Phase I Randomized Study of the Safety, Dose Escalation, and Immunogenicity of Adjuvanted Influenza A/Anhui/05 Boosting in Subjects Previously Immunized with One or Two Doses of A/Vietnam/1203/04 or in Unprimed Individuals and Compared to Placebo

DMID Protocol Number: 08-0013

Principal Investigator: Robert B. Belshe, MD
DMID Scientific Lead: Sonnie Kim Grossman, MS
DMID Clinical Project Manager: Tena Knudsen, BSN
DMID Medical Monitor: Soju Chang, MD, MPH

Version 3.0
28 May 2010
STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP): Consolidated Guideline, and the applicable regulatory requirements from United States (US) Code of Federal Regulations (CFR) (Title 45 CFR Parts 46 and Title 21 CFR including Parts 50 and 56) concerning informed consent and Institutional Review Board regulations.

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any subjects. CVs for all investigators and sub-investigators participating in this trial are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).
SIGNATURE PAGE

The signature below constitutes the investigator has read and understands 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 US federal regulations and ICH guidelines.

Principal Investigator:

Signed: ___________________________ Date: ___________________________

Name: Robert B. Belshe, MD
TABLE OF CONTENTS

Statement of Compliance .......................................................................................................ii
Signature Page ......................................................................................................................iii
Table of Contents...................................................................................................................iv
List of Abbreviations .............................................................................................................vii
Protocol Summary .................................................................................................................ix
1 KEY ROLES............................................................................................................... 1
2 Background Information and Scientific Rationale ..................................................... 3
  2.1 Background Information............................................................................ 3
  2.1.1 Public Readiness and Emergency Preparedness Act ..... 6
  2.2 Rationale ................................................................................................... 7
  2.3 Potential Risks and Benefits .................................................................. 9
    2.3.1 Potential Risks .................................................................................. 9
    2.3.2 Known Potential Benefits ............................................................. 11
3 Objectives ................................................................................................................ 12
  3.1 Study Objectives ..................................................................................... 12
    3.1.1 Primary Objectives: ........................................................................ 12
  3.2 Endpoints ................................................................................................ 12
    3.2.1 Primary endpoints .......................................................................... 12
    3.2.2 Secondary endpoints ..................................................................... 12
    3.2.3 Tertiary endpoint ........................................................................... 13
4 Study Design............................................................................................................ 14
  4.1 Substudies .............................................................................................. 16
5 Study Population and Subject Enrollment and Withdrawal..................................... 17
  5.1 Subject Inclusion Criteria ........................................................................ 17
  5.2 Subject Exclusion Criteria ..................................................................... 18
  5.3 Treatment Assignment Procedures........................................................ 20
    5.3.1 Randomization Procedures ......................................................... 20
    5.3.2 Reasons for Withdrawal ............................................................... 20
    5.3.3 Termination of Study .................................................................... 21
6 Study Intervention/Investigational Product .............................................................. 22
  6.1 Study Product Description ...................................................................... 22
    6.1.1 Acquisition ..................................................................................... 22
    6.1.2 Formulation, Packaging, and Labeling: ....................................... 22
    6.1.3 Product Storage and Stability ...................................................... 22
  6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product .......................................................... 23
  6.3 Modification of Study Intervention/Investigational Product for a Participant .......................................................... 24
