A Phase II Randomized, Double-Blinded, Controlled Study in Healthy Adults to Assess the Safety, Reactogenicity, and Immunogenicity of a Monovalent Influenza A/H7N9 Virus Vaccine Administered at Different Dosages Given With and Without MF59 Adjuvant

DMID Protocol Number: 13-0032

DMID Funding Mechanism: Vaccine Treatment and Evaluation Units

Pharmaceutical Support:

sanofi pasteur

Novartis Vaccines and Diagnostics

IND Sponsor: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

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Version: 2.0

September 30, 2013
STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with Good Clinical Practices (GCP) as required by the following:

- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997);
- National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

Compliance with these standards provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.
SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable United States of America (US) federal regulations and ICH guidelines.

Site Principal Investigator: ________________________________
Name/Title (Print)

Signature: ________________________________

Date: ____________________
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LIST OF ABBREVIATIONS

A/H1N1  Influenza A Virus of the H1N1 Subtype
A/H3N2v Influenza A Virus of the H3N2 variant Subtype
A/H5N1  Influenza A Virus of the H5N1 Subtype
A/H7N7  Influenza A Virus of the H7N7 Subtype
A/H7N9  Influenza A Virus of the H7N9 Subtype
A/H9N2  Influenza A Virus of the H9N2 Subtype
ADCC   Antibody-Dependent Cell-Mediated Cytotoxicity
AdvantageEDCSM Electronic Data Capture System
AE     Adverse Event/Adverse Experience
ASC    Antibody-Secreting Cell
BMI    Body Mass Index
BP     Blood Pressure
BPM    Beats Per Minute
CAR    Clinical Agents Repository
CDC    Centers for Disease Control and Prevention
CFR    Code of Federal Regulations
CI     Confidence Interval
CRF    Case Report Form
°C     Degrees Celsius
°F     Degrees Fahrenheit
D      Day(s)
DHHS   Department of Health and Human Services
DMID   Division of Microbiology and Infectious Diseases, NIAID, NIH
DSMB   Data and Safety Monitoring Board
ELISpot Enzyme-Linked Immunosorbent Spot
eCRF   Electronic Case Report Form
FACS   Fluorescent Activated Cell Sorter
FDA    Food and Drug Administration
FDAAA  Food and Drug Administration Amendments Act
FWA    Federalwide Assurance
GBS    Guillain-Barré Syndrome
GCP    Good Clinical Practice
GMC    Geometric Mean Concentration
GMT    Geometric Mean Titer
HA     Hemagglutinin
HAI    Hemagglutination Inhibition
HEENT  Head, Eyes, Ears, Nose, and Throat
HIV    Human Immunodeficiency Virus
IATA   International Air Transport Association
ICF    Informed Consent Form
ICH  International Conference on Harmonisation
ICMJE  International Committee of Medical Journal Editors
IEC  Independent or Institutional Ethics Committee
IgG  Immunoglobulin G
IGHV  Immunoglobulin Heavy Chain Variable
IM  Intramuscular
IND  Investigational New Drug Application
IRB  Institutional Review Board
ISM  Independent Safety Monitor
ITT  Intent-to-Treat
IUD  Intrauterine Device
LAIV  Live, Attenuated Influenza Vaccine
mAb  Monoclonal Antibody
mcg or μg  Microgram(s)
mcg/mL or μg/mL  Micrograms per milliliter
MedDRA®  Medical Dictionary for Regulatory Activities
MF59  MF59C.1 Adjuvant
mg  Milligram(s)
mg/mL  Milligrams per milliliter
mL  Milliliter(s)
mm  Millimeters
MOP  Manual of Procedures
N  Number of Subjects
Neut  Neutralizing or Neutralization
NIAID  National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH  National Institutes of Health
NLM  National Library of Medicine
NSAIDs  Non-Steroidal Anti-Inflammatory Drugs
OCRA  Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHPR  Office for Human Research Protections
OHSR  Office for Human Subjects Research
ORA  Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PBMC  Peripheral Blood Mononuclear Cell
PHI  Personal Health Information
PI  Principal Investigator
PP  Per Protocol
PREP Act  Public Readiness and Emergency Preparedness Act
QA  Quality Assurance
QC  Quality Control
RP-HPLC  Reversed-Phase High-Performance Liquid Chromatography
SAE  Serious Adverse Event/Serious Adverse Experience
SD  Standard Deviation
<table>
<thead>
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<tr>
<td>SDCC</td>
<td>Statistical and Data Coordinating Center</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
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<tr>
<td>SRID</td>
<td>Single Radial Immunodiffusion</td>
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<tr>
<td>T&lt;sub&gt;H&lt;/sub&gt;</td>
<td>CD4+ Helper T Cells</td>
</tr>
<tr>
<td>T&lt;sub&gt;FH&lt;/sub&gt;</td>
<td>T Follicular Helper Cells</td>
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<tr>
<td>US</td>
<td>United States</td>
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<td>Vaccine and Treatment Evaluation Unit(s)</td>
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PROTOCOL SUMMARY

Title: A Phase II Randomized, Double-Blinded, Controlled Study in Healthy Adults to Assess the Safety, Reactogenicity, and Immunogenicity of a Monovalent Influenza A/H7N9 Virus Vaccine Administered at Different Dosages Given With and Without MF59 Adjuvant

Phase: II

Population: Up to 700 males and non-pregnant females, 19 to 64 years old, inclusive, who are in good health and meet all eligibility criteria

Number of Sites: At least 4 Vaccine and Treatment Evaluation Unit (VTEU) sites

Study Duration: Approximately 24 months

Subject Participation Duration: Approximately 13 months

Estimated Time to Complete Enrollment: Approximately 6 weeks

Description of Agent: Two doses delivered intramuscularly approximately 21 days apart of a monovalent influenza A/H7N9 virus vaccine (hemagglutinin (HA) of A/Shanghai/2/2013 manufactured by sanofi pasteur administered at different dosages (3.75, 7.5, or 15 micrograms (mcg) of HA/0.5 milliliter (mL) dose) given with MF59C.1 (MF59) adjuvant manufactured by Novartis Vaccines and Diagnostics or without adjuvant (15 mcg of HA/0.5 mL dose and 45 mcg of HA/0.75 mL dose)

Study Objectives: Primary:

Safety:

- To assess the safety and reactogenicity of a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

Immunogenicity:

- To assess the serum antibody responses to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.
Secondary:

Safety:
- To assess unsolicited adverse events following receipt of two doses of a monovalent influenza A/H7N9 virus vaccine administered with and without MF59 adjuvant.
- To assess new-onset chronic medical conditions following receipt of two doses of a monovalent influenza A/H7N9 virus vaccine administered with and without MF59 adjuvant.

Immunogenicity:
- To assess the serum hemagglutination inhibition (HAI) antibody responses to a monovalent influenza A/H7N9 virus vaccine following receipt of one dose administered with and without MF59 adjuvant.
- To assess the serum neutralizing antibody (Neut) responses to a monovalent influenza A/H7N9 virus vaccine following receipt of each dose administered with and without MF59 adjuvant.

Exploratory:

Immunogenicity:
- To assess the effects of age, gender, body mass index, waist circumference, prior receipt of seasonal influenza vaccine, and geography (VTEU site) on serum antibody responses to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.
- To identify and quantitate vaccine-induced antibody-secreting cells (ASCs) or plasmablasts, and rapidly clone and express monoclonal antibodies (mAbs) they produce in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.
- To analyze the origin and the degree of clonality of the responding B cells by clonal analysis of the Immunoglobulin Heavy Chain Variable (IGHV) genes and the degree of somatic hypermutation within these genes in
a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of the second dose administered with and without MF59 adjuvant.

- To assess blood CD4+ Helper T Cells (T_H) and T Follicular Helper Cells (T_FH) cell responses, including T_H epitopes, in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant, and perform correlations of blood T_H and T_FH cell responses with HAI and Neut titers, CD38+CD27+ total plasmablast responses detected by Fluorescent Activated Cell Sorter (FACS) analysis, and antigen- and isotype-specific plasmablast responses by Enzyme-Linked Immunosorbent Spot (ELISpot) assay.

- To analyze the specificity of mAb against A/H7N9 and other influenza strains including evolved variants, if available, define the epitopes (at head or stalk region of HA protein) recognized by the mAb generated in response to A/H7N9 vaccination, and compare avidity and breadth of mAb generated in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of the second dose administered with and without MF59 adjuvant.

- To analyze serum antibody responses for antibody-dependent cell-mediated cytotoxicity (ADCC) in a subset of samples in response to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

**Study Outcome Measures:**

**Primary:**

**Safety:**

- Occurrence of study vaccine-related serious adverse events from the time of the first study vaccination through approximately 13 months after the first study vaccination.

- Occurrence of solicited injection site and systemic reactogenicity on the day of each study vaccination through 7 days after each study vaccination.
Immunogenicity:

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 21 days after the second study vaccination.

- Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at approximately 21 days after the second study vaccination.

Secondary:

Safety:

- Occurrence of unsolicited adverse events from the time of the first study vaccination through approximately 21 days after the last study vaccination.

- Occurrence of new-onset chronic medical conditions through 13 months after the first study vaccination.

Immunogenicity:

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 8 and 21 days after the first study vaccination.

- Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at baseline and at approximately 8 and 21 days after the first study vaccination.

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 8 days after the second study vaccination.
- Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at approximately 8 days after the second study vaccination.

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination Neut titer <1:10 and a post-vaccination Neut titer ≥1:40 or a pre-vaccination Neut titer ≥1:10 and a minimum four-fold rise in post-vaccination Neut antibody titer) at approximately 8 and 21 days after each study vaccination.

- Percentage of subjects achieving a serum Neut antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at baseline and at approximately 8 and 21 days after each study vaccination.

- Geometric Mean Titers of serum HAI and Neut antibody at baseline and at approximately 8 and 21 days after each study vaccination.

**Exploratory:**

**Immunogenicity:**

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) and Geometric Mean Titers of serum HAI antibody at baseline and at approximately 8 and 21 days after each study vaccination as a function of age, gender, body mass index, waist circumference, prior receipt of seasonal influenza vaccine, and geography (VTEU site).

- Development of serum antibody responses against antigenically drifted variants of the A/H7N9 virus in at least a subset of samples, should any variants occur and be available prior to the last subject’s Visit 07.

- Total ASC response by flow cytometry (defined as CD3-/CD20lo/CD19+/CD38hi/CD27hi cells and reported as a percentage of total lymphocytes or of total B cells) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study.
vaccination.

- Frequency of vaccine-specific ASCs measured by ELISpot assay reported as the number of influenza-specific ASCs per million peripheral blood mononuclear cells (PBMCs) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

- IGHV gene sequence analyses for B cell gene usage, clonality, and somatic hypermutation within these genes in a subset of healthy adults, 19-64 years old, at approximately 8 days after the second study vaccination.

- Frequency of blood $T_{FH}$ cell responses (reported as the number of ICOS$^+$CXCR3$^+$CXCR5$^+$ CD4$^+$ T cells) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

- Frequencies and quality of $T_H$ cells and epitope specificities in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

- Correlations of blood $T_H$ and $T_{FH}$ responses with HAI and Neut titers, plasmablast responses detected by FACS analysis, and antigen-specific plasmablast responses by ELISpot assay in a subset of healthy adults, 19-64 years old, at baseline and approximately 8 and 21 days after each study vaccination.

- Assess the specificity of mAb against A/H7N9 and other influenza strains including evolved variants, if available, compare the epitopes (at head or stalk region of HA protein) recognized by the mAb generated by A/H7N9 vaccination, and analyze the avidity and breadth of mAb generated by A/H7N9 vaccination with and without MF59 adjuvant in a subset of healthy adults, 19-64 years old, at approximately 8 days after the second study vaccination.

- Percentage of subjects demonstrating detectable levels of ADCC responses against the epitopes (at head or stalk region of HA protein) generated by A/H7N9 vaccination, and the correlation of the ADCC responses with HAI and Neut titers generated by A/H7N9 vaccination with and
without MF59 adjuvant in a subset of samples at approximately 21 days after the second study vaccination.

**Description of Study Design:**

This is a Phase II randomized, double-blinded, controlled study in up to 700 males and non-pregnant females, 19 to 64 years old, inclusive, who are in good health and meet all eligibility criteria. The study is designed to assess the safety, reactogenicity, and immunogenicity of a monovalent influenza A/H7N9 virus vaccine manufactured by sanofi pasteur administered to healthy adults at different dosages (3.75, 7.5, or 15 mcg of HA/0.5 mL dose) given with MF59 adjuvant manufactured by Novartis Vaccines and Diagnostics or without adjuvant (15 mcg of HA/0.5 mL dose and 45 mcg of HA/0.75 mL dose). The A/H7N9 vaccine was made with HA antigen derived from the influenza A/Shanghai/2/2013 virus.

Subjects will be randomly assigned to 1 of 7 groups (up to 100 subjects per group) to receive two doses of the A/H7N9 vaccine with or without MF59 adjuvant delivered intramuscularly approximately 21 days apart. The same dosage of A/H7N9 vaccine will be given to subjects at both their first and second study vaccinations.

Safety will be measured from the time of each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) through 7 days after each study vaccination by the occurrence of solicited injection site and systemic reactogenicity events. Unsolicited non-serious adverse events (AEs) will be collected from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 21 days after the last study vaccination (approximately Day 42 (Visit 05) for subjects who receive two study vaccinations; approximately Day 21 (Visit 03) for subjects who receive only one study vaccination). After approximately 21 days after the last study vaccination, non-serious AEs will be limited to new-onset chronic medical conditions, which will be documented through approximately 13 months after the first study vaccination (Visit 09). Serious adverse events (SAEs) will be collected from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 13 months after the first study vaccination (Visit 09).

Immunogenicity testing will include performing hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays on serum
obtained prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after each study vaccination (Visit 02 and Visit 04) and approximately 21 days after the second study vaccination (Visit 05).

From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 85 mL of venous blood will be drawn prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after the second study vaccination (Visit 04). Also, an additional 37 mL of venous blood will be drawn approximately 8 days after the first vaccination (Visit 02) and an additional 53 mL of venous blood will be drawn approximately 21 days after the second study vaccination (Visit 05). These additional blood samples will be used to characterize the kinetics and quality of antibody-secreting cells (ASCs) and CD4+ T cell responses by Fluorescent Activated Cell Sorter (FACS) analysis and Enzyme-Linked Immunosorbent Spot (ELISpot) assays at serial time points following receipt of the first and second dose of study vaccine as indicated above. In addition, monoclonal antibody (mAb) from 10 subjects will also be generated after single-cell sorting of ASCs at approximately 8 days after the second study vaccination (Visit 04). The origin and the degree of clonality of these mAbs will be defined, and their potential value as therapeutics for drug-resistant A/H7N9 infections will be assessed.

Novel methods for identifying and assessing alternative correlates of protection against influenza infection are needed. An antibody dependent cell-mediated cytotoxicity (ADCC) assay for H7 is in development. If successful, the potential for A/H7N9 vaccines to generate non-neutralizing antibody responses aimed against both the globular head and the stalk region of the influenza hemagglutinin protein will be assessed in a subset of samples obtained at approximately 21 days after the second study vaccination (Visit 05).
Table 1. Groups and study vaccine to be administered:

<table>
<thead>
<tr>
<th>Group (n=up to 100 subjects per Group)</th>
<th>First Dose (Day 0; Visit 01)</th>
<th>Second Dose (Day 21+3 days; Visit 03)</th>
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<tr>
<td>1</td>
<td>sanofi A/H7N9 antigen 3.75 mcg plus Novartis MF59 adjuvant</td>
<td>sanofi A/H7N9 antigen 3.75 mcg plus Novartis MF59 adjuvant</td>
</tr>
<tr>
<td>2</td>
<td>sanofi A/H7N9 antigen 7.5 mcg plus Novartis MF59 adjuvant</td>
<td>sanofi A/H7N9 antigen 7.5 mcg plus Novartis MF59 adjuvant</td>
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<tr>
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<td>sanofi A/H7N9 antigen 15 mcg plus Novartis MF59 adjuvant</td>
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<td>5</td>
<td>sanofi A/H7N9 antigen 15 mcg</td>
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<td>7</td>
<td>sanofi A/H7N9 antigen 45 mcg</td>
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Total N=up to 700 subjects
**Figure 1. Schematic of Study Design:**

**Planned Enrollment:** Total N=up to 700 subjects
males and non-pregnant females, 19 to 64 years old, inclusive

<table>
<thead>
<tr>
<th>Group</th>
<th>First Dose</th>
<th>Second Dose</th>
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<tr>
<td>7</td>
<td>45 μg no adjuvant</td>
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**Administer Study Vaccine per Group Assignment**
on Day 0 (Visit 01) and Day 21+3 days (Visit 03)

**Clinical, Safety, Reactogenicity, and Immunogenicity Assessments**

**Assessment of Final Outcome Measures**
1 KEY ROLES

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Immunology Exploratory Assays Laboratory:  
Emory VTEU site

ADCC Assay Laboratory: TBD
2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The continued emergence of novel influenza A viruses in humans—including subtypes H5N1, H3N2v, H7N7, H9N2, 2009 H1N1, and most recently H7N9, adds urgency to ongoing efforts to prepare for the next influenza pandemic (1-6). Four pandemics occurred during the last century. It was estimated that during the 1918 influenza A/H1N1 pandemic as many as 40 million deaths occurred worldwide (7). Excess mortality, high morbidity, and social disruption were all noted during the 1957 influenza A/H2N2 and the 1968 influenza A/H3N2 pandemics (8). In April 2009, a novel type of influenza virus (2009 A/H1N1) originated in pigs and spread to humans around the world becoming the first pandemic of this century. In each of these influenza pandemics, human populations lacked significant levels of pre-existing immunity to a highly transmissible form of the virus enabling it to spread rapidly. Thus, each emergence of new subtype of influenza virus in the human population has the potential to produce a global public health emergency.

Major cornerstones of influenza pandemic preparedness include enhanced surveillance aimed at the earliest possible identification of an emerging novel virus in humans and the capacity to rapidly produce sufficient quantities of safe and effective strain-specific vaccines. The threat of pandemic influenza in 1976 (swine influenza) and again in 1977 (Russian influenza) resulted in inactivated influenza virus vaccine development programs that provided important insights into variables influencing the immune responses to immunization (9, 10). Vaccine factors potentially affecting the immunogenicity of inactivated influenza virus vaccines were noted during the 1976 experience and in subsequent years. These factors included dosage of viral hemagglutinin (HA) protein in the vaccine, the number of doses administered (1 or 2), and the manufacturing methods used (whole virus, split virus, or purified surface antigen). Currently, standard-dose inactivated seasonal influenza vaccines contain 7.5 mcg HA antigen per vaccine strain (for children aged < 36 months) or 15 mcg of HA antigen (for persons aged ≥ 36 months) per vaccine strain. Host factors including age, prior priming, presence of underlying disease, and treatments of disease can all influence the immune responses to an influenza vaccine.

Serum IgG antibody to the influenza virus HA has a major role in protective immunity to influenza virus infection (11). Resistance to infection with seasonal influenza virus strains correlates directly with both serum hemagglutination inhibition (HAI) and neutralizing (Neut) antibody levels, and measurements of serum HAI and Neut antibodies are used to assess the immunogenicity of both inactivated seasonal and pandemic influenza vaccines.
One of the approaches for improving the immunogenicity of inactivated influenza vaccines is to increase the dosage of the HA antigen contained in a dose of vaccine. Studies evaluating the effect of HA dosage on the resulting immune responses to seasonal inactivated influenza vaccines have been performed over the past 35 years and have shown dose-related increases in serum and mucosal antibody responses (12-20). Higher vaccine dosage levels are also associated with the development of higher titers of serum antibodies that recognize antigenically distinct drift variants (21).

