune responses of B, CD4 and CD8 T-cells to the cell culture-based vaccine Flucelvax™ Quadrivalent, the recombinant vaccine Flublok® Quadrivalent, and the conventional egg-based vaccines Fluzone® Quadrivalent and Fluarix® Quadrivalent at approximately 7 days, 28 days, and 6 months after vaccination.
- Characterize the avidity of antibodies produced in response to the cell culture-based vaccine Flucelvax™ Quadrivalent, the recombinant vaccine Flublok® Quadrivalent, and the conventional egg-based vaccines Fluzone® Quadrivalent and Fluarix® Quadrivalent at approximately 7 and 28 days after vaccination.
- Compare immune responses among persons aged 18-64 years vaccinated with either a single dose of the cell culture-based vaccine Flucelvax™ Quadrivalent or the recombinant vaccine Flublok® Quadrivalent versus a single dose of the conventional egg-based vaccines Fluzone® Quadrivalent or Fluarix® Quadrivalent at approximately 28 days after receipt of the 2018-19 and 2019-20 vaccines as measured by
  - NAI and antibody-dependent cell-mediated cytotoxicity (ADCC) titers
  - Other relevant immunological markers

5b. STUDY ENDPOINTS (Tables 1 and 2)

Primary Endpoints

- MN responses to the *cell*-grown influenza A/H3N2 (using MDCK-SIAT or other appropriate cell line) vaccine reference viruses for each study season at approximately 28 days post-vaccination, including the following endpoints:

- Seroconversion rate (SCR) defined as the proportion of participants with paired samples that achieved ≥ 4 fold rises comparing post- versus pre-vaccination titers, and post vaccination titers ≥ 40.
- Geometric mean titers (GMT)
- MFR mean-fold rise (MFR) defined as the ratio of the post-vaccination titer value to the pre-vaccination value

- HI responses to the *cell*-grown influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria vaccine reference viruses for each study season at approximately 28 days post-vaccination, including the following endpoints:
  - SCR
  - GMT
  - MFR

Secondary Endpoints

- HI responses to *cell*-grown influenza A/H3N2 vaccine reference viruses for each study season at approximately 28 days, including the following endpoints
  - Post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160, SCR, GMT, and MFR
- HI (and MN, as appropriate) responses to *cell*-grown influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria vaccine reference viruses for each study season at approximately 28 days, including the following endpoints
  - Post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160, as measured by HI response
  - SCR, GMT, and MFR as measured by MN response, as appropriate
- HI and/or MN responses to *egg*-grown vaccine reference viruses for all vaccine viruses for each study season at approximately 28 days, including the following endpoints
  - SCR, GMT, MFR, and post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160 as measured by HI
  - SCR, GMT, and MFR as measured by MN, as appropriate
- GMT as measured by HI for all vaccine virus subtypes/lineages at 6 months post-vaccination using

- *cell*-grown vaccine reference viruses
- *egg*-grown vaccine reference viruses

Exploratory Endpoints

- HI and/or MN responses to *cell*-grown *wild-type* influenza viruses at approximately 28 days (if appropriate), including the following endpoints
  - SCR, GMT, MFR, and post-vaccination titers $\geq$ seropositive thresholds at 1:40, 1:80, 1:160 as measured by HI
  - SCR, GMT, and MFR as measured by MN, as appropriate
- GMT as measured by NAI and ADCC pre- and post-vaccination.
- Mean percentages of strain-specific T cell surface markers of activation, antibody secreting plasmablasts and memory B cells to hemagglutinin (HA), interferon-gamma (IFN- gamma), interleukin 2 (IL-2), and Tumor Necrosis Factor-alpha (TNF-alpha) responses to *wild-type* cell-grown strains and antigen-specific B and T cell repertoire, and single cell transcriptome analysis (where feasible) at approximately 7 days, 28 days, and 12 months post-vaccination.
- Frequency of RT-PCR-confirmed ILI episodes
- Indicators of immune response to vaccination based on other immunologic assays not listed above (as appropriate)

Table 1 Primary and secondary endpoints at approximately 28 days post-vaccination

	Cell-grown vaccine virus				**Egg-grown vaccine virus**			
	A/H3N2	**A/H1N1**	**B/Yam**	**B/Vic**	**A/H3N2**	**A/H1N1**	**B/Yam**	**B/Vic**
HI								
SCR								
GMT								
MFR								
Post-vaccination titers ≥ seropositive thresholds at 1:40,								
MN								
SCR								
GMT								
MFR								
	Primary endpoint			Secondary endpoint		Secondary endpoint (as appropriate)		

HI: hemagglutination inhibition; MN: microneutralization
SCR: Seroconversion rate; GMT: Geometric mean titer; MFR: Mean fold rise

Table 2 Secondary immunogenicity endpoints at approximately 6 months post-vaccination*

	Cell-grown vaccine virus				Egg-grown vaccine virus			
	A/H3N2	**A/H1N1**	**B/Yam**	**B/Vic**	**A/H3N2**	**A/H1N1**	**B/Yam**	**B/Vic**
HI								
SCR								
GMT								
MFR								
Post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160								
MN								
SCR								
GMT								
MFR								
		Secondary endpoint			Secondary endpoint (as appropriate)			

HI: hemagglutination inhibition; MN: microneutralization; NAI: neuraminidase antibody mediated inhibition; ADCC: Antibody-Dependent Cell-Mediated Cytotoxicity
SCR: Seroconversion rate; GMT: Geometric mean titer; MFR: Mean fold rise
.

6. LOCATIONS OF STUDY

- Baylor Scott & White Health, Texas A&M Health Science Center, College of Medicine, Temple, Texas
- The Center for Health Research, Kaiser Permanente Northwest, Portland, Oregon

7.0 METHODS

7.1 Study population: HCP who have direct contact with patients, including dentists and other dental health personnel, and receive medical care within two healthcare systems in Temple, TX and Portland, OR. The study will prioritize enrollment of HCP who participated in a previous study of influenza vaccine effectiveness that was conducted at both study sites during the 2010-2011 influenza season.