6.4 Accountability Procedures for the Study Intervention/Investigational Product(s) ................................................................. 24
6.5 Concomitant Medications/Treatments ........................................... 25
7 Study Schedule .................................................................................. 26
7.1 Day of Vaccination (V) .................................................................. 26
7.2 Telephone Calls: Day 2 post each vaccination (T), Window Day 1-3 .. 27
7.3 Memory Aid Review Visit: Day 8 post each vaccination (M, B), Window Day 7-9 ................................................................. 28
7.5 Final Study Visit: Telephone Call: Day 365 post last vaccination (F), Window Day 358-372 ................................................................. 29
7.6 Early Termination Visit ................................................................... 29
8 Study Procedures/Evaluations ............................................................. 31
8.1 Clinical Evaluations ........................................................................ 31
8.2 Laboratory Evaluations .................................................................. 31
8.2.1 Clinical Laboratory Evaluations .................................................... 31
8.2.2 Serum Antibody to H5 Viruses ..................................................... 32
8.2.3 Specimen Preparation, Handling, and Shipping ............................ 32
9 Assessment of Safety .......................................................................... 33
9.1 Specification of Safety Parameters ................................................... 33
9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters ................................................................. 33
9.2.1 Adverse Events, Reactogenicity, Serious Adverse Events .......... 33
9.3 Reporting Procedures ...................................................................... 38
9.3.1 Serious Adverse Event Detection and Reporting ....................... 39
9.3.2 Regulatory Reporting for Trials Under DMID-Sponsored IND ....... 40
9.3.3 Reporting of Pregnancy ............................................................... 40
9.4 Type and Duration of Follow-up of Subjects after Adverse Events ...... 40
9.5 Halting Rules .................................................................................. 40
9.6 Safety Oversight (ISM plus SMC) .................................................... 41
9.6.1 Safety Monitoring Committee (SMC) ........................................... 42
9.6.2 Independent Safety Monitor (ISM) ............................................... 42
10 Clinical Monitoring ........................................................................... 43
10.1 Site Monitoring Plan ....................................................................... 43
11 Statistical Considerations .................................................................. 44
11.1 Overview and Study Objectives ...................................................... 46
11.1.1 Primary Outcome Measures ....................................................... 47
11.2 Statistical Methods ........................................................................ 48
11.3 Demography and Baseline Characteristics ..................................... 48
11.4 Immunogenicity Analysis ............................................................... 48
11.5 Safety Analysis ............................................................................... 49
11.6 Sample size ................................................................................... 50
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7</td>
<td>Interim Analysis</td>
<td>52</td>
</tr>
<tr>
<td>12</td>
<td>Source Documents and Access to Source Data/Documents</td>
<td>53</td>
</tr>
<tr>
<td>13</td>
<td>Quality Control and Quality Assurance</td>
<td>54</td>
</tr>
<tr>
<td>14</td>
<td>Ethics/Protection of Human Subjects</td>
<td>55</td>
</tr>
<tr>
<td>14.1</td>
<td>Ethical Standard</td>
<td>55</td>
</tr>
<tr>
<td>14.2</td>
<td>Institutional Review Board</td>
<td>55</td>
</tr>
<tr>
<td>14.3</td>
<td>Informed Consent Process</td>
<td>55</td>
</tr>
<tr>
<td>14.4</td>
<td>Exclusion of Women, Minorities, and Children (Special Populations)</td>
<td>57</td>
</tr>
<tr>
<td>14.5</td>
<td>Subject Confidentiality</td>
<td>57</td>
</tr>
<tr>
<td>14.6</td>
<td>Study Discontinuation</td>
<td>57</td>
</tr>
<tr>
<td>14.7</td>
<td>Subject Compensation</td>
<td>57</td>
</tr>
<tr>
<td>14.8</td>
<td>Future Use of Stored Specimens</td>
<td>57</td>
</tr>
<tr>
<td>15</td>
<td>Data Handling and Record Keeping</td>
<td>59</td>
</tr>
<tr>
<td>15.1</td>
<td>Data Management Responsibilities</td>
<td>59</td>
</tr>
<tr>
<td>15.2</td>
<td>Data Capture Methods</td>
<td>59</td>
</tr>
<tr>
<td>15.2.1</td>
<td>Source Documents and Electronic Case Report Forms</td>
<td>59</td>
</tr>
<tr>
<td>15.3</td>
<td>Types of Data</td>
<td>60</td>
</tr>
<tr>
<td>15.4</td>
<td>Timing/Reports</td>
<td>60</td>
</tr>
<tr>
<td>15.5</td>
<td>Study Records Retention</td>
<td>60</td>
</tr>
<tr>
<td>15.6</td>
<td>Protocol Deviations</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>Publication Policy</td>
<td>62</td>
</tr>
<tr>
<td>17</td>
<td>Literature References</td>
<td>63</td>
</tr>
<tr>
<td>18</td>
<td>Supplements/Appendices</td>
<td>65</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