The safety and effect of HA dosage on the immunogenicity of inactivated vaccines made from novel influenza viruses (e.g., H9N2, H5N1, H7N7) has also been clinically evaluated. Similar to seasonal inactivated vaccines, higher dosages of HA have been associated with the generation of more frequent and higher antibody titers; however, clinical trials of inactivated H5N1 and H7N7 vaccines have shown that H5 and H7 HA proteins may be substantially less immunogenic than HAs from viruses included in seasonal vaccines or the limited inactivated H9N2 vaccines that have been studied. For example, in safety and dose-ranging immunogenicity studies of a subvirion inactivated influenza A/Vietnam (H5N1) vaccine in healthy young adults revealed that two 90-mcg doses of vaccine were required to stimulate an antibody response in approximately 57% of young healthy adults (22). An NIAID-sponsored Phase I/II trial evaluated the safety and dose-ranging immunogenicity of an inactivated influenza H7N7 vaccine in adults 18 to 40 years of age. Study participants received 2 doses of one of several unadjuvanted formulations of the vaccine (7.5 mcg, 15 mcg, 45 mcg, or 90 mcg) or placebo approximately one month apart. The vaccine was well tolerated; however, very few subjects generated any measureable antibody responses, leading the investigators to conclude that the vaccine was very weakly immunogenic (23).

The use of adjuvants is another promising approach to improve the immunogenicity of influenza vaccines. Adjuvants have the potential to increase the serum immune responses at a given dose of antigen, to decrease the amount of antigen needed in the vaccine (dose-sparing), and to improve the immune responses among some groups that generally respond poorly to inactivated antigens (e.g., immunocompromised, elderly) (24). In most influenza vaccine studies to date, the inclusion of adjuvants has also been associated with an increase in injection site reactogenicity (24). Aluminum salts are licensed as adjuvants in the United States; however, its use in subvirion H5N1 influenza vaccines has shown either no effect or a very modest enhancement of immune responses compared to non-aluminum salt containing formulations (24-26).

In contrast, oil-in-water emulsion adjuvants have shown promise in stimulating increased antibody responses to inactivated influenza vaccines containing novel HAs (27). MF59, an oil-in-water adjuvant used in Novartis Vaccines and Diagnostics’ seasonal influenza vaccine (FLUAD®) has been licensed for the elderly (65 years of age and older) in several European and non-European countries starting in 1997. Nicholson et al. evaluated the safety and immunogenicity of MF59-adjuvanted and unadjuvanted inactivated H5N3 vaccines in a clinical trial (28). Study participants received 2 doses of vaccine (7.5, 15, or 30 mcg of HA) with or
without MF59 adjuvant 3 weeks apart. Geometric mean titers of antibody and seroconversion rates were significantly higher among recipients of the adjuvanted vaccine when compared with recipients of non-adjuvanted formulations.

In response to the emergence of the 2009 H1N1 pandemic virus in early 2009, a MF59-adjuvanted egg-derived monovalent 2009 H1N1 vaccine (Focetria™) was licensed in the EU on 29 September 2009. An MF59-adjuvanted cell-derived inactivated vaccine (Celtura™) was licensed in Germany and Switzerland in November, 2009 and in Japan in January, 2010. To date, a large number of clinical trials with investigational seasonal and pandemic influenza vaccines containing MF59 adjuvant have been or are being performed in different age groups (from newborns to elderly) in Europe and the US and have shown an increased immunogenicity and a good safety and tolerability profile (27-31).

Adjuvants are expected to be a critical part of the public health response to an H5N1 pandemic if it occurs in the near term because the high dosage of unadjuvanted H5N1 vaccine needed to elicit antibody responses and the impracticality of that dosage for mass vaccination, especially if multiple administrations are required. These concerns coupled with the continued, albeit sporadic outbreaks of influenza H5N1 viruses in humans resulted in the U.S. Government stockpiling several different H5N1 vaccines over the past decade. Because of the need to be able to maximize the number of doses of immunogenic vaccine and because novel influenza infections in humans have been caused by viruses with different HA proteins, oil-in-water emulsion adjuvants, such as MF59, have been stockpiled by the Department of Health and Human Services (DHHS) as part of pandemic preparedness efforts. Since vaccines and adjuvants may be made by different manufacturers, one strategy for the U.S. Government to expand the number of potential doses of vaccine available is to determine if the vaccine from one manufacturer can be mixed prior to administration with an adjuvant produced by a different manufacturer. To test this strategy, the NIAID sponsored a Phase I clinical trial to evaluate the safety and immunogenicity of an inactivated H5N1 vaccine manufactured by sanofi pasteur administered with and without MF59 in healthy adults (32). A similar study was also conducted by NIAID using the sanofi pasteur H5N1 vaccine with AS03, another oil-in-water adjuvant produced by GlaxoSmithKline Biologicals (33). While the data from these studies are not yet publicly available, a third NIAID study in healthy adults using AS03 with a 2009 H1N1 vaccine produced by sanofi pasteur confirmed that the extemporaneously mixed adjuvant and vaccine from these different manufacturers was safe and well tolerated and that the inclusion of the adjuvant improved antibody responses (34).

On March 31st, 2013, the Chinese Center for Disease Control and Prevention confirmed that a novel avian influenza A (H7N9) virus had infected 3 humans in China causing severe disease, and on April 11th, 2013, the first report detailing the clinical features and outcomes of these patients was published (35). The individuals were hospitalized with severe bilateral pneumonia and leukopenia, and each case progressed to severe pneumonia, ARDS, and death. As of August 12th, 2013, the World Health Organization reports a total of 135 laboratory-confirmed A/H7N9 human infections including 44 deaths. The median age of the cases is 58 years with 4
cases confirmed in children. Three family clusters of at least two confirmed cases have been identified in which limited human-to-human transmission may have occurred; however, to date sustained human-to-human transmission has not been identified (3). Recent laboratory studies have shown that A/H7N9 influenza viruses readily infect cells from human respiratory tract tissue samples and can spread from ferret to ferret by droplet transmission, thereby increasing the concern about the pandemic potential of these viruses (36, 37).

2.1.1 Public Readiness and Emergency Preparedness Act

This protocol and the A/H7N9 vaccine and MF59 adjuvant tested are covered under the Public Readiness and Emergency Preparedness Act (PREP Act). Under the PREP Act, covered persons are immune from liability actions brought from the administration or use of a covered countermeasure that is the subject of a declaration. The PREP Act provides immunity for covered persons (such as manufacturers, distributors, program planners and other qualified persons who prescribe, administer or dispense the study vaccine) from tort liability, unless the injury was caused by willful misconduct.

The PREP Act also authorized a “Covered Countermeasures Process Fund” to provide compensation to eligible individuals who suffer specified injuries from administration or use of a countermeasure pursuant to the declaration. Any requests for compensation must be filed within one year of administration or use of the countermeasure. Requests would go to the HRSA Preparedness Countermeasures Injury Compensation Program (http://www.hrsa.gov/cicp/). Compensation may then be available for medical benefits, lost wages and death benefits to eligible individuals for specified injuries in accordance with regulations published by the Secretary. Eligibility for compensation and the injuries for which compensation may be available are further defined by regulation.

An individual who suffers a serious physical injury or death from administration and use of the study vaccine with or without the adjuvant must first seek compensation from the Covered Countermeasures Process Fund. A serious physical injury means an injury that is life threatening, results in, or requires medical or surgical intervention to prevent, permanent impairment of a body function or permanent damage to body structure. Any compensation will be reduced by public or private insurance or worker’s compensation available to the injured individual.

If no funds have been appropriated to the compensation program, the Secretary does not make a final determination on the individual’s request within 240 days, or if the individual decides not to accept the compensation, the injured individual or his representative may pursue a tort claim in the United States District Court for the District of Columbia, but only if the claim involves willful misconduct, is pled with particularity required under the PREP Act, verified, and accompanied by an affidavit by a physician who did not treat the individual and certified medical records. Any award is reduced by any public or private insurance or worker’s compensation available to the injured individual. Awards for non-economic damages, such as pain, suffering,
physical impairment, mental anguish, and loss of consortium are also limited. If the individual accepts compensation, or if there is no willful misconduct, then the individual does not have a tort claim that can be filed in a United States Federal or a State court.

2.2 Scientific Rationale

In the event that the lethal A/H7N9 influenza virus that emerged in humans in China acquires the ability to readily spread from person to person, it is anticipated that a large-scale national vaccination program will be initiated. Additionally, initial studies of inactivated influenza vaccines made from influenza viruses with H7 HA, indicate that an unadjuvanted vaccine may be only weakly immunogenic and that adjuvants may be needed to enhance the immune response.

Because of the prior experience with the inactivated unadjuvanted A/H7N7 vaccine that was very weakly immunogenic, it is anticipated that other H7 vaccines may need to be administered with an adjuvant to generate substantial immune responses. Therefore, DHHS is contracting with sanofi pasteur for the production of an inactivated A/H7N9 vaccine. The availability of an A/H7N9 vaccine and the stockpiled MF59 oil-in-water adjuvant provides the opportunity to assess the A/H7N9 vaccine for safety and dose-ranging immunogenicity as well as whether the inclusion of an oil-in-water emulsion adjuvant improves the immunogenicity of the A/H7N9 vaccine. The U.S. Government, through its VTEUs is uniquely positioned to conduct these types of trials which may provide critical clinical data needed to respond to the potential threat of an A/H7N9 pandemic.

The goal of this study is to assess the safety and tolerability of the study vaccine formulations and the potential of the adjuvant to enhance the immune response to the A/H7N9 vaccine in healthy adults. Other goals are to assess whether a low HA dosage results in a robust immune response, to evaluate HA antigen- and adjuvant-sparing dosing strategies, and to evaluate in at least a subset of samples the generation of serum antibody responses against antigenically drifted variants of the A/H7N9 virus should they emerge prior to the last subject’s Visit 07.

The study will also evaluate the strategy of administering an A/H7N9 vaccine from one manufacturer extemporaneously mixed prior to administration with adjuvant from a different manufacturer. Such a strategy might prove critical in the setting of a public health emergency, when stockpiled vaccine and/or adjuvant might need to be deployed rapidly for prevention of infection or for the decrease in disease severity.

Based on similar studies, we anticipate that the MF59-adjuvanted A/H7N9 vaccine will be well tolerated and that inclusion of the adjuvant will enhance the immune response. If A/H7N9 cases continue to emerge without sustained human-to-human spread and the initial safety assessment from this study is acceptable, subsequent testing of the A/H7N9 vaccine/adjuvant combination is planned in elderly and pediatric populations. If the current epidemiological profile changes and sustained human-to-human infections are reported, it is anticipated that studies in the elderly
and pediatric populations will be initiated concurrently with this study or as close to concurrently as possible.

In addition, immunology exploratory assays performed on samples collected from a subset of healthy adult subjects enrolled in this protocol will be used to characterize the kinetics and quality of antibody-secreting cells (ASCs) and CD4+ T cell responses by Fluorescent Activated Cell Sorter (FACS) analysis and Enzyme-Linked Immunosorbent Spot (ELISpot) assays at serial time points following receipt of the first and second dose of study vaccine. In addition, monoclonal antibody (mAb) from 10 subjects will also be generated after single-cell sorting of ASCs at approximately 8 days after the second study vaccination (Visit 04). The origin and the degree of clonality of these mAbs will be defined, and their potential value as therapeutics for drug-resistant A/H7N9 infections will be assessed.

Novel methods for identifying and assessing alternative correlates of protection against influenza infection are needed. An antibody dependent cell-mediated cytotoxicity (ADCC) assay for H7 is in development. If successful, the potential for A/H7N9 vaccines to generate non-neutralizing antibody responses aimed against both the globular head and the stalk region of the influenza hemagglutinin protein will be assessed in a subset of samples obtained at approximately 21 days after the second study vaccination (Visit 05).

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of this study are those associated with having blood drawn, intramuscular (IM) injection of the A/H7N9 vaccine and MF59 adjuvant combinations, possible reactions to the A/H7N9 vaccine or MF59 adjuvant, and breach of confidentiality.

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure to the draw site for several minutes. Intramuscular injection also may cause transient discomfort and fainting. Drawing blood and IM injection may also cause infection. The use of sterile technique will make infection at the site where blood will be drawn or where the study vaccination is given extremely unlikely.

The A/H7N9 vaccine has never been administered to humans or tested in animals. However, the safety profile of the A/H7N9 vaccine should be similar to the current Fluzone® vaccine. The manufacturing process used to produce the A/H7N9 vaccine is based on sanofi pasteur’s current manufacturing process for production of their licensed seasonal influenza virus vaccine Fluzone® with one modification: the HA content of bulk vaccine was determined by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC), instead of the traditional method of Single Radial Immunodiffusion (SRID) Assay. SRID could not be used to assess the HA
content, as official calibrated SRID reagents were not available from Regulatory Authorities at the time of formulation of vaccines for this study. Thus, the potential risks to subjects are anticipated to be similar to those observed for sanofi pasteur’s unadjuvanted licensed inter-pandemic (seasonal) influenza virus vaccines, and their unadjuvanted licensed 2009 A/H1N1 and A/H5N1 monovalent vaccines.

Occasionally, adult recipients of these unadjuvanted licensed, inactivated influenza virus vaccines may develop influenza-like reactions such as fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), arthralgia (joint pain), headache, and/or nausea. Some subjects may develop reactions at the injection site, including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and/or tenderness. With unadjuvanted licensed, inactivated influenza virus vaccines most of these reactions peak in intensity in the first 24 hours after vaccination and usually disappear without treatment within 1 or 2 days. Analgesics (e.g., acetaminophen, or ibuprofen or similar non-steroidal anti-inflammatory drugs (NSAIDs)) and rest may generally relieve or lessen these reactions. Bruising can sometimes occur due to the vaccination procedure.

In addition, post-marketing surveillance indicates the following adverse events of special interest (AESI) as potential risks for pandemic vaccines based on those identified for the seasonal influenza vaccines: neuritis, convulsions, severe allergic reactions, syncope, encephalitis, thrombocytopenia, vasculitis, and Guillain-Barré syndrome. Reports of these reactions were rare; however, exact incidence rates cannot be precisely calculated.

Acute and potentially life-threatening allergic reactions are also possible. Very rarely, occurring in about 1 in 4 million people given a vaccination, there can be a serious allergic reaction to a vaccine. These reactions can manifest as skin rash (hives), swelling around the mouth, throat or eyes, difficulty breathing, a fast pulse, or loss of blood pressure. If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction (death), although researchers do not expect this to occur.

During the swine influenza (H1N1) vaccine campaign of 1976, some recipients developed a paralytic illness called Guillain-Barré syndrome (GBS). GBS is an acute inflammatory neuropathy characterized by weakness, hyporeflexia or areflexia, and elevated protein concentrations in cerebrospinal fluid. The rate of GBS was significantly increased in individuals receiving the 1976 swine influenza (H1N1) vaccine at about 1 per 100,000 vaccine recipients. This syndrome has not been seen consistently with other influenza vaccines. Most persons who develop GBS recover completely, although the recovery period may be as little as a few weeks or as long as a few years. About 30% of those with GBS still have residual weakness after 3 years and about 3% may suffer a relapse of muscle weakness and tingling sensations many years after the initial attack. Intensive surveillance of GBS after administration of inactivated influenza vaccines since 1976 has shown a slight increase in risk over background
cases (more than one additional case of GBS per million persons) following vaccination, typically with onset within 6 weeks after vaccination (38). Interestingly, although vaccination rates have increased in the last 10 years the numbers of reported cases of vaccine-associated GBS have declined (39). A recent study in Canada showed that the 2009 H1N1 vaccine was associated with a small but significant risk of GBS in persons 50 years and older (40). An active, population-based surveillance study conducted during the 2009-2010 influenza season found less than 1 excess GBS case per million doses of 2009 H1N1 vaccine administered – a rate similar to that associated with some previously administered annual influenza vaccines (42-44). Another study using the Medicare system showed an elevated risk of GBS with 2009 monovalent H1N1 vaccination (incidence rate ratio = 2.41, 95% confidence interval: 1.14, 5.11; attributable risk = 2.84 per million doses administered, 95% confidence interval: 0.21, 5.48) (45). An international collaboration study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS (46). It is unknown if the administration of the currently produced A/H7N9 vaccine will result in the incidence of GBS that was seen with the 1976 vaccine product as the mechanism leading to this response has not been completely elucidated.

Because the HA content of the bulk A/H7N9 vaccine was determined by RP-HPLC, the final antigen content in each dose of study vaccine may be over or under estimated from the target formulation. While an over estimate or under estimate of the HA antigen content may impact the immune response, it is not expected to introduce a safety risk to study participants as numerous clinical studies assessing the safety of HA-containing influenza vaccine formulated to be greater than or less than Fluzone® have been shown to be safe (12, 14, 17, and 20).

As of August 30th, 2012, 39,656 subjects (5 months to 100 years of age) had received at least one dose of influenza virus vaccines mixed with MF59 adjuvant in clinical studies already completed or still ongoing. The completed trials showed that MF59 adjuvant significantly improved the immunogenicity of inactivated subunit influenza virus vaccines, with a clinically acceptable increase in the incidence of injection-site reactions (47-50). In brief, the integrated safety analysis of MF59-adjuvanted vaccines from the clinical trials database (MF59 DMF) has confirmed that MF59 is a well-tolerated and safe vaccine adjuvant. There was, as expected, a general tendency for an increased risk and report of mild and short lived solicited local and systemic reactions in subjects exposed to MF59 adjuvant, while there was a consistent indication for a similar or decreased overall risk of all unsolicited adverse events, autoimmune diseases, new onset of chronic diseases, cardiovascular diseases, serious adverse events, hospitalizations, and deaths, when compared to non MF59-adjuvanted vaccines. In addition, the cumulative safety analyses on post-marketing surveillance data, collected following the distribution of more than 66 million doses of FLUAD® (and copy products), showed that the current safety profile of an MF59-adjuvanted influenza vaccine is comparable with that of the non-adjuvanted influenza vaccine containing the same antigens. Analysis of key events, such as GBS, did not reveal a signal or risk greater than that following exposure to a conventional subunit influenza vaccine. Finally, the safety of FLUAD®, as compared with a non-adjuvanted subunit vaccine, is one of the end-points of a large post-marketing observational study currently ongoing.
The most frequently reported side effects of MF59-adjuvanted influenza vaccines have been injection site reactions and fever. Other minor systemic reactions including chills, arthralgia, asthenia, myalgia, headache, and malaise have been described. These were typically not serious reactions and the subjects generally recovered rapidly. Neurologic events (e.g., peripheral neuropathy (paresthesia)) temporally associated with the vaccine have been reported and for some of these the relationship was purely temporal. A causal association between the reported neurological symptoms (e.g., muscle aches and weakness) and these vaccines has not been established. Allergic reactions, in rare cases leading to shock, have been reported and the number was very low compared to the number of doses distributed. Data reported from the post marketing surveillance indicate that adverse reactions attributed to MF59-containing vaccines with at least reasonable suspicion were mild and self-limited, and are in line with clinical trial results, and the overall safety profile of MF59-containing vaccines did not reveal any significant risks. Adverse events with MF59-containing vaccines were as follows:

- Allergic reactions, in rare cases leading to shock, angioedema.
- Neuralgia, paresthesia, neuritis, convulsions, encephalomyelitis, and Guillain-Barré syndrome.
- Vasculitis with transient renal involvement and exudative erythema multiforme.
- Generalized skin reactions including pruritus, urticarial or non-specific rash.
- Thrombocytopenia (some very rare cases were severe with platelet counts less than 5,000 per mm$^3$), lymphadenopathy.
- Muscular weakness.
- Fibromyalgia.
- Atypical pain syndrome.