Study duration: Two years spanning two influenza seasons in the United States from August 2018 through approximately April 2020. Participants will be enrolled beginning in August 2018 (or when vaccine is available) and followed for up to approximately 18 months or through the end of influenza virus circulation during the 2019-2020 influenza season at each site. Additional enrollment may be conducted beginning in August 2019 (or when vaccine is available) to make up for participant attrition during the first year of the study with study participants enrolled in 2019 followed for approximately 6 months or through the end of influenza virus circulation during the 2019-2020 influenza season at each site.

7.2 Inclusion Criteria

- Healthcare personnel (HCP) who have direct contact with patients, including dentists and other dental health personnel
- Enrolled in Scott & White Healthcare or Kaiser Permanente health network for at least one month
- Aged 18-64 years

- Available and willing to participate in study follow-up through the end of the 2019-2020 influenza season (i.e. at least approximately 18 months if enrolled during season 1 or 6 months if enrolled during season 2)

7.3 Exclusion Criteria

- Already received an influenza vaccine during the current influenza season
- Previous hypersensitivity reaction to the study vaccines as reported by the subject
- Received any vaccine in the 4 weeks prior to the first study visit or plans to receive a vaccine (other than influenza vaccine provided through the study protocol) in the 4 weeks following the first study visit
- Currently participating in a study that involves an experimental agent (vaccine, drug, biologic, device, blood product, or medication), or has received an experimental agent within 1 month prior to enrollment in this study, or expects to receive an experimental agent during participation in this study
- Any condition that the principle investigator (PI) believes may interfere with successful completion of the study

7.4 a Withdrawal Criteria:

- Medical condition for which continued participation, in the opinion of the investigator, would pose a risk to the participant
- As deemed necessary by the principal investigator for noncompliance or other reasons
- Withdrawal of consent
- Lost to follow up defined as not returning for scheduled study visits within the defined time periods for each visit (as described in section 7.5) for visit 2 in years 1 or 2 for all participants and visit 1 in year 2 for participants enrolled in year 1 of the study.
- Receipt of influenza vaccine outside of the study and after enrollment

7.4b Discontinuation Criteria:

- Termination of the study

7.5 Implementation/monitoring procedures including data collection and analysis:

Study Design: Randomized, open-label study (Overview of stratified randomization and study activities at each site, Figures 1 and 2)

Consent/Screening/ Enrollment

The study will be open to HCP meeting the eligibility criteria at the two study sites. HCP will be recruited using site-specific methods. Consent, screening, and enrollment, and all study visits will take place at designated study site locations. All HCP who are potentially eligible for the study will receive a description of the study (purposes and procedures) and will be asked to read, sign, and date the consent form. The consent form will explain study details, risks, and benefits. Study staff will administer the consent form to HCP. The consent process will take approximately 20 minutes. In addition to receiving a copy of the full consent form, all participants will receive a brief summary of the consent form and an outlined schedule of study visits. All potential study participants will be free to decide whether or not they want to participate in the study.

From the start of enrollment, sites will make efforts to recruit participants to fill each of the four age and prior vaccination strata shown in Figure 1. If enrollment into the strata for persons with no influenza vaccination during the preceding season are not met after a certain period of time, sites may enroll participants with receipt of influenza vaccination during the preceding season into all unfilled strata if mutually agreed upon by CDC and site principle investigators. During the consent process, all participants will be asked to give consent for collection of an additional 30mL of whole blood for PBMC collection at study visit 3 outlined below if they develop RT-PCR-confirmed ILI during the influenza season.

All potential participants will take a self-administered survey or study staff may administer a survey to determine whether or not inclusion/exclusion criteria are met (Appendix B, Eligibility Screening Form). Participants will be enrolled in the study if they meet inclusion criteria and provide consent to be in the study (Appendix C, Consent Form).

Stratified enrollment will be used to ensure an even mix of age groups (18-44 years and 45-64 years) and prior vaccination status (received or did not receive influenza vaccine during the previous influenza season) (Figure 1). Eight hundred and sixty-four participants will be enrolled in year 1 (432 at each site). Additional participants will be enrolled at the start of year 2 to make up for any participants who were lost to follow-up or withdrew from the study in year 1 so that there are 864 participants (432 at each site) enrolled in the study at the start of year 2. In

order to enroll this number of participants, site investigators will plan to screen an additional 30% of potentially eligible HCP (i.e. screen approximately 560 participants at each site) during year 1.

Study Visit 1 of Year 1 [day 0 defined as day of vaccination]:

Study visit 1 may take place on the same day as study screening and consent or at a later date if screening and consent is conducted prior to study vaccine availability at the site. Participants will complete a brief questionnaire about demographic characteristics, health status (body mass index, self-perceived health status), history of chronic medical conditions during the preceding year, concomitant immunosuppressive medication use, household size, history of influenza vaccination during the preceding five years, and hours and types of patient contact encountered as part of work duties (Appendix D, Enrollment Questionnaire).

All study participants will complete a brief questionnaire to determine whether they are currently experiencing fever or other acute illness symptoms (Appendix E, Pre-vaccination Questionnaire/Vaccine Administration Form). Participants who present with moderate-severe illness with fever and/or acute illness during the study visit will not receive study vaccine until their illness has resolved. In this case, a follow up appointment will be made after resolution of the illness, at which time the participant will receive the vaccine.

Randomization: Stratified block randomization will be used at each site to ensure even allocation of age groups (18-44 years and 45-64 years) and prior vaccination status (received or did not receive influenza vaccine during the previous influenza season) to each study arm. Within each of the four strata, participants will be randomized 2:2:1:1 to receive one 0.5 mL intramuscular dose of a study vaccine: Flucelvax™ Quadrivalent, Flublok® Quadrivalent, Fluzone® Quadrivalent or Fluarix ® Quadrivalent. Randomization lists will be generated using a computerized random-number generator to select randomly permuted group blocks of twelve with four in each block assigned to Flucelvax™ Quadrivalent and Flublok® Quadrivalent and two in each block assigned to Fluzone® Quadrivalent or Fluarix ® Quadrivalent. The next available sequential study-number will be assigned to each enrolled participant upon study-entry.