There is substantial clinical experience with MF59 adjuvant outside the United States. In a very extensive set of clinical studies, MF59 has proven to be a potent vaccine adjuvant with an acceptable safety profile, resulting in the licensure of an MF59-adjuvanted subunit influenza vaccine (FLUAD$^\text{®}$) in 35 countries worldwide. The adjuvanted interpandemic seasonal influenza vaccine (FLUAD$^\text{®}$) was initially licensed for commercial sale in 1997 in Italy, and then approved with either a Mutual Recognition Procedure or a National Procedure in 14 EU countries. It is also registered in 21 other countries worldwide. This adjuvanted vaccine is currently licensed for immunization of elderly people 65 years of age and above (with the only exception of China where FLUAD$^\text{®}$ is licensed in elderly aged 60 years and above). A pediatric indication was approved in Mexico (children 6 to <36 months of age) with approval expected to follow in several other countries when adequate data is available. MF59 is also the adjuvant of EU licensed monovalent A/H1N1 swine flu subunit egg-derived (Focetria™) and of a cell-derived (Celtura™) pandemic influenza vaccines (23 and 12 million doses, respectively, have been distributed) and licensed pre pandemic vaccine (AFLUNOV™) (~30,000 doses have been commercially available).

It is unknown if the A/H7N9 vaccine poses any risks to an unborn child. Female subjects of childbearing potential who are not surgically sterile via tubal sterilization, bilateral
oophorectomy, or hysterectomy or who are not postmenopausal for ≥ 1 year must agree to practice highly effective contraception that may include, but is not limited to, abstinence from intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, and licensed hormonal methods, with use of a highly effective method of contraception for a minimum of 30 days prior to study product exposure and agree to practice highly effective contraception for the duration of study product exposure, including 2 months (defined as 60 days) after the last study vaccination. A highly effective method of contraception is defined as one which results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all female subjects of childbearing potential will be required to have a negative serum or urine pregnancy test within 24 hours prior to receiving each dose of study vaccine. If a female subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' PHI. All records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU sites. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected. Any publications from this study will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU sites for quality assurance and data analysis include groups such as National Institute of Allergy and Infectious Diseases (NIAID) and Food and Drug Administration (FDA).

There may be other unknown risks, discomforts, or side effects.

### 2.3.2 Known Potential Benefits

There are no known benefits attributable to the receipt of the A/H7N9 vaccine with and without MF59 adjuvant, but there is the prospect of benefit. It is possible that vaccination using the A/H7N9 vaccine with and without MF59 adjuvant will result in some protection against infection caused by the A/H7N9 virus. Vaccination using the A/H7N9 vaccine with and without MF59 adjuvant may or may not provide protection against a serious infection with A/H7N9 influenza, should the participant be exposed. The duration of any such protection is currently unknown. The A/H7N9 vaccine with and without MF59 adjuvant is not expected to offer protection against circulating seasonal influenza viruses. There may be pandemic preparedness benefits to society in the future if the vaccine and adjuvant being evaluated here prove to be sufficiently safe and immunogenic and can be employed if a need for widespread A/H7N9 vaccination occurs.
3 STUDY OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

Primary:

Safety:

- To assess the safety and reactogenicity of a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

Immunogenicity:

- To assess the serum antibody responses to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

Secondary:

Safety:

- To assess unsolicited adverse events following receipt of two doses of a monovalent influenza A/H7N9 virus vaccine administered with and without MF59 adjuvant.

- To assess new-onset chronic medical conditions following receipt of two doses of a monovalent influenza A/H7N9 virus vaccine administered with and without MF59 adjuvant.

Immunogenicity:

- To assess the serum hemagglutination inhibition (HAI) antibody responses to a monovalent influenza A/H7N9 virus vaccine following receipt of one dose administered with and without MF59 adjuvant.

- To assess the serum neutralizing antibody (Neut) responses to a monovalent influenza A/H7N9 virus vaccine following receipt of each dose administered with and without MF59 adjuvant.

Exploratory:

Immunogenicity:

- To assess the effects of age, gender, body mass index, waist circumference, prior receipt of seasonal influenza vaccine, and geography (VTEU site) on serum antibody
responses to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

- To identify and quantitate vaccine-induced antibody-secreting cells (ASCs) or plasmablasts, and rapidly clone and express monoclonal antibodies (mAbs) they produce in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

- To analyze the origin and the degree of clonality of the responding B cells by clonal analysis of the Immunoglobulin Heavy Chain Variable (IGHV) genes and the degree of somatic hypermutation within these genes in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of the second dose administered with and without MF59 adjuvant.

- To assess blood CD4+ Helper T Cells (T\textsubscript{H}) and T Follicular Helper Cells (T\textsubscript{FH}) cell responses, including T\textsubscript{H} epitopes, in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant, and perform correlations of blood T\textsubscript{H} and T\textsubscript{FH} cell responses with HAI and Neut titers, CD38+CD27+ total plasmablast responses detected by Fluorescent Activated Cell Sorter (FACS) analysis, and antigen- and isotype-specific plasmablast responses by Enzyme-Linked Immunosorbent Spot (ELISpot) assay.

- To analyze the specificity of mAb against A/H7N9 and other influenza strains including evolved variants, if available, define the epitopes (at head or stalk region of HA protein) recognized by the mAb generated in response to A/H7N9 vaccination, and compare avidity and breadth of mAb generated in a subset of healthy adults in response to a monovalent influenza A/H7N9 virus vaccine following receipt of the second dose administered with and without MF59 adjuvant.

- To analyze serum antibody responses for antibody-dependent cell-mediated cytotoxicity (ADCC) in a subset of samples in response to a monovalent influenza A/H7N9 virus vaccine following receipt of two doses administered with and without MF59 adjuvant.

### 3.2 Study Outcome Measures

**Primary:**

**Safety:**

- Occurrence of study vaccine-related serious adverse events from the time of the first study vaccination through approximately 13 months after the first study vaccination.
• Occurrence of solicited injection site and systemic reactogenicity on the day of each study vaccination through 7 days after each study vaccination.

Immunogenicity:

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 21 days after the second study vaccination.

• Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at approximately 21 days after the second study vaccination.

Secondary:

Safety:

• Occurrence of unsolicited adverse events from the time of the first study vaccination through approximately 21 days after the last study vaccination.

• Occurrence of new-onset chronic medical conditions through 13 months after the first study vaccination.

Immunogenicity:

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 8 and 21 days after the first study vaccination.

• Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at baseline and at approximately 8 and 21 days after the first study vaccination.

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 8 days after the second study vaccination.

• Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at approximately 8 days after the second study vaccination.
• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination Neut titer <1:10 and a post-vaccination Neut titer ≥1:40 or a pre-vaccination Neut titer ≥1:10 and a minimum four-fold rise in post-vaccination Neut antibody titer) at approximately 8 and 21 days after each study vaccination.

• Percentage of subjects achieving a serum Neut antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at baseline and at approximately 8 and 21 days after each study vaccination.

• Geometric Mean Titers of serum HAI and Neut antibody at baseline and at approximately 8 and 21 days after each study vaccination.

**Exploratory:**

**Immunogenicity:**

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) and Geometric Mean Titers of serum HAI antibody at baseline and at approximately 8 and 21 days after each study vaccination as a function of age, gender, body mass index, waist circumference, prior receipt of seasonal influenza vaccine, and geography (VTEU site).

• Development of serum antibody responses against antigenically drifted variants of the A/H7N9 virus in at least a subset of samples, should any variants occur and be available prior to the last subject’s Visit 07.

• Total ASC response by flow cytometry (defined as CD3-/CD20lo/CD19+/CD38hi/CD27hi cells and reported as a percentage of total lymphocytes or of total B cells) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

• Frequency of vaccine-specific ASCs measured by ELISpot assay reported as the number of influenza-specific ASCs per million peripheral blood mononuclear cells (PBMCs) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

• IGHV gene sequence analyses for B cell gene usage, clonality, and somatic hypermutation within these genes in a subset of healthy adults, 19-64 years old, at approximately 8 days after the second study vaccination.

• Frequency of blood **T_{Fh}** cell responses (reported as the number of ICOS+CXCR3+CXCR5+ CD4+ T cells) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.
• Frequencies and quality of TH cells and epitope specificities in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

• Correlations of blood TH and TFH responses with HAI and Neut titers, plasmablast responses detected by FACS analysis, and antigen-specific plasmablast responses by ELISpot assay in a subset of healthy adults, 19-64 years old, at baseline and approximately 8 and 21 days after each study vaccination.

• Assess the specificity of mAb against A/H7N9 and other influenza strains including evolved variants, if available, compare the epitopes (at head or stalk region of HA protein) recognized by the mAb generated by A/H7N9 vaccination, and analyze the avidity and breadth of mAb generated by A/H7N9 vaccination with and without MF59 adjuvant in a subset of healthy adults, 19-64 years old, at approximately 8 days after the second study vaccination.

• Percentage of subjects demonstrating detectable levels of ADCC responses against the epitopes (at head or stalk region of HA protein) generated by A/H7N9 vaccination, and the correlation of the ADCC responses with HAI and Neut titers generated by A/H7N9 vaccination with and without MF59 adjuvant in a subset of samples at approximately 21 days after the second study vaccination.
4 STUDY DESIGN

This is a Phase II randomized, double-blinded, controlled study in up to 700 males and non-pregnant females, 19 to 64 years old, inclusive, who are in good health and meet all eligibility criteria. The study is designed to assess the safety, reactogenicity, and immunogenicity of a monovalent influenza A/H7N9 virus vaccine manufactured by sanofi pasteur administered to healthy adults at different dosages (3.75, 7.5, or 15 mcg of HA/0.5 mL dose) given with MF59 adjuvant manufactured by Novartis Vaccines and Diagnostics or without adjuvant (15 mcg of HA/0.5 mL dose and 45 mcg of HA/0.75 mL dose). The A/H7N9 vaccine was made with HA antigen derived from the influenza A/Shanghai/2/2013 virus.

Subjects will be randomly assigned to 1 of 7 groups (up to 100 subjects per group) to receive two doses of the A/H7N9 vaccine with or without MF59 adjuvant delivered intramuscularly approximately 21 days apart. The same dosage of A/H7N9 vaccine will be given to subjects at both their first and second study vaccinations.

Safety will be measured from the time of each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) through 7 days after each study vaccination by the occurrence of solicited injection site and systemic reactogenicity events. Unsolicited non-serious adverse events (AEs) will be collected from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 21 days after the last study vaccination (approximately Day 42 (Visit 05) for subjects who receive two study vaccinations; approximately Day 21 (Visit 03) for subjects who receive only one study vaccination). After approximately 21 days after the last study vaccination, non-serious AEs will be limited to new-onset chronic medical conditions, which will be documented through approximately 13 months after the first study vaccination (Visit 09).

Serious adverse events (SAEs) will be collected from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 13 months after the first study vaccination (Visit 09).

Immunogenicity testing will include performing hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays on serum obtained prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after each study vaccination (Visit 02 and Visit 04) and approximately 21 days after the second study vaccination (Visit 05).

From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 85 mL of venous blood will be drawn prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after the second study vaccination (Visit 04). Also, an additional 37 mL of venous blood will be drawn approximately 8 days after the first vaccination (Visit 02) and an additional 53 mL of venous blood will be drawn approximately 21 days after the second study vaccination (Visit 05). These additional blood samples will be used to characterize the kinetics and quality of antibody-secreting cells (ASCs) and CD4+ T cell responses by Fluorescent Activated Cell Sorter (FACS) analysis and Enzy-
Linked Immunosorbent Spot (ELISpot) assays at serial time points following receipt of the first and second dose of study vaccine as indicated above. In addition, monoclonal antibody (mAb) from 10 subjects will also be generated after single-cell sorting of ASCs at approximately 8 days after the second study vaccination (Visit 04). The origin and the degree of clonality of these mAbs will be defined, and their potential value as therapeutics for drug-resistant A/H7N9 infections will be assessed.

Novel methods for identifying and assessing alternative correlates of protection against influenza infection are needed. An antibody dependent cell-mediated cytotoxicity (ADCC) assay for H7 is in development. If successful, the potential for A/H7N9 vaccines to generate non-neutralizing antibody responses aimed against both the globular head and the stalk region of the influenza hemagglutinin protein will be assessed in a subset of samples obtained at approximately 21 days after the second study vaccination (Visit 05).

The duration of the study for each subject will be approximately 13 months.

For additional details on study procedures and evaluations and study schedule by study visits see Sections 7 and 8 and Appendix A: Schedule of Procedures and Evaluations.

4.1 Sub studies

No sub studies are planned.
5 STUDY ENROLLMENT AND WITHDRAWAL

Up to 700 males and non-pregnant females, 19 to 64 years old, inclusive, who are in good health and meet all eligibility criteria, will be enrolled from at least 4 VTEU sites participating in this study. The target population should reflect the community at large at each of the participating VTEU sites. Estimated time to complete enrollment in this study is approximately 6 weeks. Information regarding the study may be provided to subjects who have previously participated in vaccine trials conducted at the participating VTEU sites. Other forms and/or mechanisms of recruitment may also be used. The local IRB will approve all materials prior to its use.

Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer.

5.1 Subject Inclusion Criteria

Subjects eligible to participate in this study must meet all of the following inclusion criteria:

1. Provide written informed consent prior to initiation of any study procedures.

2. Are able to understand and comply with planned study procedures and be available for all study visits.

3. Are males or non-pregnant females, 19 to 64 years old, inclusive.

4. Are in good health, as determined by vital signs (oral temperature, pulse, and blood pressure), medical history, and targeted physical examination based on medical history to ensure any existing medical diagnoses or conditions (except those in the Subject Exclusion Criteria) are stable. Subjects may be on chronic or as needed (prn)

 Stable chronic medical condition – no change in prescription medication, dose, or frequency of medication in the last 3 months (defined as 90 days) and health outcomes of the specific disease are considered to be within acceptable limits in the last 6 months (defined as 180 days). Any change that is due to change of health care provider, insurance company etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a violation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a violation of this inclusion criterion.
medications if, in the opinion of the site principal investigator or appropriate sub-
investigator, they pose no additional risk to subject safety or assessment of
reactogenicity and immunogenicity. Note: Topical, nasal, and inhaled medications (with
the exception of steroids as outlined in the Subject Exclusion Criteria (see Section 5.2)),
vitamins, and contraceptives are permitted.

5. Oral temperature is less than 100.4°F.

6. Pulse is 50 to 115 bpm, inclusive.

7. Systolic blood pressure is 85 to 150 mm Hg, inclusive.

8. Diastolic blood pressure is 55 to 95 mmHg, inclusive.

9. Female subjects of childbearing potential who are not surgically sterile via tubal
sterilization, bilateral oophorectomy, or hysterectomy or who are not postmenopausal for
≥ 1 year must agree to practice highly effective contraception that may include, but is not
limited to, abstinence from intercourse with a male partner, monogamous relationship
with a vasectomized partner, male condoms with the use of applied spermicide,
intrauterine devices, and licensed hormonal methods, with use of a highly effective
method of contraception for a minimum of 30 days prior to study product exposure and
agree to practice highly effective contraception for the duration of study product
exposure, including 2 months (defined as 60 days) after the last study vaccination. A
highly effective method of contraception is defined as one which results in a low failure
rate (i.e., less than 1% per year) when used consistently and correctly. Method of
contraception will be captured on the appropriate data collection form.

10. Female subjects of childbearing potential must have a negative urine or serum
pregnancy test within 24 hours prior to study vaccination.

5.2 Subject Exclusion Criteria

Subjects eligible to participate in this study must not meet any of the following exclusion criteria:

1. Have an acute illness within 72 hours prior to study vaccination.

2. Have any condition that, in the opinion of the site principal investigator or appropriate
sub-investigator, would place the subject at an unacceptable risk of injury, render the
subject unable to meet the requirements of the protocol, or confound the interpretation
of the results.
3. Have an acute or chronic medical condition\(^2\) that, in the opinion of the site principal investigator or appropriate sub-investigator, would render vaccination unsafe, or would interfere with the evaluation of responses.

4. Have immunosuppression as a result of an underlying illness or treatment, or use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.

5. Have known active neoplastic disease or a history of any hematologic malignancy.

6. Have known HIV, hepatitis B, or hepatitis C infection.

7. Have known hypersensitivity or allergy to eggs, egg or chicken protein, squalene-based adjuvants, or other components of the study vaccine.

8. Have a history of severe reactions following previous immunization with licensed or unlicensed influenza virus vaccines.


10. Have a history of neuralgia, paresthesia, neuritis, convulsions, or encephalomyelitis within 90 days prior to study vaccination.

11. Have a history of autoimmune disease, including, but not limited to, neuroinflammatory diseases, vasculitis, clotting disorders, dermatitis, arthritis, thyroiditis, or muscle, liver, or kidney disease.

12. Have a history of alcohol or drug abuse within 5 years prior to study vaccination.

13. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.

14. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.

15. Have taken oral or parenteral corticosteroids of any dose within 30 days prior to study vaccination.

16. Have taken high-dose inhaled corticosteroids within 30 days prior to study vaccination. High-dose defined as >800mcg/day of beclomethasone dipropionate CFC or equivalent.

\(^2\)Chronic medical condition – a medical condition persisting 3 months (defined as 90 days) or longer.
17. Received any licensed live vaccine within 30 days or any licensed inactivated vaccine within 14 days prior to the first study vaccination or planned receipt of any vaccine from the first study vaccination through the follow-up visit at approximately 21 days after the last study vaccination. This is inclusive of licensed seasonal influenza vaccines.

18. Received immunoglobulin or other blood products (with exception of Rho D immunoglobulin) within 90 days prior to study vaccination.

19. Received an experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 30 days prior to the first study vaccination, or expects to receive an experimental agent other than from participation in this study during the 13-month study period.

20. Are participating or plan to participate in another clinical trial with an interventional agent (licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication) during the 13-month study period.

21. Prior participation in a clinical trial of influenza A/H7 vaccine and assigned to a group receiving influenza A/H7 vaccine (does not apply to documented placebo recipients) or have a history of A/H7 actual or potential exposure or infection prior to the first study vaccination.

22. Plan to travel outside the U.S. (continental U.S., Hawaii and Alaska) in the time between the first study vaccination and 42 days after the first study vaccination.

23. Female subjects who are breastfeeding or plan to breastfeed at any given time from the first study vaccination until 30 days after their last study vaccination.

24. Blood donation within 30 days prior to enrollment and within 30 days after the last blood draw (only for a subset of healthy adult subjects – up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays).

5.3 Treatment Assignment Procedures

5.3.1 Enrollment and Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at each participating VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and Data Coordinating Center's (SDCC) AdvantageEDC℠ (Electronic Data Capture System).
Once consented and upon entry of demographic data and confirmation of eligibility for the trial, the subject will be enrolled. Subjects will be randomly assigned to 1 of 7 groups (up to 100 subjects per group) to receive two doses of the A/H7N9 vaccine with or without MF59 adjuvant delivered intramuscularly approximately 21 days apart. The same dosage of A/H7N9 vaccine will be given to subjects at both their first and second study vaccinations.

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC™. The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for the trial. AdvantageEDC™ will assign each subject to a dosage group after the demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the AdvantageEDC™ User’s Guide. Manual back-up procedures and instructions are provided for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

Subjects who sign the informed consent form and are randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form, and are randomized and vaccinated, and subsequently withdraw, or are withdrawn or terminated from the study, or are lost to follow-up will not be replaced.

The randomization scheme for this study is presented in the table below.
### Table 1. Groups and study vaccine to be administered:

<table>
<thead>
<tr>
<th>Group (n=up to 100 subjects per Group)</th>
<th>First Dose (Day 0; Visit 01)</th>
<th>Second Dose (Day 21+3 days; Visit 03)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sanofi A/H7N9 antigen 3.75 mcg plus Novartis MF59 adjuvant</td>
<td>sanofi A/H7N9 antigen 3.75 mcg plus Novartis MF59 adjuvant</td>
</tr>
<tr>
<td>2</td>
<td>sanofi A/H7N9 antigen 7.5 mcg plus Novartis MF59 adjuvant</td>
<td>sanofi A/H7N9 antigen 7.5 mcg plus Novartis MF59 adjuvant</td>
</tr>
<tr>
<td>3</td>
<td>sanofi A/H7N9 antigen 15 mcg plus Novartis MF59 adjuvant</td>
<td>sanofi A/H7N9 antigen 15 mcg plus Novartis MF59 adjuvant</td>
</tr>
<tr>
<td>4</td>
<td>sanofi A/H7N9 antigen 15 mcg plus Novartis MF59 adjuvant</td>
<td>sanofi A/H7N9 antigen 15 mcg</td>
</tr>
<tr>
<td>5</td>
<td>sanofi A/H7N9 antigen 15 mcg</td>
<td>sanofi A/H7N9 antigen 15 mcg plus Novartis MF59 adjuvant</td>
</tr>
<tr>
<td>6</td>
<td>sanofi A/H7N9 antigen 15 mcg</td>
<td>sanofi A/H7N9 antigen 15 mcg</td>
</tr>
<tr>
<td>7</td>
<td>sanofi A/H7N9 antigen 45 mcg</td>
<td>sanofi A/H7N9 antigen 45 mcg</td>
</tr>
</tbody>
</table>

Total N=up to 700 subjects
5.3.2 Masking Procedures

This is a double-blind study.

Subjects, investigators, study personnel performing any study-related assessments following study vaccine administration, and laboratory personnel performing antibody assays will be blinded to group assignment.

The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., pharmacists performing study vaccination preparations and unblinded study vaccine administrators) at the participating VTEU sites.

The unblinded study vaccine administrator is a study clinician licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

The Data and Safety Monitoring Board (DSMB) may receive data in aggregate and presented by group. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion.

5.3.3 Reasons for Withdrawal and Discontinuation of Treatment

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty.

A subject may withdraw or be withdrawn from the study for any of the following reasons:

- Medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of the study, or would interfere with the evaluation of responses.

- Subject no longer meets eligibility criteria.

- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.

- Subject withdrawal of consent.

- Subject lost to follow-up.
• Termination of the study.

• New information becomes available that makes further participation unsafe.

The second study vaccination will not be administered to a subject if any of the following criteria are met:

• Medical condition for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would pose a risk to the subject or would be likely to confound interpretation of the results.

• Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity. For subjects with injection site or systemic signs or symptoms, or with an acute illness, including an oral temperature greater than or equal to 100.4°F, the second study vaccination should be postponed/deferred until signs, symptoms, or acute illness have resolved and if within the acceptable protocol-specified window for that visit. If outside this window, the DMID Medical Officer must first approve the second study vaccination and the documentation of approval should be filed in the subject’s chart.

• Any unresolved or continuing solicited or unsolicited Grade 3 adverse event. An unresolved or continuing Grade 1 or Grade 2 adverse event is permissible unless, in the opinion of the site principal investigator or appropriate sub-investigator, it would render study vaccination unsafe or interfere with the evaluation of responses.

• Grade 3 adverse event that occurs without alternative etiology in the 7 days following the first study vaccination.

• Severe or sustained reaction or disability related to the first study vaccination.

• New onset of illness or condition that meets exclusion criteria.

• Subject no longer meets eligibility criteria.

• As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.

• Subject refusal of further study vaccination.

• Subject withdrawal of consent.

• Subject lost to follow-up.

• Termination of the study.
- New information becomes available that makes further participation unsafe.

5.3.4 Handling of Withdrawals and Discontinuation of Treatment

The primary reason for withdrawal from the study will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 8.4. Although subjects are free to withdraw at any time or may be withdrawn by the site principal investigator or appropriate sub-investigator at any time, subjects who receive at least one dose of study vaccine will be encouraged to remain in the study for follow-up safety assessments and collection of venous blood samples for immunogenicity testing. Every attempt will be made to follow all adverse events, including solicited injection site and systemic reactions, serious adverse events, and new-onset chronic medical conditions ongoing at the time of early withdrawal to resolution.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls, e-mails, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject’s records.

See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study vaccine will not be replaced. Subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine may be replaced.

5.3.5 Termination of Study

Although the study Sponsor has every intention of completing the study, it reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to DSMB review and recommendation and at the discretion of DMID.
6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

A/H7N9 Vaccine

sanofi pasteur has developed a monovalent influenza A/H7N9 virus vaccine. The manufacturing process for the production of this monovalent influenza A/H7N9 virus vaccine is similar to the manufacturing process used to produce the licensed Influenza Virus Vaccine Fluzone® family of products, except for a minor modification in the Phosphate Buffered Saline (PBS) diluent in the formulation step that was made according to previous experiences of manufacturing of monovalent pandemic vaccines. This monovalent inactivated split influenza virus vaccine was made with HA antigen derived from the influenza A/Shanghai/2/2013 virus. Licensed release testing specifications were maintained, with a modification to the potency test. The HA content of bulk vaccine was determined by RP-HPLC, instead of the traditional method of SRID Assay. SRID could not be used to assess the HA content, as official calibrated SRID reagents were not available from Regulatory Authorities at the time of formulation of vaccine for this study.

MF59 Adjuvant

The MF59C.1 (MF59) adjuvant developed by Novartis Vaccines and Diagnostics is an oil-in-water emulsion composed of a small amount of squalene (a natural lipid derived from the shark), stabilized by the addition of two emulsifiers [a water-soluble surfactant (polysorbate 80, also known as Tween 80) and an oil-soluble surfactant (sorbitan trioleate, also known as Span 85)], and a low ionic strength buffer. Biodegradable squalene oil is a non-steroid, natural metabolite of cholesterol, a normal component of cell membranes, catabolized by humans, and not associated with the production of specific antibodies.

6.1.1 Acquisition

A/H7N9 vaccine will be provided by sanofi pasteur under contract to DHHS.

MF59 adjuvant will be provided by Novartis Vaccines and Diagnostics under contract to DHHS.

Sterile empty vials will be obtained from Allergy Laboratories, Inc. by the DMID Clinical Agents Repository (CAR), Fisher BioServices.

A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials for vaccine preparation will be provided through the DMID CAR to the participating VTEU sites prior to the start of the study. Should the
site principal investigator require additional A/H7N9 vaccine, MF59 adjuvant, or sterile empty vials during this trial, further instructions are provided in the protocol-specific MOP.

6.1.2 Formulation, Storage, Packaging, and Labeling

A/H7N9 Vaccine

The monovalent influenza A/H7N9 virus vaccine is supplied as a sterile, clear, and slightly opalescent suspension in single-dose vials containing:

- 7.5 mcg HA per 0.5 mL
- 15 mcg HA per 0.5 mL
- 30 mcg HA per 0.5 mL

Each vial contains a fill volume of 0.7 mL. It contains no preservative (i.e., non-thimerosal). The vials containing study product must be stored at 2°C to 8°C (35.6°F to 46.4°F). Do not freeze.

MF59 Adjuvant

The MF59 adjuvant is supplied as an oil-in-water milky emulsion in single-use vials containing a fill volume of 0.7 mL. The vials containing study product must be stored at 2°C to 8°C (35.6°F to 46.4°F), protected from light. Do not freeze.

Each of these study products will be labeled according to manufacturer specifications and include the statement “Caution: New Drug – Limited by Federal Law to Investigational Use.”

Further details are included in the respective Investigator’s Brochures for the A/H7N9 vaccine and MF59 adjuvant, as well as in the protocol-specific MOP.

Sterile empty vials will be provided as 3 mL with 13 mm latex-free stoppers.

6.1.3 Study Product Storage and Stability Procedures

The temperature of the storage unit must be recorded daily (excluding non-business days and holidays as applicable), monitored during the duration of the trial per the participating VTEU sites’ standard operating procedures, and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as ‘Do Not Use’ (until further notice). The pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental deep-freezing or disruption of the cold chain, the affected
study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CAR or destroy it on site. Additional instructions for quarantine are provided in the protocol-specific MOP.

6.2 Dosage, Preparation, and Administration of Study Intervention/Investigational Product

See Appendices B-H for detailed information on the preparation, labeling, storage, and administration of study vaccine for each dosage group. Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration.

Visually inspect the A/H7N9 vaccine and MF59 adjuvant upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined at 2°C to 8°C (35.6°F to 46.4°F) and labeled as ‘Do Not Use’ (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CAR or destroy it on site. If the A/H7N9 vaccine or MF59 adjuvant is unusable, study personnel will use another vial from the study supply. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Also, visually inspect the A/H7N9 vaccine plus MF59 adjuvant admixture prior to use. The admixture will be milky in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it. The affected A/H7N9 vaccine plus MF59 adjuvant admixture must be quarantined at room temperature and labeled as ‘Do Not Use’ (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the A/H7N9 vaccine plus MF59 adjuvant admixture can be used. If it cannot be used, the site will receive
specific instructions on how to send the A/H7N9 vaccine plus MF59 adjuvant admixture to the DMID CAR or destroy it on site. If the A/H7N9 vaccine plus MF59 adjuvant admixture is unusable, the participating VTEU sites’ pharmacist will prepare another vial. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Once mixed, the A/H7N9 vaccine plus MF59 adjuvant admixture must be stored at room temperature in an upright position, but does not need to be protected from light, and must be used within 8 hours. Once mixed, the A/H7N9 unadjuvanted vaccine admixture for the 45 mcg/dose study group (Group 7) must be stored at 2°C to 8°C (35.6°F to 46.4°F) and must be used within 8 hours.

Study vaccine administration will be performed by an unblinded study clinician licensed to administer medications/vaccines. Each dose of study vaccine will be administered via a single IM injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Aseptic technique will be used for the withdrawal and administration of each dose of study vaccine using a disposable sterile needle appropriate in length for each subject and a disposable sterile 1-mL syringe. See the protocol-specific MOP for information on how to administer IM injections. Each dose of study vaccine must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered. Note: Each 0.5 mL dose of MF59-adjuvanted study vaccine contains one- 0.25 mL dose of MF59 adjuvant.

6.3 Modification of Study Intervention/Investigational Product for a Subject

There will be no dose modifications. If a subject’s second study vaccination is deferred, it should be rescheduled to occur within the acceptable protocol-specified window for that visit. If this period elapses, the site must obtain prior approval from the DMID Medical Officer to administer the second study vaccination and the documentation of approval should be filed in the subject’s chart. Subjects who do not receive the second study vaccination will be asked to return for safety assessments and for scheduled venous blood sample collections for immunogenicity testing and will be followed for the duration of the study – see the protocol-specific MOP for further details.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

After receipt of the A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials, the site principal investigator is responsible for its distribution and disposition, and has ultimate responsibility for study product accountability. Logs of receipt, temperature, maintenance, and disposal must be
maintained in the study file. The study product accountability records and dispensing logs will also capture vial numbers, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, MF59 adjuvant, and admixture, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor’s monitoring staff will verify the participating VTEU sites’ study product accountability records and dispensing logs per the site monitoring plan.

Used and unused vials of A/H7N9 vaccine, MF59 adjuvant, and admixture will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, MF59 adjuvant, and admixture. Final disposition of the unused A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

### 6.5 Assessment of Subject Compliance with Study Investigational Product

Subject compliance is not anticipated to be an issue. Deviations from the dose schedule may only occur as described in Section 6.3.

### 6.6 Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken within 30 days prior to signing the informed consent form through approximately 21 days after the second study vaccination (Visit 05), or approximately 21 days after the first study vaccination (Visit 03) for those who only receive one dose of study vaccine, or early termination (if prior to 21 days after the last study vaccination), whichever occurs first. Prescription and over-the-counter drugs will be included as well as vitamins and supplements.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Medications that might interfere with the evaluation of the investigational product should not be used unless absolutely necessary. Medications in this category include, but are not limited to, the prohibited medications per the Subject Exclusion Criteria (see Section 5.2).
7 STUDY PROCEDURES AND EVALUATIONS

7.1 Clinical Evaluations

Complete medical history will be obtained by interview of the subjects on Day 0 (Visit 01) prior to the first study vaccination. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits after the first study vaccination, an interim medical history will be obtained by interview of the subjects noting any changes since the previous visit. The interim medical history should include an assessment for symptoms suggestive of an autoimmune disorder.

Medications history (concomitant medications) will include a review of all current medications and medications taken within 30 days prior to signing the informed consent form through approximately 21 days after the second study vaccination (Visit 05), or approximately 21 days after the first study vaccination (Visit 03) for those who only receive one dose of study vaccine, or early termination (if prior to 21 days after the last study vaccination), whichever occurs first. Prescription and over-the-counter drugs will be included as well as vitamins and supplements. Assessment of eligibility will also include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5).

On Day 0 (Visit 01) prior to the first study vaccination, a targeted physical examination, if indicated based on subject’s complete medical history, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. At follow-up visits after the first study vaccination, a targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on subject’s interim medical history. Targeted physical examinations should include an assessment for signs suggestive of an autoimmune disorder.

Vital signs (oral temperature, pulse, and blood pressure) will be collected prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)). Vital signs assessed on Day 0 (Visit 01) prior to the first study vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Height and weight will be collected on Day 0 (Visit 01) prior to the first study vaccination for the calculation of Body Mass Index (BMI). Waist circumference will also be measured immediately above the iliac crest and recorded in inches per the NIH guidelines (51). See the protocol-specific MOP for additional details.
Reactogenicity assessments will include an assessment of solicited adverse events occurring on the day of each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) through 7 days after each study vaccination, which includes an assessment of injection site reactions including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness as well as systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, and nausea.

Subjects will be observed in the clinic for at least 20 minutes after each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)). The study vaccination site will be examined, and any AE/SAEs will be assessed and recorded on the appropriate data collection form prior to discharge from the clinic. The study vaccination site will also be examined approximately 8 days after each study vaccination (Visit 02 and Visit 04).

All subjects will complete a subject memory aid on the day of each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) through 7 days after each study vaccination. Subject memory aids will be reviewed with the subject for adverse events approximately 2 days after each study vaccination (Visit 01A and Visit 03A) via phone call as well as approximately 8 days after each study vaccination (Visit 02 and Visit 04) via clinic visit.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

Urine or serum pregnancy tests will be performed by the local or site laboratory within 24 hours prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) on all female subjects of childbearing potential. Results must be negative and known prior to randomization on Day 0 (Visit 01) and administration of each study vaccination to be eligible for participation in the study and receipt of each dose of study vaccine, respectively.

7.2.2 Special Assays or Procedures

Immunogenicity

Assays to determine serum levels of HAI and Neut antibodies will be performed at Southern Research. Venous blood samples (approximately 10 mL) for HAI and Neut antibody assays will be collected from each subject prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after each study vaccination (Visit 02 and Visit 04) and approximately 21 days after the second study.
vaccination (Visit 05). Subjects who withdraw early will have HAI and Neut antibody assays run on available sera.

In addition, immunology exploratory assays will be performed on samples collected from a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays). An additional 85 mL of venous blood will be drawn prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after the second study vaccination (Visit 04). Also, an additional 37 mL of venous blood will be drawn approximately 8 days after the first vaccination (Visit 02) and an additional 53 mL of venous blood will be drawn approximately 21 days after the second study vaccination (Visit 05). These additional blood samples will be used to characterize the kinetics and quality of antibody-secreting cells (ASCs) and CD4⁺ T cell responses by Fluorescent Activated Cell Sorter (FACS) analysis and Enzyme-Linked Immunosorbent Spot (ELISpot) assays at serial time points following receipt of the first and second dose of study vaccine as indicated above. In addition, monoclonal antibody (mAb) from 10 subjects will also be generated after single-cell sorting of ASCs at approximately 8 days after the second study vaccination (Visit 04). The origin and the degree of clonality of these mAbs will be defined, and their potential value as therapeutics for drug-resistant A/H7N9 infections will be assessed.

Additionally, an ADCC assay for H7 is in development. If successful, ADCC may be assessed in a subset of samples collected at approximately 21 days after the second study vaccination (Visit 05), but no additional venous blood will be collected from subjects.

Any laboratory involved with immunology exploratory assays or the ADCC assay will remain blinded to the HAI and Neut antibodies results performed at Southern Research.

The volume of venous blood to be collected for HAI and Neut antibody assays and immunology exploratory assays (ASCs, mAb, and CD4⁺ T cell response assays) is presented in the table below.
<table>
<thead>
<tr>
<th>Study Visit Number</th>
<th>V01</th>
<th>V01A*</th>
<th>V02</th>
<th>V03</th>
<th>V03A*</th>
<th>V04</th>
<th>V05</th>
<th>V06*</th>
<th>V07*</th>
<th>V08*</th>
<th>V09*</th>
<th>Total</th>
</tr>
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<tr>
<td>Study Day post first study vaccination</td>
<td>D0</td>
<td>D2±1d</td>
<td>D8+2d</td>
<td>D21+3d</td>
<td>D23</td>
<td>D29</td>
<td>D42</td>
<td>D81</td>
<td>D141</td>
<td>D201</td>
<td>D386</td>
<td></td>
</tr>
<tr>
<td>Study Day post second study vaccination</td>
<td></td>
<td>D0</td>
<td>D2±1d</td>
<td>D8+2d</td>
<td>D21+3d</td>
<td>D60±7d</td>
<td>D120±14d</td>
<td>D180±14d</td>
<td>D365±14d</td>
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<td></td>
</tr>
<tr>
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<td>X</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50 mL</td>
</tr>
<tr>
<td>Immunology Exploratory Assays*</td>
<td>85</td>
<td>37</td>
<td>85</td>
<td></td>
<td>85</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>345 mL</td>
</tr>
</tbody>
</table>

* Phone call assessment.

¹ All blood drawn prior to study vaccination.

* An additional volume of venous blood will be drawn at Visits 01, 02, 03, 04, and 05 from a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays).
7.2.3 Specimen Preparation, Handling, and Shipping

7.2.3.1 Instruction for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP.

7.2.3.2 Specimen Shipment

Specimen shipment will occur at intervals during the course of the study following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the protocol-specific MOP.

Specimens for HAI and Neut antibody assays will be shipped from the participating VTEU sites to the DMID CAR, and then provided by the DMID CAR to Southern Research in a blinded manner as they become available to the DMID CAR. Shipment of the Visit 01, Visit 02, Visit 03, Visit 04, and Visit 05 serum samples collected from 25 subjects per dosage group will be expedited to Southern Research for analysis.

Specimens for the immunology exploratory assays will be provided by and remain at the Emory VTEU site.

Specimens for the ADCC assay will be shipped from the participating VTEU sites to the DMID CAR, and then provided by the DMID CAR to the ADCC assay laboratory once it has been identified.

Further instructions for specimen shipment are included in the protocol-specific MOP.
8 STUDY SCHEDULE

Complete study schedule details listed by study visit are described below. Refer also to Sections 4 and 7 and Appendix A: Schedule of Procedures and Evaluations.

8.1 Enrollment/Baseline Visit

8.1.1 Visit 01, Day 0, Dose 1, Clinic Visit

- Subjects will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures, including administration of the first study vaccination.

- Eligibility criteria will be reviewed with subjects prior to the first study vaccination. Subject receipt of licensed 2012-2013 and 2013-2014 seasonal influenza vaccine, what type (LAIV or inactivated), and approximate date of vaccination will be recorded on the appropriate data collection form, if known. Note: Prior receipt of licensed 2013-2014 seasonal influenza vaccine is not an exclusion criterion, as long as it has been administered within the allowable window (see Section 5).

- Complete medical history will be obtained by interview of subjects prior to the first study vaccination to assure eligibility.

- All concomitant medications taken within 30 days prior to signing the informed consent form will be recorded on the appropriate data collection form prior to the first study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the first study vaccination. Vital signs assessed on Day 0 (Visit 01) prior to the first study vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- Collect height and weight prior to the first study vaccination for the calculation of BMI. Also obtain waist circumference measurement (recorded in inches) immediately above the iliac crest. See the protocol-specific MOP for additional details.