'Opt-In Consent for Longitudinal PBMC Collection:

After participants have been randomized, they may be invited to participate in longitudinal PBMC collection that requires collection of an additional 30mL of blood at study visits 1, 2, and 3 plus an additional study visit 1b as outlined below until up to 25 participants per study arm at

each site have been fully enrolled in this activity. Participants are considered fully enrolled and contributing to the sample size goals for longitudinal PBMC collection once they have completed PBMC collection at study visits 1 and 1b. During year 2 of the study, recruitment methods for PBMC collection may be modified to target selected participants based on their immune responses to vaccination or natural infection during year 1 of the study. A new consent will be required for additional longitudinal PBMC collection activities in year 2.

Blood Draw: All study participants will have 20 ml of venous blood drawn at the first study visit for serology testing. In addition, participants who consent to longitudinal PBMC collection at all study visits will have an additional 30 ml of venous blood drawn for collection of PBMCs to assess cell-mediated immune responses to vaccination.

Vaccine administration: Prior to receiving the influenza vaccine, all study participants will receive an influenza vaccine fact sheet which outlines potential risks, side effects, and benefits of the vaccine. Participants will receive a single (0.5ml) dose of study vaccine administered in the deltoid muscle of the arm opposite the blood draw. Vaccine administration will be performed by a qualified nurse trained in the delivery of the study vaccines and documented on Appendix E, Pre-vaccination Questionnaire/Vaccine Administration Form).

Blinding: Both participants and study investigators will be aware of study arm assignments with the exception of laboratory investigators who will be blinded to assignment until testing is completed, as appropriate.

Post vaccination safety monitoring: Since all study vaccines are licensed for use in participants meeting the eligibility criteria and safety and tolerability of each vaccine have been established in prior studies, data on reactogenicity events and adverse events will not be solicited. However, study staff will provide participants with a phone number to call in case they have any questions after vaccination.

Study Visit 1b of Year 1 [days 6-9]

Blood Draw: Participants who had blood collected at day 0 for collection of PBMCs will be instructed to return on day 7 (acceptable range days 6-9) to have 30 ml of venous blood drawn for collection of PBMCs to assess cell-mediated immune responses to vaccination.

Study Visit 2 of Year 1 [optimal timing days 21-35 (acceptable timing days 21-62)]

Blood Draw: All study participants will have 20 ml of venous blood drawn for serology testing. Participants who had blood collected at day 0 for collection of PBMCs will also have an additional 30 ml of venous blood drawn for collection of PBMCs.

Study Visit 3 of Year 1 [optimal timing days 180-190 (acceptable timing days 180-210) or after influenza circulation has ended]

Blood Draw: All study participants will have 20 ml of venous blood drawn for serology testing. Participants who had blood collected at day 0 for collection of PBMCs and participants who had an episode of RT-PCR-confirmed ILI will also have an additional 30 ml of venous blood drawn for collection of PBMCs at study visit 3.

Study Visit 1 of Year 2 [day 0]

All participants will complete a follow-up questionnaire during study visit 1 of Year 2 (Appendix D, Enrollment Questionnaire). All study participants will also complete a brief questionnaire to determine whether they are currently experiencing fever or other acute illness symptoms (Appendix E, Pre-vaccination Questionnaire/Vaccine Administration Form). Participants who present with moderate-severe illness with fever and/or acute illness during the study visit will not receive study vaccine until their illness has resolved. In this case, a follow up appointment will be made after resolution of the illness, at which time the participant will receive the vaccine.

Randomization of Participants from Year 1 of the Study: Participants from year 1 of the study will remain in the same study arm to which they were assigned by randomization in year 1 (i.e. during study visit 1 of year 1). Study nurses administering vaccine will consult participants' study files to confirm which study vaccine participants from year 1 received during the first year of the study.

Randomization of Newly Enrolled Participants in Year 2: Study staff will review participant allocation across age and prior vaccination strata within each study arm to determine the number of new participants that need to be recruited to maintain even allocation across each group. Stratified block randomization with randomly permuted group blocks of appropriate size will then be used at each site to randomize participants as needed into each of the four study arms to make up for attrition from year 1 enrollment.

Blood Draw: All study participants will have 20 ml of venous blood drawn at the first study visit for serology testing. In addition, the participants selected and consented for longitudinal

collection of PBMCs will have an additional 30 ml of venous blood drawn for collection of PBMCs to assess cell-mediated immune responses to vaccination.

Vaccine administration: Study participants will receive the study vaccine to which they are assigned. The same procedures for vaccine administration outlined above for study visit 1 in year 1 will be followed for vaccine administration during this study visit.

Study Visits 1b, 2, and 3 of Year 2

Study visits 1b, 2, and 3 during year 2 will be conducted at the same time points (i.e. days post-vaccination or after influenza virus circulation has ended) as in year 1 and will include the same study activities as in year 1.

Monitoring of Adverse Events

Because all four study vaccines are licensed for use in the study population and previous studies demonstrated a good safety profile[30-33] in adults for each vaccine, active monitoring for adverse events will not be conducted as part of this trial. Study staff will refer participants who passively report a potential adverse event to their primary care provider for further evaluation as needed.

Active Surveillance for Influenza-Like Illness

For the purposes of active surveillance, ILI will be defined as new onset of cough or worsening of chronic cough during the preceding seven days. Active and passive surveillance for ILI will be conducted during the influenza seasons at each site defined as the time period when influenza viruses are detected through local (e.g. hospital) and/or state surveillance. At enrollment, participants will be advised that they will receive notification from the study team once the influenza season begins at their site and will be instructed to report any illness meeting the ILI case definition to study staff as soon as they develop symptoms. Sites may opt to give participants small items such as refrigerator magnets with the study phone number to remind participants of how to report ILI episodes. Participants will also receive an orientation about self-collection of mid-turbinate nasal swabs and a self-collection swab kit. Throughout the influenza season, study staff will also contact participants via telephone call, email, or text messaging on a weekly basis to ask whether participants have experienced onset of ILI during the preceding seven days. Participants who have illness meeting the ILI case definition will be asked to self-collect a mid-turbinate nasal swab on the same day and deliver the swab to the study site within 3 days of reporting the ILI episode where the day of illness report is defined as

day 0 according to standard operating procedures. If participants prefer to have study staff collect the mid-turbinate nasal swab, they will be instructed to come to the study site or receive a home visit from study staff for swab collection within 3 days of reporting the ILI episode where the day of illness report is defined as day 0. Participants will return to active surveillance 14 days after illness onset. At the end of the active surveillance period during each study year, study staff will complete an Active Surveillance Tracking Form (Appendix J) for each participant to capture aggregate indicators of participant response to surveillance contacts, including the number of weeks that the participant was under active surveillance, the number of surveillance contacts that the participant received, the number of contacts to which the participant responded, and the number of ILI episodes reported by the participant.