- A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed prior to the first study vaccination by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the
site principal investigator or sub-investigator, if indicated based on review of complete medical history.

- A urine or serum pregnancy test will be performed within 24 hours prior to the first study vaccination on all female subjects of childbearing potential. Results must be negative and known prior to randomization and first study vaccination.

- Approximately 10 mL of venous blood will be collected prior to the first study vaccination for baseline HAI and Neut antibody assays.

- From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 85 mL of venous blood will be collected prior to the first study vaccination for baseline immunology exploratory assays.

- Subjects will be enrolled in AdvantageEDC\textsuperscript{SM} and randomly assigned to a dosage group prior to the first study vaccination.

- Subjects will receive a single dose of study vaccine via IM injection in the deltoid muscle of the preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 20 minutes after the first study vaccination. The first study vaccination site will be examined, and any AE/SAEs will be assessed and recorded on the appropriate data collection form prior to discharge from the clinic.

- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their temperature around the same time each day. Subjects will be instructed on how to use the memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after the first study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, s/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

### 8.2 Follow-up Visits

Follow-up visits are scheduled in reference to dosing dates as indicated for each visit window.
8.2.1 Visit 01A, Day 2, Phone Call
(Window: Day 2±1 day post first study vaccination)

Study personnel will contact subjects by phone to solicit any AE/SAE and concomitant medication information and review information on the memory aid.

8.2.2 Visit 02, Day 8, Clinic Visit
(Window: Day 8+2 days post first study vaccination)

- Obtain interim medical history, including an assessment for symptoms suggestive of an autoimmune disorder, by interview of subjects and note any changes since the previous visit.

- Study personnel will review the memory aid information with subjects and assess and record all AE/SAEs and concomitant medications on the appropriate data collection form.

- A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.

- Examine the first study vaccination site.

- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays.

- From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 37 mL of venous blood will be collected for immunology exploratory assays.

8.2.3 Visit 03, Day 21, Dose 2, Clinic Visit
(Window: Day 21+3 days post first study vaccination or Day 0, Dose 2)

- Eligibility criteria will be reviewed with subjects prior to the second study vaccination to assure continued eligibility.

- Obtain interim medical history, including an assessment for symptoms suggestive of an autoimmune disorder, by interview of subjects prior to the second study vaccination and note any changes since the previous visit.
All concomitant medications will be recorded on the appropriate data collection form prior to the second study vaccination.

All AE/SAEs will be assessed and recorded on the appropriate data collection form prior to the second study vaccination.

Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the second study vaccination. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed prior to the second study vaccination by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.

A urine or serum pregnancy test will be performed within 24 hours prior to the second study vaccination on all female subjects of childbearing potential. Results must be negative and known prior to the second study vaccination.

Approximately 10 mL of venous blood will be collected prior to the second study vaccination for HAI and Neut antibody assays.

From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 85 mL of venous blood will be collected prior to the second study vaccination for immunology exploratory assays.

Subjects will receive a single dose of study vaccine via IM injection in the deltoid muscle of the preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 20 minutes after the second study vaccination. The second study vaccination site will be examined, and any AE/SAEs will be assessed and recorded on the appropriate data collection form prior to discharge from the clinic.

Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their temperature around the same time each day. Subjects will be instructed on how to use the memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after the second study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, s/he will give further instructions on the
proper course of action, including a return to the clinic for immediate evaluation if appropriate.

8.2.4 Visit 03A, Day 23, Phone Call  
(Window: Day 2±1 day post second study vaccination)

Study personnel will contact subjects by phone to solicit any AE/SAE and concomitant medication information and review information on the memory aid.

8.2.5 Visit 04, Day 29, Clinic Visit  
(Window: Day 8+2 days post second study vaccination)

- Obtain interim medical history, including an assessment for symptoms suggestive of an autoimmune disorder, by interview of subjects and note any changes since the previous visit.
- Study personnel will review the memory aid information with subjects and assess and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.
- Examine the second study vaccination site.
- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays.
- From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 85 mL of venous blood will be collected for immunology exploratory assays.

8.2.6 Visit 05, Day 42, Clinic Visit  
(Window: Day 21+3 days post second study vaccination)

- Obtain interim medical history, including an assessment for symptoms suggestive of an autoimmune disorder, by interview of subjects and note any changes since the previous visit.
- All concomitant medications will be recorded on the appropriate data collection form.

- A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.

- All AE/SAEs will be assessed and recorded on the appropriate data collection form.

- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays.
  - An ADCC assay may be performed on a subset of these samples, but no additional venous blood will be collected.

- From a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays), an additional 53 mL of venous blood will be collected for immunology exploratory assays.

8.2.7 Visit 06, Day 81, Follow-up Phone Call  
(Window: Day 60±7 days post second study vaccination)

Subjects will be contacted by phone to query for safety events. Adverse events limited to new-onset chronic medical conditions and SAEs that have occurred since the previous visit will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

8.2.8 Visit 07, Day 141, Follow-up Phone Call  
(Window: Day 120±14 days post second study vaccination)

Subjects will be contacted by phone to query for safety events. Adverse events limited to new-onset chronic medical conditions and SAEs that have occurred since the previous visit will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

8.2.9 Visit 08, Day 201, Follow-up Phone Call  
(Window: Day 180±14 days post second study vaccination)

Subjects will be contacted by phone to query for safety events. Adverse events limited to new-onset chronic medical conditions and SAEs that have occurred since the previous visit will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.
8.3 Final Visit

8.3.1 Visit 09, Day 386, Follow-up Phone Call
(Window: Day 365±14 days post second study vaccination)

Subjects will be contacted by phone to query for safety events. Adverse events limited to new-onset chronic medical conditions and SAEs that have occurred since the previous visit will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

8.4 Early Termination Visit (if needed)

The following activities will be performed at the early termination visit for subjects who withdraw, or are withdrawn or terminated from the study:

- Obtain interim medical history, including an assessment for symptoms suggestive of an autoimmune disorder, by interview of subjects and note any changes since the previous visit.

- All concomitant medications will be recorded on the appropriate data collection form (if prior to 21 days after the last study vaccination).

- Information regarding AE/SAEs will be assessed and recorded on the appropriate data collection form (AEs will be limited to new-onset chronic medical conditions and SAEs if after 21 days after the last study vaccination).

- A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.

- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays (if prior to Visit 05).

8.5 Unscheduled Visit (if needed)

Unscheduled visits may occur at any time during the study. Any of the following activities may be performed:

- Review memory aid (if within 8 days after the last study vaccination).

- Review concomitant medications (if prior to 21 days after the last study vaccination).
- Review adverse events (if prior to 21 days after the last study vaccination).
- Review serious adverse events.
- Review new-onset chronic medical conditions (if after 21 days after the last study vaccination).
- Obtain interim medical history, including an assessment for symptoms suggestive of an autoimmune disorder, by interview of subjects and note any changes since the previous visit (if indicated).
- A targeted physical examination, including an assessment for signs suggestive of an autoimmune disorder, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.
- Examine study vaccination site (if within 8 days after the last study vaccination).
9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Study vaccine-related serious adverse events occurring from the time of the first study vaccination through approximately 13 months after the first study vaccination.

2. Solicited Adverse Events – reactogenicity events occurring on the day of each study vaccination through 7 days after each study vaccination:
   a) Injection site reactions including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness.
   b) Systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, and nausea.

3. Unsolicited Adverse Events – non-serious adverse events occurring from the time of the first study vaccination through approximately 21 days after the last study vaccination.

4. New-onset chronic medical conditions occurring through approximately 13 months after the first study vaccination.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

**Adverse Event (AE):** International Conference on Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited local (injection site) and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data
collection form and electronic case report form (eCRF). Information to be collected for unsolicited AEs includes event description, date of onset, licensed study physician’s assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator), date of resolution of the event, seriousness and outcome. AEs while on study will be documented appropriately regardless of relationship. AEs will be followed to resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it will be recorded as an AE.

AEs must be graded for severity and assessed for relationship to study product (see definitions below). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

**Severity of Event:** AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol-defined grading system (see Section 9.2.2). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- **Mild (Grade 1):** Events require minimal or no treatment and do not interfere with the subject’s daily activities.

- **Moderate (Grade 2):** Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.

- **Severe (Grade 3):** Events interrupt the subject’s usual daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

**Relationship to Study Product:** The study physician’s assessment of an AE’s relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:
- **Related** – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- **Not Related** – There is not a reasonable possibility that the administration of the study product caused the event.

### 9.2.2 Reactogenicity

Reactogenicity events are AEs that are known to occur with this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

<table>
<thead>
<tr>
<th>Local (Injection Site) Reactogenicity Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local (Injection Site) Reaction</strong></td>
</tr>
<tr>
<td>Pain – experienced without touching the injection site (spontaneous discomfort)</td>
</tr>
<tr>
<td>Tenderness – hurts only when injection site is touched or the arm is moved</td>
</tr>
<tr>
<td>Pruritus (Itching)</td>
</tr>
<tr>
<td>Ecchymosis (Bruising)*</td>
</tr>
<tr>
<td>Erythema (Redness)*</td>
</tr>
<tr>
<td>Induration (Hardness)/Swelling*</td>
</tr>
</tbody>
</table>

* Will be also measured in mm but size will not be used as halting criteria.
Ecchymosis (bruising), erythema (redness), and induration (hardness)/swelling as analyzed by measurement will be graded as follows:

**Local (Injection Site) Reactogenicity Measurements**

<table>
<thead>
<tr>
<th>Local (Injection Site) Reaction</th>
<th>Small</th>
<th>Medium (20 mm – 50 mm)</th>
<th>Large (&gt;50 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecchymosis (Bruising)*</td>
<td>&lt;20 mm</td>
<td>20 mm – 50 mm</td>
<td>&gt;50 mm</td>
</tr>
<tr>
<td>Erythema (Redness)*</td>
<td>&lt;20 mm</td>
<td>20 mm – 50 mm</td>
<td>&gt;50 mm</td>
</tr>
<tr>
<td>Induration (Hardness)/Swelling*</td>
<td>&lt;20 mm</td>
<td>20 mm – 50 mm</td>
<td>&gt;50 mm</td>
</tr>
</tbody>
</table>

* Will not be used as halting criteria.

**Subjective Systemic Reactogenicity Grading**

<table>
<thead>
<tr>
<th>Systemic (Subjective)</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feverishness (Chills/Shivering/Sweating)</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
<tr>
<td>Fatigue (Tiredness)</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
<tr>
<td>Malaise (General Unwell Feeling)</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
<tr>
<td>Myalgia (Body Aches/Muscular Pain)*</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
<tr>
<td>Arthralgia (Joint Pain)*</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
<tr>
<td>Headache</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
<tr>
<td>Nausea</td>
<td>No interference</td>
<td>Some interference</td>
<td>Significant interference,</td>
</tr>
<tr>
<td></td>
<td>with daily activity</td>
<td>with daily activity</td>
<td>prevents daily activity</td>
</tr>
</tbody>
</table>

* Not at injection site.
Oral temperature# will be graded as follows:

**Quantitative Systemic Reactogenicity Grading**

<table>
<thead>
<tr>
<th>Systemic (Quantitative)</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever* - oral†</td>
<td>38.0°C – 38.4°C</td>
<td>38.5°C – 38.9°C</td>
<td>&gt;38.9°C</td>
</tr>
<tr>
<td></td>
<td>100.4°F – 101.1°F</td>
<td>101.2°F – 102.0°F</td>
<td>&gt;102.0°F</td>
</tr>
</tbody>
</table>

# Oral temperature assessed on Day 0 (Visit 01) prior to the first study vaccination will be considered as baseline.

* Note: A fever can be considered not related to the study product if an alternative etiology can be documented.

† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

### 9.2.3 Additional Adverse Event Severity Grading

Pulse and blood pressure# will be graded as follows:

<table>
<thead>
<tr>
<th>Physiologic Parameter</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradycardia - beats per minute</td>
<td>45 – 49</td>
<td>40 – 44</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Tachycardia - beats per minute</td>
<td>116 – 130</td>
<td>131 – 155</td>
<td>&gt;155</td>
</tr>
<tr>
<td>Hypotension (systolic) mm Hg</td>
<td>80 – 84</td>
<td>75 – 79</td>
<td>&lt;75</td>
</tr>
<tr>
<td>Hypotension (diastolic) mm Hg</td>
<td>50 – 54</td>
<td>45 – 49</td>
<td>&lt;45</td>
</tr>
<tr>
<td>Hypertension (systolic) mm Hg</td>
<td>151 – 155</td>
<td>156 – 160</td>
<td>&gt;160</td>
</tr>
<tr>
<td>Hypertension (diastolic) mm Hg</td>
<td>96 – 100</td>
<td>101 – 105</td>
<td>&gt;105</td>
</tr>
</tbody>
</table>

# Pulse and blood pressure assessed on Day 0 (Visit 01) prior to the first study vaccination will be considered as baseline.

### 9.2.4 Serious Adverse Events

**Serious Adverse Event (SAE):** An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:
• Death,

• a life-threatening adverse event*,

• inpatient hospitalization or prolongation of existing hospitalization,

• a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

• a congenital anomaly/birth defect.

• Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

• All events described as Guillain-Barré syndrome will also be considered SAEs.

* Life-threatening adverse event. An adverse event is considered “life-threatening” if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

SAEs will be:

• Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

• Recorded on the appropriate SAE form and eCRF.

• Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

• Reviewed and evaluated by an Independent Safety Monitor (ISM), the DSMB (periodic review unless related), DMID, and the IRB.
9.2.5 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for reporting all AE/SAEs that are observed or reported during the study, regardless of the relationship to study product. AE/SAEs, abnormal clinical laboratory test values, or abnormal clinical findings will be documented, reported, and followed appropriately.

9.3 Reporting Procedures

Solicited injection site and systemic reactogenicity events will be documented from the time of each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) through 7 days after each study vaccination.

Unsolicited non-serious AEs will be documented from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 21 days after the last study vaccination (approximately Day 42 (Visit 05) for subjects who receive two study vaccinations; approximately Day 21 (Visit 03) for subjects who receive only one study vaccination). After approximately 21 days after the last study vaccination, non-serious AEs will be limited to new-onset chronic medical conditions, which will be documented through approximately 13 months after the first study vaccination (Visit 09).

SAEs will be documented from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 13 months after the first study vaccination (Visit 09).

9.3.1 Serious Adverse Events

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group at the following address:

DMID Pharmacovigilance Group  
Clinical Research Operations and Management Support (CROMS)  
6500 Rock Spring Dr. Suite 650  
Bethesda, MD 20814, USA  
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)  
SAE FAX: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)  
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into AdvantageEDC®. Please see the protocol-specific MOP for details regarding this procedure.
Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site principal investigator or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating site principal investigators (i.e., all principal investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator’s IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor’s initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as “not related” to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC SM on the Pregnancy Report form. No further study vaccinations will be administered to pregnant subjects, but with the subject’s permission all study mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the subject’s permission.
9.4 **Type and Duration of Follow-up of Subjects after Adverse Events**

AEs will be followed from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 21 days after the last study vaccination (approximately Day 42 (Visit 05) for subjects who receive two study vaccinations; approximately Day 21 (Visit 03) for subjects who receive only one study vaccination). After approximately 21 days after the last study vaccination, AEs will be limited to new-onset chronic medical conditions, which will be followed through approximately 13 months after the first study vaccination (Visit 09).

SAEs will be followed from the time of the first study vaccination (Day 0 (Visit 01)) through resolution even if this extends beyond the study-reporting period (approximately 13 months after the first study vaccination (Visit 09)). Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

If the site principal investigator or appropriate sub-investigator becomes aware of an acute febrile illness and the site principal investigator or appropriate sub-investigator decides to bring the subject in for an evaluation to determine etiology, then the site principal investigator or appropriate sub-investigator, at their own discretion, can determine if specific viral testing should be done by either culture or PCR to determine if the infectious agent was influenza and what strain.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 **Halting Rules**

Further enrollment and study vaccinations will be halted for DSMB review/recommendation if any of the following are reported:

- Any death occurring within 21 days after administration of a study vaccination that was not the result of trauma or accident, regardless of relatedness to study vaccination.
- Any subject experiences ulceration, abscess, or necrosis at the injection site related to study product administration.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of study product that is considered related to study product.
- Two or more subjects experience generalized urticaria within 72 hours after administration of study product that is considered related to study product.
• Any subject experiences a study vaccine-related SAE from the time of the first study vaccination through the subject’s last study visit.

• Any subject experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product.

• Any subject develops an autoimmune disease after administration of study product.

The study will also be halted for DSMB review/recommendation if, within 7 days after administration of each study vaccination, any of the following occurs:

• 7% or more of subjects (with a minimum of 5) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related injection site reaction.

• 7% or more of subjects (with a minimum of 5) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related subjective systemic reaction, for which the severity (grade) is corroborated by study personnel.

• 7% or more of subjects (with a minimum of 5) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related quantitative systemic reaction.

Grading scales for solicited local (injection site) and systemic (subjective and quantitative) reactions are included in Section 9.2.2.

If any of the halting rules are met following any subject receipt of the first or second study vaccination, the study will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the DSMB to proceed.

DMID retains the authority to suspend additional enrollment and study interventions/administration of study product during the entire study, as applicable.

9.6 Safety Oversight

9.6.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs in real time and other AEs as needed and provide an independent assessment to DMID. Each participating VTEU site will have an ISM with experience in infectious diseases or internal medicine, in close proximity to the participating VTEU site, and have the authority to readily access study participant records.
9.6.2 Data and Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to the study. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The DSMB will review study progress and participant, clinical, safety, reactogenicity, and immunogenicity data at the following time points:

- At specified times during the course of study as defined in the DSMB Charter.
- Monthly until the last subject completes Visit 05.
- Ad hoc when a halting rule is met, for immediate concerns regarding observations during the study, or as needed.

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. Procedures for DSMB reviews/meetings will be defined in the charter. The DSMB will review applicable data to include, but not limited to, study progress and participant, clinical, safety, reactogenicity, and immunogenicity data which may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing, solicited and unsolicited AE/SAEs, and HAI and Neut antibody assays results. Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by group. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion. The DSMB will meet and review this data at scheduled time points or ad hoc as needed during the study as defined in the DSMB charter. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate the study.

DMID or the DSMB chair may convene the DSMB on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The DMID Medical Monitor is empowered to stop enrollment and study vaccinations if adverse events that meet the halting criteria are reported. The DMID Medical Monitor and the ISM will be responsible for reviewing SAEs in real time. The DSMB will review SAEs on a regular basis and ad hoc during the study.
10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that the study is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the study site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.
11 STATISTICAL CONSIDERATIONS

11.1 Introduction

The goal of this study is to assess the safety and tolerability of the study vaccine formulations and the potential of the adjuvant to enhance the immune response to the A/H7N9 vaccine in healthy adults. Other goals are to assess whether a low HA dosage results in a robust immune response, to evaluate HA antigen- and adjuvant-sparing dosing strategies, and to evaluate in at least a subset of samples the generation of serum antibody responses against antigenically drifted variants of the A/H7N9 virus should they emerge prior to the last subject’s Visit 07.

The study will also evaluate the strategy of administering an A/H7N9 vaccine from one manufacturer extemporaneously mixed prior to administration with adjuvant from a different manufacturer. Such a strategy might prove critical in the setting of a public health emergency, when stockpiled vaccine and/or adjuvant might need to be deployed rapidly for prevention of infection or for the decrease in disease severity.

Note that study groups 3 and 4 and study groups 5 and 6 receive the identical first dose of study vaccine, sanofi A/H7N9 antigen 15 mcg plus Novartis MF59 adjuvant and sanofi A/H7N9 antigen 15 mcg, respectively. Therefore, results prior to the second study vaccination will be analyzed by collapsing study groups 3 and 4 and study groups 5 and 6, respectively.