In addition, electronic surveillance for participant visits for medically-attended acute respiratory illness (MAARI) will be conducted daily Monday through Friday during the influenza season. When study staff identify MAARI episodes, they will contact participants via telephone call ideally within 1 day, especially for MAARI episodes identified on Monday through Thursday, but no later than within 3 days to confirm that the participant's illness met the case definition for ILI. Participants with ILI will be asked to self-collect a mid-turbinate nasal swab (or have a staff-collected swab if the participant prefers) using the same procedures as outlined above for active ILI surveillance.

Electronic Medical Record Extraction/Abstraction

When feasible, sites investigators will extract (or abstract if necessary) data from participants' electronic medical records and state immunization registries on influenza vaccination history during at least the preceding five years to include, date of receipt, vaccine product name, vaccine lot number, and route of administration (Appendix G, EMR Chart Extraction/Abstraction Form). Participants may be asked to sign release of medical information forms to allow access to vaccination records as needed to meet site-specific data access requirements.

Protocol Deviations

Study staff will report protocol deviations using a Protocol Deviation Log for each participant (Appendix H). Sites may also complete additional protocol deviation forms as required by their local IRBs. Sites will report deviations to their local IRBs according to site-specific reporting requirements.

Data Linkage with Previous Study of Influenza Vaccine Effectiveness among HCP during the 2010-2011 Influenza Season

For participants who previously participated in a CDC-sponsored study of influenza vaccine effectiveness that was conducted at both study sites during the 2010-2011 influenza season, study investigators may link data from the previous study with data collected during the current study.

Protocol Completion or Termination

Study staff will complete a protocol completion or termination form for each participant at the time the participant either completes all protocol procedures or at the time of termination if early termination occurs (Appendix I, Study Status Change Form).

Site Monitoring Plan:

CDC will conduct site monitoring through teleconference calls and site visits (as needed) to ensure that human subject protection, study procedures, laboratory procedures, vaccine administration, and data collection procedures are high quality and meet guidelines, and that the study is conducted in accordance with the protocol and sponsor standard operating procedures. CDC will summarize requests to address any areas for improvement or modification that are identified during teleconference calls or site visits through written communication to site PIs.

Figure 1 Stratified randomization and sample size goals per site, N=432

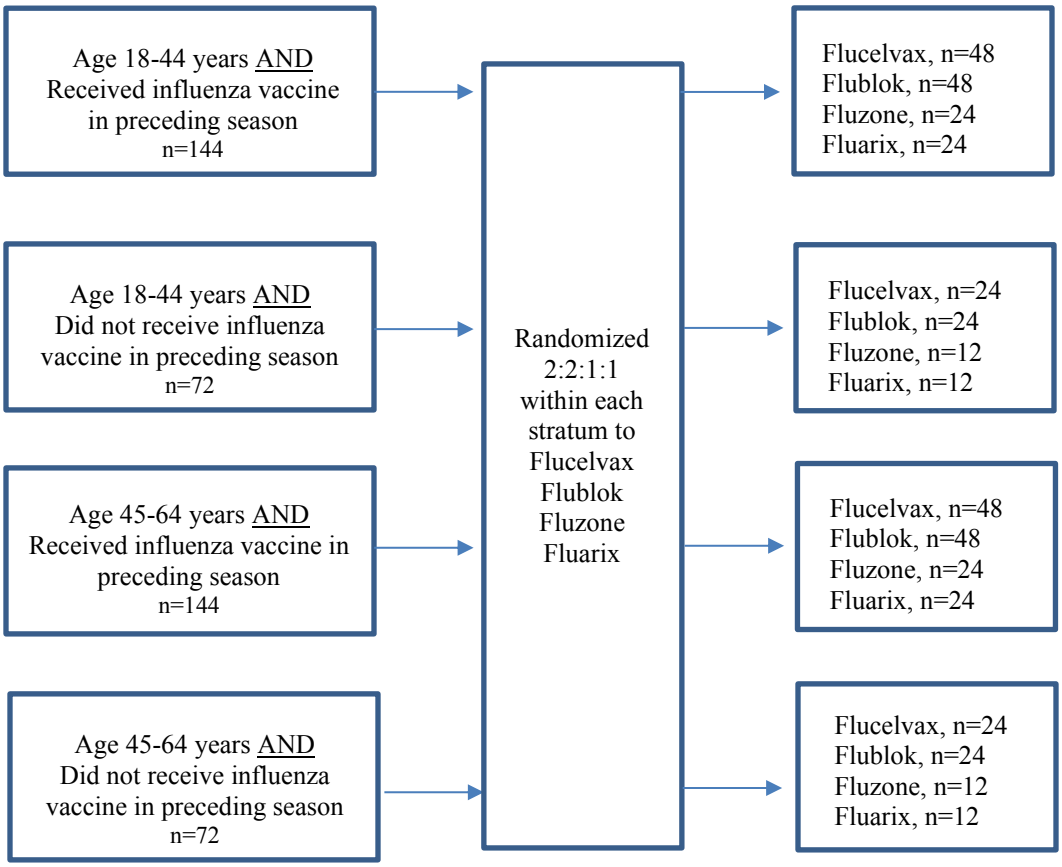
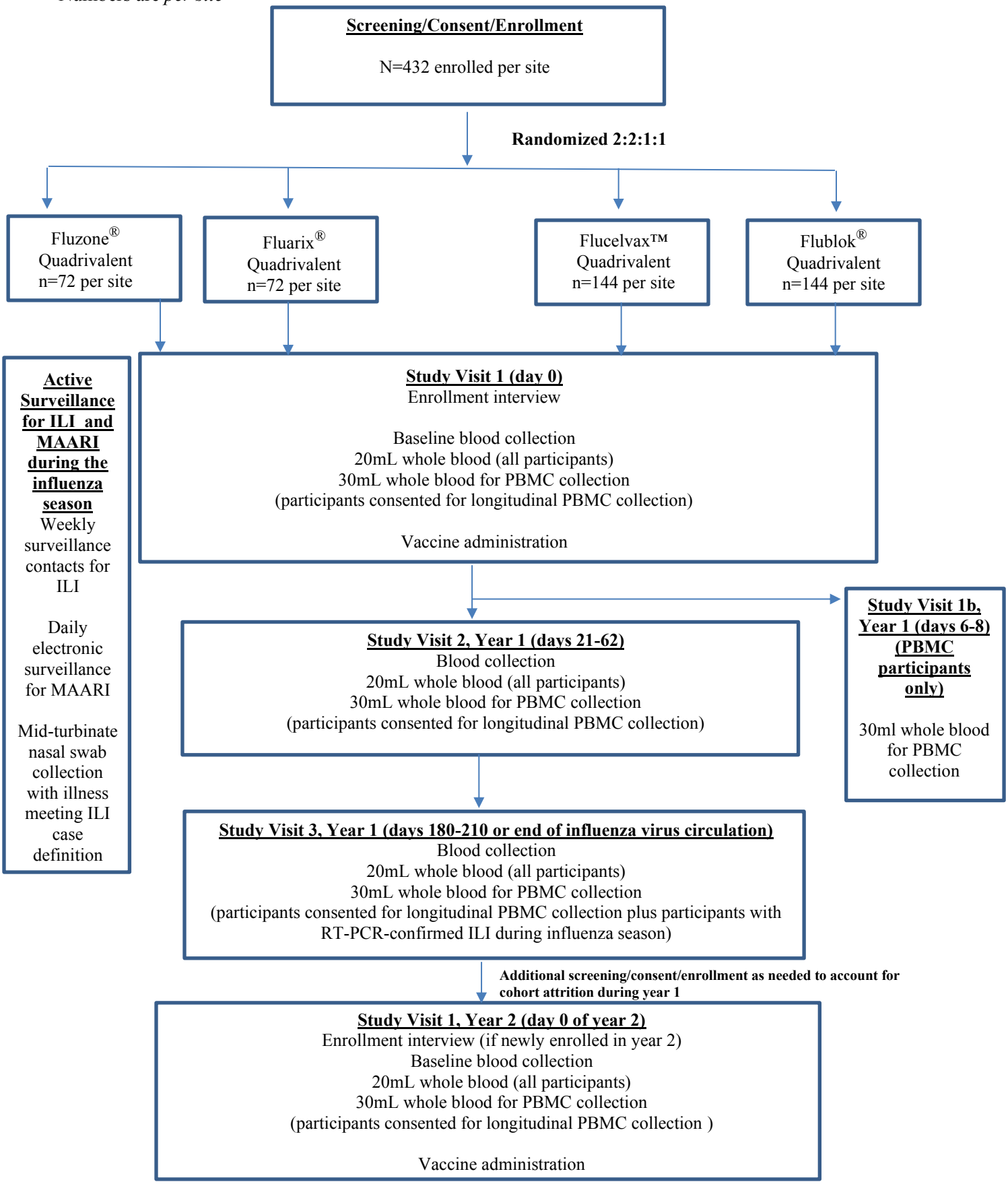