11.2 Study Hypotheses

This is a Phase II randomized, double-blinded, controlled study and is not designed to test a specific hypothesis. Rather, it is intended to assess the safety, reactogenicity, and immunogenicity of two doses of monovalent A/H7N9 vaccine delivered intramuscularly approximately 21 days apart at different dosages (3.75, 7.5, or 15 mcg of HA/0.5 mL dose) given with MF59 adjuvant manufactured by Novartis Vaccines and Diagnostics or without adjuvant (15 mcg of HA/0.5 mL dose and 45 mcg of HA/0.75 mL dose). The sample size facilitates the formal testing of selected hypotheses as discussed in Section 11.4 Sample Size Considerations.

11.3 Study Outcome Measures

Primary:

Safety:
• Occurrence of study vaccine-related serious adverse events from the time of the first study vaccination through approximately 13 months after the first study vaccination.

• Occurrence of solicited injection site and systemic reactogenicity on the day of each study vaccination through 7 days after each study vaccination.

**Immunogenicity:**

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 21 days after the second study vaccination.

• Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at approximately 21 days after the second study vaccination.

**Secondary:**

**Safety:**

• Occurrence of unsolicited adverse events from the time of the first study vaccination through approximately 21 days after the last study vaccination.

• Occurrence of new-onset chronic medical conditions through 13 months after the first study vaccination.

**Immunogenicity:**

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 8 and 21 days after the first study vaccination.

• Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at baseline and at approximately 8 and 21 days after the first study vaccination.

• Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 8 days after the second study vaccination.
- Percentage of subjects achieving a serum HAI antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at approximately 8 days after the second study vaccination.

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination Neut titer <1:10 and a post-vaccination Neut titer ≥1:40 or a pre-vaccination Neut titer ≥1:10 and a minimum four-fold rise in post-vaccination Neut antibody titer) at approximately 8 and 21 days after each study vaccination.

- Percentage of subjects achieving a serum Neut antibody titer of 1:40 or greater against the A/H7N9 antigen contained in the study vaccine at baseline and at approximately 8 and 21 days after each study vaccination.

- Geometric Mean Titers of serum HAI and Neut antibody at baseline and at approximately 8 and 21 days after each study vaccination.

**Exploratory:**

**Immunogenicity:**

- Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) and Geometric Mean Titers of serum HAI antibody at baseline and at approximately 8 and 21 days after each study vaccination as a function of age, gender, body mass index, waist circumference, prior receipt of seasonal influenza vaccine, and geography (VTEU site).

- Development of serum antibody responses against antigenically drifted variants of the A/H7N9 virus in at least a subset of samples, should any variants occur and be available prior to the last subject’s Visit 07.

- Total ASC response by flow cytometry (defined as CD3-/CD20lo/CD19+/CD38hi/CD27hi cells and reported as a percentage of total lymphocytes or of total B cells) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

- Frequency of vaccine-specific ASCs measured by ELISpot assay reported as the number of influenza-specific ASCs per million peripheral blood mononuclear cells (PBMCs) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

- IGHV gene sequence analyses for B cell gene usage, clonality, and somatic hypermutation within these genes in a subset of healthy adults, 19-64 years old, at approximately 8 days after the second study vaccination.
• Frequency of blood T<sub>FH</sub> cell responses (reported as the number of ICOS+CXCR3+CXCR5+ CD4+ T cells) in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

• Frequencies and quality of T<sub>H</sub> cells and epitope specificities in a subset of healthy adults, 19-64 years old, at baseline and at approximately 8 and 21 days after each study vaccination.

• Correlations of blood T<sub>H</sub> and T<sub>FH</sub> responses with HAI and Neut titers, plasmablast responses detected by FACS analysis, and antigen-specific plasmablast responses by ELISpot assay in a subset of healthy adults, 19-64 years old, at baseline and approximately 8 and 21 days after each study vaccination.

• Assess the specificity of mAb against A/H7N9 and other influenza strains including evolved variants, if available, compare the epitopes (at head or stalk region of HA protein) recognized by the mAb generated by A/H7N9 vaccination, and analyze the avidity and breadth of mAb generated by A/H7N9 vaccination with and without MF59 adjuvant in a subset of healthy adults, 19-64 years old, at approximately 8 days after the second study vaccination.

• Percentage of subjects demonstrating detectable levels of ADCC responses against the epitopes (at head or stalk region of HA protein) generated by A/H7N9 vaccination, and the correlation of the ADCC responses with HAI and Neut titers generated by A/H7N9 vaccination with and without MF59 adjuvant in a subset of samples at approximately 21 days after the second study vaccination.

11.4 Sample Size Considerations

11.4.1 Study Design

This is a Phase II randomized, double-blinded, controlled study in up to 700 males and non-pregnant females, 19 to 64 years old, inclusive, who are in good health and meet all eligibility criteria. The study is designed to assess the safety, reactogenicity, and immunogenicity of a monovalent influenza A/H7N9 virus vaccine manufactured by sanofi pasteur administered to healthy adults at different dosages (3.75, 7.5, or 15 mcg of HA/0.5 mL dose) given with MF59 adjuvant manufactured by Novartis Vaccines and Diagnostics or without adjuvant (15 mcg of HA/0.5 mL dose and 45 mcg of HA/0.75 mL dose). The A/H7N9 vaccine was made with HA antigen derived from the influenza A/Shanghai/2/2013 virus.

Subjects will be randomly assigned to 1 of 7 groups (up to 100 subjects per group) to receive two doses of the A/H7N9 vaccine with or without MF59 adjuvant delivered intramuscularly.
approximately 21 days apart. The same dosage of A/H7N9 vaccine will be given to subjects at both their first and second study vaccinations.

Safety will be measured from the time of each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) through 7 days after each study vaccination by the occurrence of solicited injection site and systemic reactogenicity events. Unsolicited non-serious adverse events (AEs) will be collected from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 21 days after the last study vaccination (approximately Day 42 (Visit 05) for subjects who receive two study vaccinations; approximately Day 21 (Visit 03) for subjects who receive only one study vaccination). After approximately 21 days after the last study vaccination, non-serious AEs will be limited to new-onset chronic medical conditions, which will be documented through approximately 13 months after the first study vaccination (Visit 09). Serious adverse events (SAEs) will be collected from the time of the first study vaccination (Day 0 (Visit 01)) through approximately 13 months after the first study vaccination (Visit 09).

Immunogenicity testing will include performing hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays on serum obtained prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after each study vaccination (Visit 02 and Visit 04) and approximately 21 days after the second study vaccination (Visit 05).

Immunology exploratory assay testing will be performed on samples from a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation) obtained prior to each study vaccination (Day 0 (Visit 01) and approximately Day 21 (Visit 03)) as well as approximately 8 days after each study vaccination (Visit 02 and Visit 04) and approximately 21 days after the second study vaccination (Visit 05).

Additionally, an ADCC assay for H7 is in development. If successful, ADCC may be assessed in a subset of samples collected at approximately 21 days after the second study vaccination (Visit 05).

### 11.4.2 Study Population

The study population for this protocol is up to 700 males and non-pregnant females, 19 to 64 years old, inclusive, who are in good health and meet all eligibility criteria. The subjects will be recruited from the general population at the participating VTEU sites that have substantial experience conducting large influenza vaccine studies. For example, 3 VTEUs recruited 452 subjects in 2 stages in a total of 3 weeks for a similar study of an alternate (unadjuvanted) formulation of the A/H5N1 vaccine in healthy adults aged 18 to 64 years (22).
11.4.3 Subject Enrollment and Follow-up

Based on the accrual rate for similar studies, it seems reasonable to expect that the VTEUs will be able to enroll the study in a timely fashion. Prior experience suggests that there will be at most a 2-3% loss to follow-up. As discussed below, sample size calculations will conservatively assume a 5% loss. Subjects who sign the informed consent form and are randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form, and are randomized and vaccinated, and subsequently withdraw, or are withdrawn or terminated from the study, or are lost to follow-up will not be replaced.

Follow-up will consist of 2 segments. The first encompasses the core data for this study and will consist of results for all visits through approximately 21 days after the second study vaccination (Visit 05). The second segment consists of safety follow-up assessments through approximately 13 months after the first study vaccination (Visit 09).

11.4.4 Sample Size

The sample size for this study was selected to obtain preliminary estimates in a time critical manner. While this study is not designed to test any specific null hypothesis, the following illustrates the precision and power that is available for some simple comparisons.

The following table indicates the probability of observing one or more event, such as an adverse event of a particular type, for a single study vaccine group (n=95) or for all groups combined (n=665):

<table>
<thead>
<tr>
<th>&quot;True&quot; Unknown Population Probability of an Event</th>
<th>Probability of Observing 1 or more Events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=95</td>
</tr>
<tr>
<td>0.1%</td>
<td>9.1%</td>
</tr>
<tr>
<td>0.3%</td>
<td>24.8%</td>
</tr>
<tr>
<td>0.5%</td>
<td>37.9%</td>
</tr>
<tr>
<td>1.0%</td>
<td>61.5%</td>
</tr>
<tr>
<td>2.0%</td>
<td>85.3%</td>
</tr>
<tr>
<td>3.0%</td>
<td>94.5%</td>
</tr>
</tbody>
</table>

Therefore, when the true event rate is 3.0%, there is 94.5% power to observe one or more events in a single study vaccine group. For the full sample, there is an 89.1% chance of observing one or more event when the true event rate is as low as 0.3%.

Binomial confidence intervals are widest (have the least precision) when the response rate is 50.0%. When the observed rate is 50% and the sample sizes are 95 (single study vaccine
group) or 190 (two study vaccine groups with same first dose combined), then the 95% asymptotic confidence intervals are (39.9%, 60.1%) and (44.2%, 55.8%) respectively. These results are not power computations. They are only provided as an indication of the worst case scenario for observed binomial confidence intervals.

The table below illustrates the minimum detectable differences between two proportions (e.g., attaining an HAI titer ≥1:40) using a Z-test with pooled variance when rates in one group are 50.0% or 75.0%. Due to the lack of experience with A/H7N9 vaccines, these rates were selected to provide a “worst case” scenario (50%) and a contrasting rate (75%).

<table>
<thead>
<tr>
<th>Assumed Response Rate in Group 1</th>
<th>Sample Size per Group in Two Group Comparison</th>
<th>Power=80%</th>
<th>Power=90%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum Detectable Difference (Group 2 – Group 1)</td>
<td>Response Rate in Group 2</td>
</tr>
<tr>
<td>0.50</td>
<td>95</td>
<td>0.20</td>
<td>0.70</td>
</tr>
<tr>
<td>0.75</td>
<td>95</td>
<td>0.15</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Therefore, even in the comparison of two study vaccine groups of size n=95 where one group has a 0.50 response rate, there will be 90% power to detect a significant difference in a comparison group with response rate of 0.73, i.e., a difference of 0.23. At the opposite extreme, there is 80% power to detect a difference of 0.15 when one group has a 0.75 response rate.

### 11.5 Planned Interim Analyses

Interim analyses will only be used to terminate the trial in the event that unanticipated safety concerns deemed to be of sufficient concern require such action by the sponsor. These assessments will not be made on the basis of testing a formal statistical hypothesis; therefore, p-value adjustment will not be made to any analyses. A DSMB will be convened by DMID to review study progress and participant, clinical, safety, reactogenicity, and immunogenicity data.

#### 11.5.1 Interim Safety Review

Interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing, and solicited and unsolicited AE/SAEs. Additional data may be requested by the DSMB, and interim
statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by group. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion. The DSMB will meet and review this data at scheduled time points or ad hoc as needed during the study as defined in the DSMB charter. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate the study.

Additionally, the study will be monitored to determine if any of the halting rules described in Section 9.5 are met.

**11.5.2 Interim Immunogenicity Review**

An analysis of serum antibody responses comparing venous blood samples collected on Visit 01, Visit 02, Visit 03, Visit 04, and Visit 05 is planned for 25 subjects per dosage group when their samples are available. This information will be examined due to the extraordinary need to inform public health decision makers in their preparations for response to A/H7N9. Emergent public health needs may also dictate interim safety, reactogenicity, and/or immunogenicity analysis be performed on all available information at any time during the trial. If this occurs, immunogenicity data will be analyzed as results are available from Southern Research. Although immunogenicity results will not be used to make any decisions concerning the conduct of this trial, they may be used to make decisions on activities external to this trial, e.g., designing future trials of the A/H7N9 vaccine with and without MF59 adjuvant in other age groups.

Immune responses will be summarized in terms of strain-specific A/H7N9 HAI and Neut antibody titers and the relationship to study vaccine dosage, adjuvant, and ordering of adjuvants. It is anticipated that all analyses will be carried out in parallel for both assays. Interim analyses will focus on rates of HAI titers ≥1:40, seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) and Geometric Mean Titers (GMTs).

**11.6 Final Analysis Plan**

The final analysis will be performed and clinical study report completed when all primary and secondary safety endpoint data and all primary immunogenicity endpoint data are available. Any available data from the secondary and exploratory immunogenicity endpoints may also be included. The clinical study report will be amended as additional secondary and exploratory immunogenicity endpoint data become available.
11.6.1 Analysis Populations

The Safety Analysis population includes all eligible subjects who received at least one dose of study vaccine.

The intent-to-treat (ITT) population includes all eligible subjects who received at least one dose of study vaccine and contributed both pre- and at least one post-study vaccination blood samples for testing for which valid results were reported.

The per protocol (PP) population excludes subjects who did not receive both doses of study vaccine or who had major protocol deviations, such as receipt of non-study vaccines during the timeframe prohibited by the protocol or receipt of the second study vaccination substantially out of window.

In the case of miss-randomization subjects will be analyzed according to the study product actually received for all analysis populations.

11.6.2 Safety Data

Solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the reactogenicity rates. The possibility of a dose- or adjuvant-response relationship will be explored using logistic regression. Analyses will be conducted separately for each study vaccination. Both study vaccination doses will also be compared, for example, to determine if the response to the first dose of study vaccine is predictive of the response to the second dose of study vaccine.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class. The number of SAEs is likely to be small in this study and will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (vaccinations and AEs), severity, relatedness, and outcome for each event. Additionally, the rate and exact 95% confidence intervals of AEs in aggregate, as well as by MedDRA® categories, will be computed.

11.6.3 Immunogenicity Data (HAI and Neut)

Immune responses will be summarized in terms of strain-specific A/H7N9 HAI and Neut antibody titers and the relationship to study vaccine dosage, adjuvant, and ordering of adjuvants. It is anticipated that all analyses will be carried out in parallel for both assays. Analyses will include rates of HAI titers ≥1:40, seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) and Geometric Mean
Titers (GMTs). Descriptive summary statistics (e.g., means and 95% confidence intervals) will be provided for all assays and time points. Group-specific and appropriate pair-wise comparisons (differences and Geometric Fold Rises) will be summarized. Plots such as reverse cumulative distributions will be presented.

Dose-response models will be developed. HAI and Neut antibody responses will each be compared for the A/H7N9 vaccine with and without MF59 adjuvant using log transformed data for linear models. Logistic regression will similarly be used to examine the relationship between the proportion of responders and dosage and adjuvant. Longitudinal models will be used to determine the impact of successive doses on immune responses.

The data will also be explored to examine the possible impact of available covariates such as baseline immune responses, age, gender, body mass index, waist circumference, prior receipt of seasonal influenza vaccine, and geography as determined by participating VTEU sites.

All or at least a subset of samples may also be tested for cross-reactive immune responses using drifted variants of the A/H7N9 virus should they emerge prior to the last subject’s Visit 07. If such assays are performed, the results will be summarized using descriptive statistics, correlations with A/H7N9 responses, and association with study vaccine dose and adjuvant as part of the Clinical Study Report (CSR) or as an addendum if the CSR has already been finalized.

**11.6.4 Exploratory Immunogenicity Data**

Immunology exploratory assays, including ASCs and CD4+ T cell responses by FACS analysis and ELISpot assays, will be performed on samples collected from a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays) at Visit 01, Visit 02, Visit 03, Visit 04, and Visit 05.

In addition, mAb from 10 subjects will also be generated after single-cell sorting of ASCs at approximately 8 days after the second study vaccination (Visit 04). The origin and the degree of clonality of these mAbs will be defined, and their potential value as therapeutics for drug-resistant A/H7N9 infections will be assessed.

Additionally, an ADCC assay for H7 is in development. If successful, ADCC may be assessed in a subset of samples collected at approximately 21 days after the second study vaccination (Visit 05).

Analysis of the exploratory immunogenicity data will be primary descriptive. Data will be summarized by treatment arm. Correlation between exploratory endpoints, HAI titers, and Neut titers will be assessed.
11.6.5 Missing Values and Outliers

All attempts will be made to collect all data per protocol. No imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.
12 DATA COLLECTION FORMS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating VTEU site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Each participating VTEU site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical study records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.
13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating VTEU site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all trial-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.
14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site principal investigator will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site principal investigator’s Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and informed consent form will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DMID. The IRB Federal Wide Assurance number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site principal investigator for submission to the IRB.

14.3 Informed Consent Process

14.3.1 Informed Consent

The site principal investigator will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual’s trial participation. Before any study procedures are performed, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products, including the nature and risks of the trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including
specifically their serum samples. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them, and have the opportunity to discuss the trial with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits are given to subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain the research study to subjects and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering study product.

DMID will provide the site principal investigator, in writing, any new information that significantly impacts the subjects' risk of receiving the investigational product. This new information will be communicated by the site principal investigator to subjects who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an informed consent form must be signed. Subjects will be given a copy of all informed consent forms that they sign.

By signing the informed consent form, subjects agree to complete all evaluations required by the trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from the trial for any reason.

The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all adults who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations.

It is unknown if the A/H7N9 vaccine poses any risks to an unborn child. Female subjects of childbearing potential who are not surgically sterile via tubal sterilization, bilateral
oophorectomy, or hysterectomy or who are not postmenopausal for ≥ 1 year must agree to practice highly effective contraception that may include, but is not limited to, abstinence from intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, and licensed hormonal methods, with use of a highly effective method of contraception for a minimum of 30 days prior to study product exposure and agree to practice highly effective contraception for the duration of study product exposure, including 2 months (defined as 60 days) after the last study vaccination. A highly effective method of contraception is defined as one which results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all female subjects of childbearing potential will be required to have a negative serum or urine pregnancy test within 24 hours prior to receiving each dose of study vaccine. If a female subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

Children will not be included in this trial as presently there are no safety or efficacy data in adults.

14.5 Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the participating site principal investigators, their study personnel, the sponsor(s), and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the Sponsor and all data and information generated by the participating VTEU site as part of the trial (other than a subject’s medical records) will be kept confidential by the site principal investigator and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the trial; (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16. If a written contract for the conduct of the trial which includes confidentiality provisions inconsistent with this statement is executed, that contract’s confidentiality provisions shall apply rather than this statement.
The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site principal investigator. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The participating VTEU sites will permit access to such records.

14.6 Study Discontinuation

If the trial is discontinued, subjects who sign the informed consent form, and are randomized and vaccinated will continue to be followed for safety assessments. No further study vaccinations will be administered.

14.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for taking part in this trial.

Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB’s policies and procedures, and subject to IRB approval.

If it is determined by the participating VTEU site and the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating VTEU site, such as giving emergency medications to stop immediate allergic reactions to the study vaccine. No financial compensation will be provided to the subject by the participating VTEU site for any injury suffered due to participation in this trial.

14.8 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. Some samples may be stored at the local site and some at a central clinical storage facility. Samples may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject’s confidentiality.