Figure 2 Study activity flow diagram*

*Numbers are *per site*



Statistical Analysis:

Humoral antibody responses as measured by HI (and MN, NAI and ADCC, as appropriate) at baseline (day 0), approximately 28 days and 6 months post-vaccination will be compared between participants in each study arm using Fluzone® Quadrivalent and Fluarix® Quadrivalent as the comparator groups. Participants in the Fluzone® Quadrivalent and Fluarix® Quadrivalent groups initially will be evaluated separately for the primary endpoints of SCR and GMT at 28 days post-vaccination. If the absolute difference in SCR is <15% and there is a $\leq$2 fold difference in post-vaccination GMT between participants in the Fluzone® Quadrivalent and Fluarix® Quadrivalent arms, then participants in these two study arms will be combined for the final analysis of the primary endpoints at 28 days post-vaccination and analyses of secondary endpoints. A difference in post-vaccination GMT of >2 fold between study arms will be considered particularly noteworth for evaluation of the primary endpoint of GMT at 28 days post-vaccination. Ninety-five percent confidence intervals will be calculated for relevant antibody response measures as outlined in the study endpoints at each time point (day 0 and approximately 28 days and 6 months post-vaccination). Post-vaccination titers $\geq$ seropositive thresholds at 1:40, 1:80, 1:160, SCR, GMTs, and MFRs at each time point post-vaccination will be compared between study arms using ANOVA. In addition, a sensitivity analysis will be run using ANCOVA with baseline titers as a covariate.

A sub-group analysis to assess prior vaccination status (number of influenza vaccinations received during the preceding five years) as an effect modifier of differences in GMTs between study arms at 28 days will also be conducted using ANCOVA.

Cell-mediated immune responses at baseline and approximately 7 days, 28 days, and 6 months (or at the end of influenza virus circulation) will be compared between participants in each study arm. Non-parametric tests will be used to carry out comparisons of cell-mediated immunity studies given the small sample size.

All primary analyses will be conducted as intention-to-treat. All tests will be 2-tailed with a level of significance of .05.

Power/Sample size calculations:

Study sample size was determined based on available resources. Assuming a Type 1 error of 5%, a Type 2 error of 20%, an overall sample size of 864 with 288 participants in the Flucelvax® Quadrivalent and Flublok® Quadrivalent arms and 144 participants in each of the Fluarix® Quadrivalent and Fluzone® Quadrivalent arms would provide adequate statistical power to detect a 30% relative difference in SCR between study arms if the SCR among Fluarix®

Quadrivalent and Fluzone® Quadrivalent is ≥50%. Using the same assumptions and sample size would also provide adequate statistical power to detect a difference in post-vaccination GMT of ≥2 fold between study arms if post-vaccination GMT is ≥20. Table 3 provides estimated sample sizes required for varying SCR among recipients of Fluzone Quadrivalent or Fluarix Quadrivalent and differences in SCR between Flucelvax™ Quadrivalent and Flublok® Quadrivalent recipients compared to Fluarix Quadrivalent or Fluzone Quadrivalent recipients.

Based on prior experience enrolling participants at the two study sites, we will assume that 10% of participants from year 1 of the study will drop out by study visit 1 of year 2 of the study. To account for participant loss due to drop out, we will plan to enroll up to approximately 90 additional participants in year 2.

Table 3. Sample size calculations to detect specified differences in SCR between either Flucelvax™ Quadrivalent or Flublok® Quadrivalent compared to Fluzone Quadrivalent or Fluarix Quadrivalent at approximately 28 days post-influenza vaccination

SCR at 28 days post-vaccination to either Fluzone or Fluarix	Relative difference in SCR at 28 days post-vaccination comparing either Flucelvax or Flublok to Fluzone or Fluarix	n per Fluzone or Fluarix arm	n per Flucelvax or Flublok arm
40%	10%	2050	2871
40%	20%	530	742
40%	30%	232	325
40%	40%	132	185
50%	10%	1350	1890
50%	20%	335	470
50%	30%	145	203
50%	40%	80	112
60%	10%	866	1215
60%	20%	208	291
60%	30%	88	123
60%	40%	44	64

SCR: Seroconversion rate. Assumes Fluzone and Fluarix vaccine groups are not collapsed.

Laboratory Protocol:

Specimen Collection/Processing/Storage:

- Serologic studies: 20 mL of blood will be collected from all participants via venipuncture in 10 ml serum separator tubes with clot activator and gel according to CDC guidelines (Appendix K, Human Sera Collection Guidelines for Influenza Serology). Each tube will be labeled or barcode linked with the participant ID number, study visit number, and date of collection. See Table 4 for specimen collection schedule. Collected blood will be stored at

4°C immediately either by placing the sample on ice, in a 4°C refrigerator, or in a cooler with cold packs. Blood may be stored at 4°C for up to 18 hours. Sometime between 1 hour and 18 hours after collection, the blood collection tube will be centrifuged to separate the clotted blood from the serum and the serum removed to a clean tube labeled or barcode linked with the study participant ID number, study visit number, and date of collection. The clotted blood will be discarded. The serum will then be divided into approximately 8-10 aliquots of 100 microliters/aliquot into labeled tubes. After aliquoting, the serum samples will be immediately stored at -20°C or colder.

- PBMC studies: 30 ml of whole blood will be collected at each study visit from participants who consent to longitudinal PBMC collection and at study visit 3 from participants who have RT-PCR-confirmed ILI (see table 2 for specimen collection schedule) and placed in vacutainer tubes containing ethylenediaminetraacetic acid (EDTA) or heparin. PBMCs will be isolated from whole blood and divided into approximately six aliquots of 5x10 million cells/aliquot according to CDC Guidelines for Freezing Human PBMCs (Appendix L). Specimens will be processed within 24 hours of collection. PBMCs will be shipped to the Influenza Laboratory at CDC in Atlanta, Georgia, United States.
- Mid-turbinate nasal swabs will be collected either by participant self-collection or staff collection from participants meeting the study case definition for ILI using flocked dacron swabs. Swabs will be placed immediately into viral transport media. Participants who self-collect swabs will be asked to store the swab in viral transport media in their refrigerators and deliver the swab to the study site within 72 hours of specimen collection. Once participant self-collected swabs are received at the study site, they will be processed, aliquoted and tested or frozen at -70 Celsius within 72 hours according to study standard operating procedures. Swabs that are collected by study staff will be stored immediately at 4°C for no more than 72 hours prior to processing, aliquoting and testing or freezing at -70 Celsius according to study standard operating procedures.
- Participant specimens (blood and respiratory samples) will be stored indefinitely for future use at a CDC-designated facility for future testing to evaluate aspects of respiratory infection and/or immune responses to influenza vaccine or influenza virus infection, as needed.
- Participants will also be asked if they would like to be contacted about future research studies related to this study.

Table 4. Specimen Collection Timing and Specifications

Study Visit	Collection Type	Collection Amount	Testing Purpose	Testing Location
Day 0	Serum	20 ml	HI and MN studies NAI and ADCC studies (as appropriate)	CDC Atlanta or a CDC-designated lab CDC Atlanta
	*PBMCs	30 ml total	Cellular immune responses (B, CD4 and CD8) to influenza vaccine and antibody avidity	CDC Atlanta
6-9 days	*PBMCs	30 ml total	Cellular immune responses (B, CD4 and CD8) to influenza vaccine and antibody avidity	CDC Atlanta
21-35 days (acceptable range 21-62 days)	Serum	20 ml	HI and MN studies NAI and ADCC studies (as appropriate)	CDC Atlanta or a CDC-designated lab CDC Atlanta
	*PBMCs	30 ml total	Cellular immune responses (B, CD4 and CD8) to influenza vaccine and antibody avidity	CDC Atlanta
180-190 (acceptable range 180-210 days)	Serum	20 ml	HI MN, NAI, and ADCC studies (as appropriate)	CDC Atlanta or a CDC-designated lab CDC Atlanta
	*†PBMCs	30 ml total	Cellular immune responses (B, CD4 and CD8) to influenza vaccine	CDC Atlanta
Illness visit during influenza season**	Mid-turbinate nasal swab	N/A	Reverse-transcription polymerase chain reaction for influenza A/H1N1, A/H3N2, B/Yamagata, B/Victoria	Baylor Scott and White Health and Oregon State Public Health Laboratory

*From participants who consent to longitudinal PBMC collection, estimated up to 100 per site.
†Participants with reverse-transcription polymerase chain reaction-confirmed influenza-like illness during the study will have 30mL of whole blood drawn at study visit 3 for PBMC collection.
**For influenza-like illness defined as illness with new onset of cough or worsening of chronic cough during the preceding 7 days.

Specimen Testing:

Sera will be tested by HI and MN to measure antibody titers against cell-grown and egg-grown vaccine reference viruses at approximately 28 days post-vaccination compared to baseline samples drawn at day 0 prior to vaccination during each study season. Sera collected at approximately 6 months post-vaccination or at the end of influenza virus circulation at each site will be tested by HI (and MN, if appropriate) to evaluate duration of immune response. Sera may also be tested by HI and MN against cell-grown wild-type influenza strains, as appropriate. MDCK-SIAT cells or another appropriate cell line will be used to cultivate cell-grown virus strains. A subset of sera from each study arm may also be tested by NAI, ADCC, and other immunologic assays as appropriate to measure activity against antigens represented in the study vaccines at day 0 and at approximately 28 days and 6 months post-vaccination or at the end of

influenza virus circulation at each site. Sera collected at days 0 and approximately 6 months post-vaccination or at the end of influenza virus circulation at each site from participants who have RT-PCR-confirmed ILI during the study period may also be tested by a broad array of assays that may include HI, MN, NAI, ADCC and other assays as appropriate.