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject’s samples will NOT be kept in their health records.
Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of the trial. The subject's decision can be changed at any time prior to the end of the trial by notifying the study doctors or nurses in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.
15 DATA HANDLING AND RECORD KEEPING

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRF and provided by the SDCC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from the data collection forms should be consistent with the data collection forms or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the participating VTEU sites under the supervision of the respective site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

The SDCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), reactogenicity, and immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.
15.3 Types of Data

Data for this study will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

15.4 Timing/Reports

A final report will be prepared following the availability of all the safety, reactogenicity, and immunogenicity data. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and immunogenicity summary reports may be generated for the DSMB.

After full analysis and final reporting is complete, and upon request and DMID approval, the SDCC will provide the participating VTEU sites with a summary of results by treatment group and/or subject treatment assignments. In this regard, the participating VTEU sites requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5 Study Records Retention

Study records and reports, including, but not limited to, case report forms (CRFs), source documents, informed consent forms (except for future use informed consent forms), laboratory test results, and medication inventory records, shall be retained for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. The site must contact DMID for authorization prior to the destruction of any study records. Informed consent forms for future use will be maintained as long as the sample exists.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or other study personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.
These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the SDCC’s AdvantageEDC℠.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per its guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.
16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine’s PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

Following completion of the study, the lead principal investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

Results of any exploratory immunogenicity analysis will not be published prior to publication of the primary immunogenicity results for this study.

It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication.

For trials in which DMID is not the IND/IDE sponsor, or there is no IND/IDE, and DMID does not provide data management services, it is the responsibility of the investigator to register the trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

Refer to:
- Public Law 110-85, Section 801, Clinical Trial Databases
17 LITERATURE REFERENCES


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APPENDICES

Appendix A: Schedule of Procedures and Evaluations
Appendix B: Preparation, Labeling, Storage, and Administration of Study Vaccine for Group 1
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## APPENDIX A: SCHEDULE OF PROCEDURES AND EVALUATIONS

<table>
<thead>
<tr>
<th>Study Visit Number</th>
<th>V01</th>
<th>V01A*</th>
<th>V02</th>
<th>V03</th>
<th>V03A*</th>
<th>V04</th>
<th>V05</th>
<th>V06*</th>
<th>V07*</th>
<th>V08*</th>
<th>V09*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Day post first study vaccination</td>
<td>D0</td>
<td>D2±1d</td>
<td>D8±2d</td>
<td>D21+3d</td>
<td>D23</td>
<td>D29</td>
<td>D42</td>
<td>D81</td>
<td>D141</td>
<td>D201</td>
<td>D386</td>
</tr>
<tr>
<td>Study Day post second study vaccination</td>
<td>D0</td>
<td>D2±1d</td>
<td>D8±2d</td>
<td>D21+3d</td>
<td>D60±7d</td>
<td>D120±14d</td>
<td>D180±14d</td>
<td>D365±14d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Early Termination
- X (if prior to 21 days after last study vaccination)

### Procedure

- **Obtain Informed Consent**: X†
- **Review Eligibility Criteria**: X†
- **Medical History**: X† X† X† X† X† X† X† X†
- **Concomitant Medications**: X† X† X† X† X† X† X† X†
- **Vital Signs**: X† X† X† (Oral Temperature, Pulse, and BP)
- **Height and Weight**: X†
- **Waist Circumference**: X†
- **Targeted Physical Examination**: X†
- **Urine or Serum Pregnancy Test**: X
- **Venous Blood Collection for HA1 and Neut Antibody Assays**: X† X†
- **Venous Blood Collection for Immunology Exploratory Assays**: X† X† X† X† X†
### Study Visit Number

<table>
<thead>
<tr>
<th>Study Visit Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>V01</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>Study Day post first study vaccination</td>
</tr>
<tr>
<td>D0</td>
</tr>
<tr>
<td>Study Day post second study vaccination</td>
</tr>
<tr>
<td>D0</td>
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### Early Termination

<table>
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<th>Procedure</th>
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</thead>
<tbody>
<tr>
<td>Enrollment in AdvantageEDCSM and Randomization</td>
</tr>
<tr>
<td>Study Vaccination</td>
</tr>
<tr>
<td>Examine Study Vaccination Site</td>
</tr>
<tr>
<td>20-minute Evaluation Period After Study Vaccination</td>
</tr>
<tr>
<td>Distribute Memory Aid and Study-Related Materials</td>
</tr>
<tr>
<td>Review Memory Aid</td>
</tr>
<tr>
<td>AE/SAE Assessment</td>
</tr>
</tbody>
</table>

* Phone call assessment.
† Prior to study vaccination.
‡ Complete medical history by interview of subjects to be obtained on Day 0 (Visit 01) prior to the first study vaccination and interim medical history by interview of subjects to be obtained at follow-up visits after the first study vaccination.
§ All current medications and medications taken within 30 days prior to signing the informed consent form.
% Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
$ Vital signs assessed on Day 0 (Visit 01) prior to the first study vaccination will be considered as baseline.
** Waist circumference is measured immediately above the iliac crest and recorded in inches.
() Targeted physical examination if indicated based on review of complete or interim medical history.
^ Must be performed on all female subjects of childbearing potential within 24 hours prior to each study vaccination and results must be negative and known prior to each study vaccination.

& Inclusive of reactogenicity assessments performed on the day of each study vaccination through 7 days after each study vaccination.

# New-onset chronic medical conditions and SAEs if after 21 days after the last study vaccination.

1 Only for a subset of healthy adult subjects (up to 75 volunteers, 19-64 years old, enrolled at the Emory VTEU site, who consent to blood donation for the immunology exploratory assays).
APPENDIX B: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF STUDY VACCINE FOR GROUP 1

1. Study Vaccine Preparation

**Group 1 First and Second Doses (3.75 mcg A/H7N9 Antigen + MF59 Adjuvant)**

Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration. Admixture of A/H7N9 vaccine and MF59 adjuvant by the pharmacist must be performed under a laminar flow hood using aseptic technique according to USP 797 guidelines.

1-inch, 23- or 25-gauge needles that are attached to disposable 1-mL sterile syringes should be used for study vaccine preparation. Once used, needles and syringes should be disposed of as biohazardous material in accordance with institutional guidelines.

The sterile empty vials provided for this study do NOT have a vacuum. Therefore, after injecting study product into a vial for admixing, the same volume of air from the dead space must be removed.

Visually inspect the A/H7N9 vaccine and MF59 adjuvant upon receipt and prior to use. The A/H7N9 vaccine suspension will be clear and slightly opalescent in appearance. The MF59 adjuvant oil-in-water emulsion will be milky in appearance. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). At the completion of admixture preparation and prior to study vaccine administration, visually inspect the A/H7N9 vaccine plus MF59 adjuvant admixture. The admixture will be milky in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

Used and unused vials of A/H7N9 vaccine, MF59 adjuvant, and admixture will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, MF59 adjuvant, and admixture. Final disposition of the unused A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

1.1 Preparation procedure for 3.75 mcg A/H7N9 antigen + MF59 adjuvant (Group 1 First and Second Doses)

1.1.1 Remove one vial of 7.5 mcg/0.5 mL A/H7N9 antigen and one vial of MF59 adjuvant from the refrigerator.

1.1.2 Record the Vial Numbers of the 7.5 mcg/0.5 mL A/H7N9 antigen vial and MF59 adjuvant vial on the appropriate Study Product Accountability Record for each vial.

1.1.3 Gently invert the 7.5 mcg/0.5 mL A/H7N9 antigen vial 5 to 7 times. Using aseptic technique, puncture the septum top of the vial with a 1-inch, 23- or 25-gauge needle attached to a disposable 1-mL sterile syringe.
1.1.4 Withdraw 0.5 mL from the vial containing 7.5 mcg/0.5 mL A/H7N9 antigen. Using aseptic technique, slowly inject the entire volume into a 3-mL sterile empty vial.

1.1.5 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial containing the A/H7N9 antigen, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.6 Using aseptic technique, puncture the septum top of the MF59 adjuvant vial with a new 1-inch, 23- or 25-gauge needle attached to a new disposable 1-mL sterile syringe.

1.1.7 Withdraw 0.5 mL from the MF59 adjuvant vial. Using aseptic technique, slowly inject the entire volume into the 3-mL sterile mixing vial containing the A/H7N9 antigen.

1.1.8 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial now containing both the A/H7N9 antigen and MF59 adjuvant, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.9 Gently invert the 3-mL sterile mixing vial now containing the A/H7N9 antigen plus MF59 adjuvant admixture 5 to 7 times. Do not shake vial. This is the final mixed vial for Group 1.

1.1.10 Label this final mixed vial in accordance with Section 2 of this appendix.

1.1.11 A single 0.5 mL dose from this final mixed vial contains 3.75 mcg of A/H7N9 antigen plus one dose of MF59 adjuvant. Only 1 dose should be drawn from this final mixed vial.

1.1.12 Refer to Sections 3 and 4 of this appendix for further instructions. Record the Final Mixed Vial Number and the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for each final mixed vial.

2. Labeling of Final Mixed Vials

All final mixed vials must be labeled. Contents on the label must adhere to local regulations and institutional procedures. At a minimum, the following items listed must be included on the label for each final mixed vial:

- Vial number, which is assigned sequentially by the pharmacist as the final mixed vials are prepared (number uniquely, do not start over at 1 or repeat numbers)
- Protocol number
- Product name (identifying contents) and strength
- Preparation and/or expiration date and time
- Caution: For Investigational Use only

3. Storage and Stability of Final Mixed Vials

Final mixed vials must be stored at room temperature in an upright orientation and must be used within 8 hours.
4. Study Vaccine Administration

Study vaccine administration will be performed by an unblinded study clinician who is licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. Each dose of study vaccine will be administered via a single intramuscular injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of Procedures for information on how to administer intramuscular injections.

All subjects in Group 1 will receive a single 0.5 mL dose of study vaccine from the final mixed vial via intramuscular injection on the day of each vaccination. Gently invert the final mixed vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the final mixed vial. Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe. The single dose must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered. Note: Each 0.5 mL dose of MF59-adjuvanted study vaccine contains one-0.25 mL dose of MF59 adjuvant.

Needles and syringes will be disposed of in Sharps containers immediately after administration.

The Study Product Accountability Records and Dispensing Logs will capture vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, MF59 adjuvant, and admixture, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.
APPENDIX C: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF STUDY VACCINE FOR GROUP 2

1. Study Vaccine Preparation

Group 2 First and Second Doses (7.5 mcg A/H7N9 Antigen + MF59 Adjuvant)

Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration. Admixture of A/H7N9 vaccine and MF59 adjuvant by the pharmacist must be performed under a laminar flow hood using aseptic technique according to USP 797 guidelines.

1-inch, 23- or 25-gauge needles that are attached to disposable 1-mL sterile syringes should be used for study vaccine preparation. Once used, needles and syringes should be disposed of as biohazardous material in accordance with institutional guidelines.

The sterile empty vials provided for this study do NOT have a vacuum. Therefore, after injecting study product into a vial for admixing, the same volume of air from the dead space must be removed.

Visually inspect the A/H7N9 vaccine and MF59 adjuvant upon receipt and prior to use. The A/H7N9 vaccine suspension will be clear and slightly opalescent in appearance. The MF59 adjuvant oil-in-water emulsion will be milky in appearance. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). At the completion of admixture preparation and prior to study vaccine administration, visually inspect the A/H7N9 vaccine plus MF59 adjuvant admixture. The admixture will be milky in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

Used and unused vials of A/H7N9 vaccine, MF59 adjuvant, and admixture will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, MF59 adjuvant, and admixture. Final disposition of the unused A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

1.1 Preparation procedure for 7.5 mcg A/H7N9 antigen + MF59 adjuvant (Group 2 First and Second Doses)

1.1.1 Remove one vial of 15 mcg/0.5 mL A/H7N9 antigen and one vial of MF59 adjuvant from the refrigerator.

1.1.2 Record the Vial Numbers of the 15 mcg/0.5 mL A/H7N9 antigen vial and MF59 adjuvant vial on the appropriate Study Product Accountability Record for each vial.

1.1.3 Gently invert the 15 mcg/0.5 mL A/H7N9 antigen vial 5 to 7 times. Using aseptic technique, puncture the septum top of the vial with a 1-inch, 23- or 25-gauge needle attached to a disposable 1-mL sterile syringe.
1.1.4 Withdraw 0.5 mL from the vial containing 15 mcg/0.5 mL A/H7N9 antigen. Using aseptic technique, slowly inject the entire volume into a 3-mL sterile empty vial.

1.1.5 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial containing the A/H7N9 antigen, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.6 Using aseptic technique, puncture the septum top of the MF59 adjuvant vial with a new 1-inch, 23- or 25-gauge needle attached to a new disposable 1-mL sterile syringe.

1.1.7 Withdraw 0.5 mL from the MF59 adjuvant vial. Using aseptic technique, slowly inject the entire volume into the 3-mL sterile mixing vial containing the A/H7N9 antigen.

1.1.8 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial now containing both the A/H7N9 antigen and MF59 adjuvant, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.9 Gently invert the 3-mL sterile mixing vial now containing the A/H7N9 antigen plus MF59 adjuvant admixture 5 to 7 times. Do not shake vial. This is the final mixed vial for Group 2.

1.1.10 Label this final mixed vial in accordance with Section 2 of this appendix.

1.1.11 A single 0.5 mL dose from this final mixed vial contains 7.5 mcg of A/H7N9 antigen plus one dose of MF59 adjuvant. Only 1 dose should be drawn from this final mixed vial.

1.1.12 Refer to Sections 3 and 4 of this appendix for further instructions. Record the Final Mixed Vial Number and the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for each final mixed vial.

2. Labeling of Final Mixed Vials

All final mixed vials must be labeled. Contents on the label must adhere to local regulations and institutional procedures. At a minimum, the following items listed must be included on the label for each final mixed vial:

- Vial number, which is assigned sequentially by the pharmacist as the final mixed vials are prepared (number uniquely, do not start over at 1 or repeat numbers)
- Protocol number
- Product name (identifying contents) and strength
- Preparation and/or expiration date and time
- Caution: For Investigational Use only

3. Storage and Stability of Final Mixed Vials

Final mixed vials must be stored at room temperature in an upright orientation and must be used within 8 hours.
4. **Study Vaccine Administration**

Study vaccine administration will be performed by an unblinded study clinician who is licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. Each dose of study vaccine will be administered via a single intramuscular injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of Procedures for information on how to administer intramuscular injections.

All subjects in Group 2 will receive a single 0.5 mL dose of study vaccine from the final mixed vial via intramuscular injection on the day of each vaccination. Gently invert the final mixed vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the final mixed vial. Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe. The single dose must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered. Note: Each 0.5 mL dose of MF59-adjuvanted study vaccine contains one- 0.25 mL dose of MF59 adjuvant.

Needles and syringes will be disposed of in Sharps containers immediately after administration.

The Study Product Accountability Records and Dispensing Logs will capture vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, MF59 adjuvant, and admixture, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.
APPENDIX D: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF STUDY VACCINE FOR GROUP 3

1. Study Vaccine Preparation

**Group 3 First and Second Doses (15 mcg A/H7N9 Antigen + MF59 Adjuvant)**

Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration. Admixture of A/H7N9 vaccine and MF59 adjuvant by the pharmacist must be performed under a laminar flow hood using aseptic technique according to USP 797 guidelines.

1-inch, 23- or 25-gauge needles that are attached to disposable 1-mL sterile syringes should be used for study vaccine preparation. Once used, needles and syringes should be disposed of as biohazardous material in accordance with institutional guidelines.

The sterile empty vials provided for this study do NOT have a vacuum. Therefore, after injecting study product into a vial for admixing, the same volume of air from the dead space must be removed.

Visually inspect the A/H7N9 vaccine and MF59 adjuvant upon receipt and prior to use. The A/H7N9 vaccine suspension will be clear and slightly opalescent in appearance. The MF59 adjuvant oil-in-water emulsion will be milky in appearance. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). At the completion of admixture preparation and prior to study vaccine administration, visually inspect the A/H7N9 vaccine plus MF59 adjuvant admixture. The admixture will be milky in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

Used and unused vials of A/H7N9 vaccine, MF59 adjuvant, and admixture will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, MF59 adjuvant, and admixture. Final disposition of the unused A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

1.1 Preparation procedure for 15 mcg A/H7N9 antigen + MF59 adjuvant (Group 3 First and Second Doses)

1.1.1 Remove one vial of 30 mcg/0.5 mL A/H7N9 antigen and one vial of MF59 adjuvant from the refrigerator.

1.1.2 Record the Vial Numbers of the 30 mcg/0.5 mL A/H7N9 antigen vial and MF59 adjuvant vial on the appropriate Study Product Accountability Record for each vial.

1.1.3 Gently invert the 30 mcg/0.5 mL A/H7N9 antigen vial 5 to 7 times. Using aseptic technique, puncture the septum top of the vial with a 1-inch, 23- or 25-gauge needle attached to a disposable 1-mL sterile syringe.
1.1.4 Withdraw 0.5 mL from the vial containing 30 mcg/0.5 mL A/H7N9 antigen. Using aseptic technique, slowly inject the entire volume into a 3-mL sterile empty vial.

1.1.5 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial containing the A/H7N9 antigen, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.6 Using aseptic technique, puncture the septum top of the MF59 adjuvant vial with a new 1-inch, 23- or 25-gauge needle attached to a new disposable 1-mL sterile syringe.

1.1.7 Withdraw 0.5 mL from the MF59 adjuvant vial. Using aseptic technique, slowly inject the entire volume into the 3-mL sterile mixing vial containing the A/H7N9 antigen.

1.1.8 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial now containing both the A/H7N9 antigen and MF59 adjuvant, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.9 Gently invert the 3-mL sterile mixing vial now containing the A/H7N9 antigen plus MF59 adjuvant admixture 5 to 7 times. Do not shake vial. This is the final mixed vial for Group 3.

1.1.10 Label this final mixed vial in accordance with Section 2 of this appendix.

1.1.11 A single 0.5 mL dose from this final mixed vial contains 15 mcg of A/H7N9 antigen plus one dose of MF59 adjuvant. Only 1 dose should be drawn from this final mixed vial.

1.1.12 Refer to Sections 3 and 4 of this appendix for further instructions. Record the Final Mixed Vial Number and the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for each final mixed vial.

2. Labeling of Final Mixed Vials

All final mixed vials must be labeled. Contents on the label must adhere to local regulations and institutional procedures. At a minimum, the following items listed must be included on the label for each final mixed vial:

- Vial number, which is assigned sequentially by the pharmacist as the final mixed vials are prepared (number uniquely, do not start over at 1 or repeat numbers)
- Protocol number
- Product name (identifying contents) and strength
- Preparation and/or expiration date and time
- Caution: For Investigational Use only

3. Storage and Stability of Final Mixed Vials

Final mixed vials must be stored at room temperature in an upright orientation and must be used within 8 hours.
4. Study Vaccine Administration

Study vaccine administration will be performed by an unblinded study clinician who is licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. Each dose of study vaccine will be administered via a single intramuscular injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of Procedures for information on how to administer intramuscular injections.

All subjects in Group 3 will receive a single 0.5 mL dose of study vaccine from the final mixed vial via intramuscular injection on the day of each vaccination. Gently invert the final mixed vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the final mixed vial. Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe. The single dose must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered. Note: Each 0.5 mL dose of MF59-adjuvanted study vaccine contains one 0.25 mL dose of MF59 adjuvant.

Needles and syringes will be disposed of in Sharps containers immediately after administration.

The Study Product Accountability Records and Dispensing Logs will capture vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, MF59 adjuvant, and admixture, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.
APPENDIX E: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF STUDY VACCINE FOR GROUP 4

1. Study Vaccine Preparation

Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration.

Visually inspect the A/H7N9 vaccine and MF59 adjuvant upon receipt and prior to use. The A/H7N9 vaccine suspension will be clear and slightly opalescent in appearance. The MF59 adjuvant oil-in-water emulsion will be milky in appearance. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s).