Four parameters will be analyzed for HI: SCR, GMT, MFR, and post-vaccination titers $\geq$ seropositive thresholds at 1:40, 1:80, and 1:160. Three parameters will be analyzed for MN: SCR, GMT and MFR. SCR will be defined as the proportion of participants with paired samples that achieved ≥ 4 fold rise comparing post vs pre-vaccination titers and post vaccination titers $\geq$ 1:40. To allow for calculation of HI GMTs, a titer of 1:5 will be assigned to vaccine non-responders. MFR will be defined as the mean of the ratio of post-vaccination HI/MN titer and pre-vaccination HI/MN titer for each subject.

PBMCs will be tested with B and T-cell assays to understand vaccine-induced B, CD4 and CD8 T-cell responses. Immune responses will be quantified and characterized using flurochrome-conjugated HA-probes for H1 and H3 HA components of A viruses and HA-probes for B (Victoria and Yamagata) viruses. If reduced binding to sialic acid (RBS) mutants for B viruses is observed, an intracellular cytokine staining protocol will be used on influenza-stimulated PBMCs. IFN-gamma and TNF-alpha, IL-2 and other T-cell-based cytokines will be examined. The mean percentages of influenza strain-specific plasmablasts and memory B cells and cytokine secreting CD4 and CD8 T-cells will be compared between participants in each study arm. Where feasible, HLA-typing and tetramer based antigen-specific T cell analysis, vaccine component (HA, NA, M2e and stem)-specific B and T cell repertoire and single cell transcriptome will be assessed. Responses will be examined at baseline and at approximately 7 days, 28 days, and 6 months post-vaccination. Influenza HA or NA-specific B cells may also be isolated from a subset of PBMC samples using flow cytometry and single-cell sorting. RNA libraries will then be prepared from these B cells, and the sequence of the heavy and light chain variable regions will be analyzed using next-generation sequencing (NGS).

Mid-turbinate nasal swabs will be divided into 3 aliquots for testing for influenza viruses by RT-PCR using primers for influenza B, A/H1N1, and A/H3N2 viruses. Specimens may also be cultured for influenza viruses or undergo additional influenza virus testing such as for influenza viral load using quantitative RT-PCR methods and for antiviral resistance using neuraminidase inhibition assays and sequencing. Remaining samples will be stored at -70 Celsius at a CDC-designated facility.

7.6

Vaccine Product: Four vaccine products will be used in this trial: Fluzone® Quadrivalent, Fluarix® Quadrivalent, Flucelvax™ Quadrivalent, and Flublok® Quadrivalent. CDC or study sites will purchase vaccine. All four vaccines are inactivated influenza vaccines that are approved for use in adults aged ≥18 years in the United States. All four vaccine products will contain vaccine strains representative of the following four strains in the 2018-2019 formulation: an A/Michigan/45/2015 (H1N1)pdm09-like virus; an A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus; a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage); and a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage).

Fluarix® Quadrivalent is a split-virus/subvirion vaccine manufactured by GlaxoSmithKline. It is formulated from influenza virus grown on embryonated chicken eggs. Vaccine virus is harvested, concentrated and purified using zonal centrifugation, and then inactivated with sodium deoxycholate and formaldehyde. The resulting vaccine suspension is clear and is available for use in adults as prefilled single-dose 0.5mL syringes. Each dose is formulated to contain 15 μg of HA per strain. Each 0.5mL dose of vaccine also contains octoxynol-10 (TRITON X-100) up to 0.115 mg, α-353 tocopheryl hydrogen succinate up to 0.135 mg, and polysorbate 80 (Tween 80) up to 0.550 mg. Each dose may also contain residual amounts of hydrocortisone up to 0.0016 mcg, gentamicin sulfate up to 0.15 mcg, ovalbumin 355 up to 0.050 mcg, formaldehyde up to 5 mcg, and sodium deoxycholate up to 65 mcg.[33]

Fluzone® Quadrivalent is a split-virus/subvirion vaccine manufactured by Sanofi Pasteur. It is formulated from influenza virus grown on embryonated chicken eggs. Vaccine virus is harvested, inactivated with formaldehyde, purified by zonal centrifugation using a sucrose gradient, split by Triton® X-100, and then further purified to a split-virus/subvirion form. The resulting vaccine suspension is clear and is available for use in adults as a prefilled single-dose 0.5mL syringe or a 5mL multi-dose vial; the vaccine is administered intramuscularly. Each dose is formulated to contain 15 μg of HA per strain. Each 0.5mL dose of vaccine also contains sodium phosphate-buffered isotonic sodium chloride solution, up to 100mcg of formaldehyde, and up to 250mcg of octylphenol ethoxylate. Multi-dose vials also contain the preservatives, thimerosal and mercury. The most common reactions occurring after vaccine administration in adults are pain at the injection site, myalgia, headache, and malaise. The majority of these reactions are mild to moderate.[32]

Flucelvax™ Quadrivalent is a subunit vaccine manufactured by Seqirus Inc. The vaccine is formulated from influenza virus cultivated in MDCK cells. Vaccine virus is harvested,

inactivated with ß-propiolactone, split by cetyltrimethylammonium bromide, and then further purified to a subunit form. The resulting vaccine suspension is clear and is available for use in adults as a prefilled single-dose 0.5mL syringe for intramuscular administration. Each dose is formulated to contain 15 μg of HA per strain. Each 0.5mL dose of vaccine also contains phosphate buffered saline and up to 8.4mcg of MDCK cell protein, up to 120mcg of protein other than HA, up to 10ng of MDCK cell DNA, up to 1125 mcg of polysorbate, up to 13.5mcg of cetyltrimethlyammonium bromide, and up to 0.5mcg of β-propiolactone. The vaccine does not contain preservatives. The most common reactions occurring after vaccine administration in adults are pain or erythema at the injection site, headache, fatigue, myalgia, and malaise. The majority of these reactions are mild to moderate.[31]