Used and unused vials of A/H7N9 vaccine, MF59 adjuvant, and admixture will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, MF59 adjuvant, and admixture. Final disposition of the unused A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

Group 4 First Dose (15 mcg A/H7N9 Antigen + MF59 Adjuvant)

Admixture of A/H7N9 vaccine and MF59 adjuvant by the pharmacist must be performed under a laminar flow hood using aseptic technique according to USP 797 guidelines.

1-inch, 23- or 25-gauge needles that are attached to disposable 1-mL sterile syringes should be used for study vaccine preparation. Once used, needles and syringes should be disposed of as biohazardous material in accordance with institutional guidelines.

The sterile empty vials provided for this study do NOT have a vacuum. Therefore, after injecting study product into a vial for admixing, the same volume of air from the dead space must be removed.

At the completion of admixture preparation and prior to study vaccine administration, visually inspect the A/H7N9 vaccine plus MF59 adjuvant admixture. The admixture will be milky in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

1.1 Preparation procedure for 15 mcg A/H7N9 antigen + MF59 adjuvant (Group 4 First Dose)

1.1.1 Remove one vial of 30 mcg/0.5 mL A/H7N9 antigen and one vial of MF59 adjuvant from the refrigerator.

1.1.2 Record the Vial Numbers of the 30 mcg/0.5 mL A/H7N9 antigen vial and MF59 adjuvant vial on the appropriate Study Product Accountability Record for each vial.

1.1.3 Gently invert the 30 mcg/0.5 mL A/H7N9 antigen vial 5 to 7 times. Using aseptic technique, puncture the septum top of the vial with a 1-inch, 23- or 25-gauge needle attached to a disposable 1-mL sterile syringe.
1.1.4 Withdraw 0.5 mL from the vial containing 30 mcg/0.5 mL A/H7N9 antigen. Using aseptic technique, slowly inject the entire volume into a 3-mL sterile empty vial.

1.1.5 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial containing the A/H7N9 antigen, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.6 Using aseptic technique, puncture the septum top of the MF59 adjuvant vial with a new 1-inch, 23- or 25-gauge needle attached to a new disposable 1-mL sterile syringe.

1.1.7 Withdraw 0.5 mL from the MF59 adjuvant vial. Using aseptic technique, slowly inject the entire volume into the 3-mL sterile mixing vial containing the A/H7N9 antigen.

1.1.8 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial now containing both the A/H7N9 antigen and MF59 adjuvant, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.9 Gently invert the 3-mL sterile mixing vial now containing the A/H7N9 antigen plus MF59 adjuvant admixture 5 to 7 times. **Do not shake vial.** This is the final mixed vial for Group 4 First Dose.

1.1.10 Label this final mixed vial in accordance with Section 2 of this appendix.

1.1.11 A single 0.5 mL dose from this final mixed vial contains 15 mcg of A/H7N9 antigen plus one dose of MF59 adjuvant. Only 1 dose should be drawn from this final mixed vial.

1.1.12 Refer to Sections 3 and 4 of this appendix for further instructions. Record the Final Mixed Vial Number and the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for each final mixed vial.

**Group 4 Second Dose (15 mcg A/H7N9 Antigen–Unadjuvanted)**

Admixing of vials is **NOT** required for the 15 mcg A/H7N9 antigen–unadjuvanted dose.

1.2 Preparation procedure for 15 mcg A/H7N9 antigen–unadjuvanted *(Group 4 Second Dose)*

1.2.1 Remove one vial of 15 mcg/0.5 mL A/H7N9 antigen from the refrigerator.

1.2.2 Record the Vial Number of the 15 mcg/0.5 mL A/H7N9 antigen vial on the appropriate Study Product Accountability Record.

1.2.3 A single 0.5 mL dose from this vial contains 15 mcg of A/H7N9 antigen. Only 1 dose should be drawn from this vial. Vials are labeled as per manufacturer label. No additional labeling is required unless as per the site-specific pharmacy SOP.
1.2.4 Refer to Sections 3 and 4 of this appendix for further instructions. Record the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for this vial.

2. Labeling of Final Mixed Vials

All final mixed vials must be labeled. Contents on the label must adhere to local regulations and institutional procedures. At a minimum, the following items listed must be included on the label for each final mixed vial:

- Vial number, which is assigned sequentially by the pharmacist as the final mixed vials are prepared (number uniquely, do not start over at 1 or repeat numbers)
- Protocol number
- Product name (identifying contents) and strength
- Preparation and/or expiration date and time
- Caution: For Investigational Use only

3. Study Vaccine Storage and Stability

Final mixed vials of A/H7N9 antigen plus MF59 adjuvant admixture must be stored at room temperature in an upright orientation and must be used within 8 hours.

Unadjuvanted A/H7N9 antigen vials must be stored at 2°C to 8°C (35.6°F to 46.4°F).

4. Study Vaccine Administration

Study vaccine administration will be performed by an unblinded study clinician who is licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. Each dose of study vaccine will be administered via a single intramuscular injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of Procedures for information on how to administer intramuscular injections.

All subjects in Group 4 First Dose will receive a single 0.5 mL dose of 15 mcg A/H7N9 antigen plus MF59 adjuvant admixture from the final mixed vial via intramuscular injection on the day of the first vaccination. Gently invert the final mixed vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the final mixed vial. Note: Each 0.5 mL dose of MF59-adjuvanted study vaccine contains one- 0.25 mL dose of MF59 adjuvant.

All subjects in Group 4 Second Dose will receive a single 0.5 mL dose of 15 mcg A/H7N9 antigen–unadjuvanted via intramuscular injection on the day of the second vaccination. Gently invert the vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the vial.

Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe. The single dose must be administered within 30 minutes of drawing into a syringe, and the prepared syringe must be stored at room temperature until administered.
Needles and syringes will be disposed of in Sharps containers immediately after administration.

The Study Product Accountability Records and Dispensing Logs will capture vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, MF59 adjuvant, and admixture, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.
APPENDIX F: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF STUDY VACCINE FOR GROUP 5

1. Study Vaccine Preparation

Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration.

Visually inspect the A/H7N9 vaccine and MF59 adjuvant upon receipt and prior to use. The A/H7N9 vaccine suspension will be clear and slightly opalescent in appearance. The MF59 adjuvant oil-in-water emulsion will be milky in appearance. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s).

Used and unused vials of A/H7N9 vaccine, MF59 adjuvant, and admixture will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, MF59 adjuvant, and admixture. Final disposition of the unused A/H7N9 vaccine, MF59 adjuvant, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

Group 5 First Dose (15 mcg A/H7N9 Antigen–Unadjuvanted)

Admixing of vials is NOT required for the 15 mcg A/H7N9 antigen–unadjuvanted dose.

1.1 Preparation procedure for 15 mcg A/H7N9 antigen–unadjuvanted (Group 5 First Dose)

1.1.1 Remove one vial of 15 mcg/0.5 mL A/H7N9 antigen from the refrigerator.

1.1.2 Record the Vial Number of the 15 mcg/0.5 mL A/H7N9 antigen vial on the appropriate Study Product Accountability Record.

1.1.3 A single 0.5 mL dose from this vial contains 15 mcg of A/H7N9 antigen. Only 1 dose should be drawn from this vial. Vials are labeled as per manufacturer label. No additional labeling is required unless as per the site-specific pharmacy SOP.

1.1.4 Refer to Sections 3 and 4 of this appendix for further instructions. Record the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for this vial.

Group 5 Second Dose (15 mcg A/H7N9 Antigen + MF59 Adjuvant)

Admixture of A/H7N9 vaccine and MF59 adjuvant by the pharmacist must be performed under a laminar flow hood using aseptic technique according to USP 797 guidelines.

1-inch, 23- or 25-gauge needles that are attached to disposable 1-mL sterile syringes should be used for study vaccine preparation. Once used, needles and syringes should be disposed of as biohazardous material in accordance with institutional guidelines.

The sterile empty vials provided for this study do NOT have a vacuum. Therefore, after injecting study product into a vial for admixing, the same volume of air from the dead space must be removed.
At the completion of admixture preparation and prior to study vaccine administration, visually inspect the A/H7N9 vaccine plus MF59 adjuvant admixture. The admixture will be milky in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

1.2 Preparation procedure for 15 mcg A/H7N9 antigen + MF59 adjuvant (Group 5 Second Dose)

1.2.1 Remove one vial of 30 mcg/0.5 mL A/H7N9 antigen and one vial of MF59 adjuvant from the refrigerator.

1.2.2 Record the Vial Numbers of the 30 mcg/0.5 mL A/H7N9 antigen vial and MF59 adjuvant vial on the appropriate Study Product Accountability Record for each vial.

1.2.3 Gently invert the 30 mcg/0.5 mL A/H7N9 antigen vial 5 to 7 times. Using aseptic technique, puncture the septum top of the vial with a 1-inch, 23- or 25-gauge needle attached to a disposable 1-mL sterile syringe.

1.2.4 Withdraw 0.5 mL from the vial containing 30 mcg/0.5 mL A/H7N9 antigen. Using aseptic technique, slowly inject the entire volume into a 3-mL sterile empty vial.

1.2.5 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial containing the A/H7N9 antigen, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.2.6 Using aseptic technique, puncture the septum top of the MF59 adjuvant vial with a new 1-inch, 23- or 25-gauge needle attached to a new disposable 1-mL sterile syringe.

1.2.7 Withdraw 0.5 mL from the MF59 adjuvant vial. Using aseptic technique, slowly inject the entire volume into the 3-mL sterile mixing vial containing the A/H7N9 antigen.

1.2.8 Withdraw 0.5 mL of air from the dead space of the sterile mixing vial now containing both the A/H7N9 antigen and MF59 adjuvant, then remove the needle from the sterile mixing vial. Dispose of the needle and syringe appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.2.9 Gently invert the 3-mL sterile mixing vial now containing the A/H7N9 antigen plus MF59 adjuvant admixture 5 to 7 times. Do not shake vial. This is the final mixed vial for Group 5 Second Dose.

1.2.10 Label this final mixed vial in accordance with Section 2 of this appendix.

1.2.11 A single 0.5 mL dose from this final mixed vial contains 15 mcg of A/H7N9 antigen plus one dose of MF59 adjuvant. Only 1 dose should be drawn from this final mixed vial.

1.2.12 Refer to Sections 3 and 4 of this appendix for further instructions. Record the Final Mixed Vial Number and the dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log for each final mixed vial.
2. Labeling of Final Mixed Vials

All final mixed vials must be labeled. Contents on the label must adhere to local regulations and institutional procedures. At a minimum, the following items listed must be included on the label for each final mixed vial:

- Vial number, which is assigned sequentially by the pharmacist as the final mixed vials are prepared (number uniquely, do not start over at 1 or repeat numbers)
- Protocol number
- Product name (identifying contents) and strength
- Preparation and/or expiration date and time
- Caution: For Investigational Use only

3. Study Vaccine Storage and Stability

Unadjuvanted A/H7N9 antigen vials must be stored at 2°C to 8°C (35.6°F to 46.4°F).

Final mixed vials of A/H7N9 antigen plus MF59 adjuvant admixture must be stored at room temperature in an upright orientation and must be used within 8 hours.

4. Study Vaccine Administration

Study vaccine administration will be performed by an unblinded study clinician who is licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. Each dose of study vaccine will be administered via a single intramuscular injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of Procedures for information on how to administer intramuscular injections.

All subjects in Group 5 First Dose will receive a single 0.5 mL dose of 15 mcg A/H7N9 antigen—unadjuvanted via intramuscular injection on the day of the first vaccination. Gently invert the vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the vial.

All subjects in Group 5 Second Dose will receive a single 0.5 mL dose of 15 mcg A/H7N9 antigen plus MF59 adjuvant admixture from the final mixed vial via intramuscular injection on the day of the second vaccination. Gently invert the final mixed vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the final mixed vial. Note: Each 0.5 mL dose of MF59-adjuvanted study vaccine contains one- 0.25 mL dose of MF59 adjuvant.

Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe. The single dose must be administered within 30 minutes of drawing into a syringe, and the prepared syringe must be stored at room temperature until administered.

Needles and syringes will be disposed of in Sharps containers immediately after administration.
The Study Product Accountability Records and Dispensing Logs will capture vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, MF59 adjuvant, and admixture, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.
APPENDIX G: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF
STUDY VACCINE FOR GROUP 6

1. Study Vaccine Preparation

   **Group 6 First and Second Doses (15 mcg A/H7N9 Antigen–Unadjuvanted)**

   Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same
day of study vaccine administration. Admixing of vials is **NOT** required for the 15 mcg A/H7N9
antigen–unadjuvanted dose.

   Visually inspect the A/H7N9 vaccine upon receipt and prior to use. The suspension will be clear and
slightly opalescent in appearance. If it appears to have been damaged, contaminated or discolored,
contain visible particulate matter, or if there are any concerns regarding its integrity, do **NOT** use it.

   Used and unused vials of A/H7N9 vaccine will be retained until monitored and released for disposition
as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine. Final
disposition of the unused A/H7N9 vaccine will be determined by DMID and communicated to the
participating VTEU sites by the DMID Clinical Project Manager.

   **1.1 Preparation procedure for 15 mcg A/H7N9 antigen–unadjuvanted (Group 6 First and
Second Doses)**

   1.1.1 Remove one vial of 15 mcg/0.5 mL A/H7N9 antigen from the refrigerator.

   1.1.2 Record the Vial Number of the 15 mcg/0.5 mL A/H7N9 antigen vial on the appropriate
Study Product Accountability Record.

   1.1.3 A single 0.5 mL dose from this vial contains 15 mcg of A/H7N9 antigen. Only 1 dose
should be drawn from this vial. Vials are labeled as per manufacturer label. No
additional labeling is required unless as per the site-specific pharmacy SOP.

   1.1.4 Refer to Sections 2 and 3 of this appendix for further instructions. Record the
dispensation of the single 0.5 mL dose on the appropriate Study Product Dispensing Log
for this vial.

2. Study Vaccine Storage and Stability

   Unadjuvanted A/H7N9 antigen vials must be stored at 2°C to 8°C (35.6°F to 46.4°F).

3. Study Vaccine Administration

   Study vaccine administration will be performed by an unblinded study clinician who is licensed to
administer medications/vaccines and may also participate in dose preparation, but will not be involved
in study-related assessments or have subject contact for data collection following study vaccine
administration. Each dose of study vaccine will be administered via a single intramuscular injection
given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will
be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of
Procedures for information on how to administer intramuscular injections.
All subjects in Group 6 will receive a single 0.5 mL dose of 15 mcg A/H7N9 antigen–unadjuvanted via intramuscular injection on the day of each vaccination. Gently invert the vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the vial. Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe. The single dose must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered.

Needles and syringes will be disposed of in Sharps containers immediately after administration.

The Study Product Accountability Records and Dispensing Logs will capture vial numbers, date of study vaccine preparation/administration, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of A/H7N9 vaccine, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.
APPENDIX H: PREPARATION, LABELING, STORAGE, AND ADMINISTRATION OF STUDY VACCINE FOR GROUP 7

1. Study Vaccine Preparation

**Group 7 First and Second Doses (45 mcg A/H7N9 Antigen–Unadjuvanted)**

Study vaccine preparation will be performed by the participating VTEU sites’ pharmacist on the same day of study vaccine administration. Admixture of A/H7N9 vaccines by the pharmacist must be performed under a laminar flow hood using aseptic technique according to USP 797 guidelines.

1-inch, 23- or 25-gauge needles that are attached to disposable 1-mL sterile syringes should be used for study vaccine preparation. Once used, needles and syringes should be disposed of as biohazardous material in accordance with institutional guidelines.

Visually inspect the A/H7N9 vaccine upon receipt and prior to use. The suspension will be clear and slightly opalescent in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

At the completion of admixture preparation and prior to study vaccine administration, visually inspect the unadjuvanted A/H7N9 vaccine admixture. The admixture will be clear and slightly opalescent in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it.

Used and unused vials of A/H7N9 vaccine will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine. Final disposition of the unused A/H7N9 vaccine will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

1.1 Preparation procedure for 45 mcg A/H7N9 antigen–unadjuvanted *(Group 7 First and Second Doses)*

1.1.1 Remove two vials of 30 mcg/0.5 mL A/H7N9 antigen from the refrigerator.

1.1.2 Record the Vial Numbers for both of the 30 mcg/0.5 mL A/H7N9 antigen vials on the appropriate Study Product Accountability Record.

1.1.3 Gently invert both 30 mcg/0.5 mL A/H7N9 antigen vials 5 to 7 times.

1.1.4 Using aseptic technique, puncture the septum top of the **first** vial containing 30 mcg/0.5 mL A/H7N9 antigen with a 1-inch, 23- or 25-gauge needle attached to a disposable 1-mL sterile syringe.

1.1.5 Withdraw 0.5 mL from the **first** vial containing 30 mcg/0.5 mL A/H7N9 antigen. Using aseptic technique, slowly inject the entire volume **into the second vial** containing 30 mcg/0.5 mL A/H7N9 antigen.

1.1.6 Remove the needle from the **second** A/H7N9 antigen vial now containing both the contents from the first 30 mcg/0.5 mL A/H7N9 antigen vial **PLUS** the contents of the second 30 mcg/0.5 mL A/H7N9 antigen vial. Dispose of the needle and syringe.
appropriately in accordance with institutional guidelines for disposal of sharp instruments with biohazardous material.

1.1.7 Gently invert the second A/H7N9 antigen vial now containing the unadjuvanted A/H7N9 antigen admixture 5 to 7 times. Do not shake vial. This is the final mixed vial for Group 7.

1.1.8 Label this final mixed vial in accordance with Section 2 of this appendix.

1.1.9 A single 0.75 mL dose from this final mixed vial contains 45 mcg of A/H7N9 antigen. Only 1 dose should be drawn from this final mixed vial.

1.1.10 Refer to Sections 3 and 4 of this appendix for further instructions. Record the Final Mixed Vial Number and the dispensation of the single 0.75 mL dose on the appropriate Study Product Dispensing Log for each final mixed vial.

2. Labeling of Final Mixed Vials

All final mixed vials must be labeled. Contents on the label must adhere to local regulations and institutional procedures. At a minimum, the following items listed must be included on the label for each final mixed vial:

- Vial number, which is assigned sequentially by the pharmacist as the final mixed vials are prepared (number uniquely, do not start over at 1 or repeat numbers)
- Protocol number
- Product name (identifying contents) and strength
- Preparation and/or expiration date and time
- Caution: For Investigational Use only

3. Storage and Stability of Final Mixed Vials

Final mixed vials of unadjuvanted A/H7N9 antigen admixture must be stored at 2°C to 8°C (35.6°F to 46.4°F) and must be used within 8 hours.

4. Study Vaccine Administration

Study vaccine administration will be performed by an unblinded study clinician who is licensed to administer medications/vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. Each dose of study vaccine will be administered via a single intramuscular injection given in the deltoid muscle of the subjects’ preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Refer to the protocol-specific Manual of Procedures for information on how to administer intramuscular injections.

All subjects in Group 7 will receive a single 0.75 mL dose of study vaccine from the final mixed vial via intramuscular injection on the day of each vaccination. Gently invert the final mixed vial 5 to 7 times immediately before the single dose is withdrawn. Only one dose should be withdrawn from the final mixed vial. Aseptic technique will be used for the withdrawal and administration of the single dose using a needle appropriate in length for each subject and a disposable sterile 1-mL syringe.
The single dose must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered.

Needles and syringes will be disposed of in Sharps containers immediately after administration.

The Study Product Accountability Records and Dispensing Logs will capture vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study products, including the amount of study vaccine from the final mixed vial and the amount of A/H7N9 vaccine from the 30 mcg/0.5 mL A/H7N9 antigen vial, whether administered or not, must be documented on the appropriate Study Product Accountability Record or Dispensing Log.