Flublok® Quadrivalent is a recombinant vaccine manufactured by Sanofi Pasteur. The vaccine is formulated from purified HA protein from cell-culture isolates of the vaccine reference virus grown using baculovirus vector technology in an insect cell line (*expres*SF+®) from cells of the fall armyworm. HA is removed from cells using Triton® X-100 and then purified by chromatography. The resulting vaccine suspension is clear and is available for use in adults as a prefilled single-dose 0.5mL syringe for intramuscular administration. Each dose is formulated to contain 45 μg of HA per strain. Each 0.5mL dose of vaccine also contains 4.4mg of sodium chloride, 0.195mcg of monobasic sodium phosphate, 1.3mg of dibasic sodium phosphate, and 27.5mcg of polysorbate 20. The vaccine may also contain up to 19mcg of baculovirus and *Spodoptera frugiperda* cell proteins, up to 10ng of baculovirus and cellular DNA, and up to 100mcg of Triton X-100.[30]

Vaccine products will not be modified for this study. The vaccines must be stored at 2° C to 8° C [35.6° F to 46.4° F] until needed. Vaccine products should not be frozen and should be protected from natural light. Documentation of proper vaccine storage will be monitored daily and maintained during the duration of the trial. In the event of accidental deep-freezing or disruption of the cold chain, study vaccine will not be administered.

8.0 Ethical Considerations

8.1 Possible Risks

The potential discomforts from study participation include having blood drawn, intramuscular injection of vaccine, and possible reactions to vaccine. Blood samples will be taken from all participants at each study visit. Participants may experience some minimal pain and/or bruising at the site of the blood draw. To prevent or lessen bruising, study staff will apply pressure or ask participants to apply pressure to the draw site for several minutes after the blood

draw. There is also a slight risk of infection associated with blood draws. Study staff will swab the site with alcohol and use sterile equipment to make infection at the site where blood is drawn or where vaccination is given extremely unlikely.

All study vaccines are licensed for participants who meet the eligibility criteria for this study. Occasionally, adult recipients of influenza vaccines may develop influenza-like reactions such as fever, body aches, headache, malaise, myalgia, and/or nausea. If present, these symptoms usually occur soon after vaccination and may last up to 1-2 days post vaccination. Some participants may develop reactions at the site of vaccination (redness, swelling, pain, or tenderness). Analgesics such as ibuprofen or acetaminophen and rest will generally relieve or moderate these symptoms. Acute and potentially life-threatening allergic reactions are also possible. Severe reactions from influenza vaccine are estimated to occur in < 1 per 4 million persons vaccinated. Signs of a severe allergic reaction may include shortness of breath, wheezing, hives, hoarseness, difficulty swallowing, swollen face/ tongue/ pharynx, tachycardia, and dizziness. During the swine influenza campaign in the United States in 1976, about 1 per 100,000 vaccine recipients developed a paralytic illness called Guillain-Barré Syndrome. This level of risk has not been seen with other influenza vaccines. Most persons who develop Guillain-Barré syndrome recover completely. There may be other unknown side effects.

8.2 Provisions for protecting privacy/ confidentiality:

All data collected in this study will be kept confidential. All written information will be stored in locked cabinets with limited access, and electronic information will be stored on secured servers. Blood samples that will be sent to collaborating institutions for processing and analysis will not contain any personal identification information. Collaborating institutions will destroy any stored data after completion of the study and data analysis. During the study, only a few members of the research team will have access to personal identifying information about the study participants. All members of the research team, including the study investigators, research nurses, research assistants, and research coordinators, will have access to de-identified data during the study. Individuals who are not a part of the research team will not have access to the study information or data while the study is taking place. With an official letter and a clearly stated objective, scientists who are not a part of the research team may receive access to de-identified data.

8.3 Consent forms for study volunteers: Written informed consent will be obtained for all potential participants prior to enrollment. In understandable language, trained project staff will explain study procedures to potential participants and discuss the advantages and disadvantages of participating. Participants will be given a copy of the full consent form. Participants will be

asked to read the full consent before agreeing to be in the study and providing their signature. Study coordinators will emphasize the voluntary nature of the study, the possible benefits and outcomes, alternatives to participation, confidentiality of participation, and the participant's right to refuse and/or withdraw from the study at any time. It will be explained to participants that discontinuation of participation or choosing not to participate will not affect their professional standing.

9.0 Study Oversight, Management, and Guidance for Decision Making

A steering committee consisting of representatives from US CDC and each study site will provide high level input into this project. The steering committee will review and approve a data usage agreement as guidance for decision making by the steering committee prior to the start of the study. The day-to-day project management will occur through CDC with the assistance of a contract research organization (to be determined); the steering committee will be consulted on over-arching project issues including final protocol decisions, adjudicating any protocol deviations that might occur, reviewing and confirming analysis plans, and making final decisions on analyses, manuscripts, and authorship as needed. Upon the completion of all study deliverables and after a suitable moratorium, external parties may request de-identified study data from the steering committee as specified in U.S. Government Data Sharing guidelines.

10.0 References

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Appendix A: Schedule of Events*

Study Visit	Screening/ Enrollment	Visit 1, Year 1	Visit 1b**, Year 1	Visit 2, Year 1	Visit 3, Year 1	Visit 1, Year 2
Study Day		0	6-9	21-62	180-210	0
Appendix B: Eligibility Screening Form	X					
Appendix C: Consent Form	X					X if needed
Appendix D: Enrollment Questionnaire		X				X
Appendix E: Pre-vaccination Questionnaire/Vaccine Administration Form		X				X
Appendix F1: Blood Specimen Collection/Tracking Form		X	X	X	X	X
Appendix F2: Influenza-like Illness Data Collection and Respiratory Specimen Collection Form		(X)				
Appendix G: EMR Chart Extraction/Abstraction Form		X				X
Appendix H: Protocol Deviation Log		(X)				
Appendix I: Study Status Change Form		X				
Appendix J: Active Surveillance Tracking Form		X				

Parentheses indicate that forms will be completed as needed.
*Study visits 1b, 2, and 3 are not shown for year 2. Activities are the same as for the analogous visit in year 1.