A/H5N1 Influenza A Virus of the H5N1 Subtype
AE Adverse Event/Adverse Experience
CFR Code of Federal Regulations
CIOMS Council for International Organizations of Medical Sciences
CONSORT Consolidated Standards of Reporting Trials
CFR Code of Federal Regulations
CRO Contract Research Organization
DCC Data Coordinating Center
DHHS Department of Health and Human Services
DMID Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
eCRF Electronic Case Report Form
FDA Food and Drug Administration
FWA Federalwide Assurance
GCP Good Clinical Practice
GMT Geometric Mean Titer
HA Hemagglutination
HAI Hemagglutination Inhibition Assay
HIPAA Health Insurance Portability and Accountability Act
IB Investigator’s Brochure
ICF Informed Consent Form
ICH International Conference on Harmonisation
ICMJE International Committee of Medical Journal Editors
IDES Internet Data Entry System
IEC Independent or Institutional Ethics Committee
IM Intramuscular
IND Investigational New Drug Application
IRB Institutional Review Board
ISM Independent Safety Monitor
JAMA Journal of the American Medical Association
μg Micrograms
MedDRA Medical Dictionary for Regulatory Activities
MN Microneutralization
MOP Manual of Procedures
N Number (typically refers to subjects)
NDA New Drug Application
NEJM New England Journal of Medicine
NIAID National Institute of Allergy and Infectious Diseases, NIH, DHHS
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH</td>
<td>National Institutes of Health, DHHS</td>
</tr>
<tr>
<td>OCRA</td>
<td>Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS</td>
</tr>
<tr>
<td>OHRP</td>
<td>Office for Human Research Protections</td>
</tr>
<tr>
<td>OHSR</td>
<td>Office for Human Subjects Research</td>
</tr>
<tr>
<td>ORA</td>
<td>Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS</td>
</tr>
<tr>
<td>PHI</td>
<td>Protected Health Information</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event/Serious Adverse Experience</td>
</tr>
<tr>
<td>SMC</td>
<td>Safety Monitoring Committee</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>VTEU</td>
<td>Vaccine and Treatment Evaluation Unit (sites)</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
PROTOCOL SUMMARY

Title: Phase I Randomized Study of the Safety, Dose Escalation, and Immunogenicity of Adjuvanted Influenza A/Anhui/05 Boosting in Subjects Previously Immunized with One or Two Doses of A/Vietnam/1203/04 or in Unprimed Individuals and Compared to Placebo

Phase: I

Population: Up to 735 healthy adults, aged 18 through 49*, who either were previously enrolled and vaccinated in DMID 07-0019 or are H5 vaccine naive healthy volunteers.

* Subjects previously enrolled in 07-0019 will be eligible for 08-0013 even if they are older than 49 years of age at the time of enrollment into 08-0013.

Number of Sites: Up to 16 sites will enroll subjects in this study

Study Duration: Approximately 18 months.

Subject Participation Duration: Subjects in Groups 8-9 from DMID 07-0019 who will roll-over to participate in this study for approximately 12 months, and newly enrolled, healthy H5 vaccine naïve volunteers (Group 10) who will participate for approximately 13 months, from Day 0 of this study.

- Group 8, who received A/Vietnam/1203/04 90 mcg vaccine as prime (Day 0 in DMID 07-0019), will receive a Booster dose of A/Anhui/05 vaccine (A) with or without MF59 adjuvant in DMID 08-0013 on Day 0 (post Day 365 following the subject’s receipt of the first vaccination in DMID 07-0019).

- Group 9, who received two doses of A/Vietnam/1203/04 90 mcg vaccine as prime (on Days 0, 28 in DMID 07-0019), will receive a Booster dose of A/Anhui/05 vaccine (A) with or without MF59 adjuvant in DMID 08-0013 on Day 0 (post Day 365 following the subject’s receipt of the first vaccination in DMID 07-0019).

- Group 10, an unprimed control and dose response group of H5 vaccine naive volunteers, will be added in DMID 08-0013 to receive two doses of A/Anhui/05
vaccine (A) with or without MF59 adjuvant or placebo on Day 0 and Day 28.

- All Groups will participate for approximately 12 months after the last vaccination.

**Description of Agent or Intervention:**

Group 8 and 9 subjects will receive one of two possible dosages administered by intramuscular injection:

- A/Anhui/05 vaccine with or without MF59 adjuvant. Approximately 20 subjects will be randomized to receive one of 2 possible doses, in each dose assignment: 3.75 mcg $\text{MF}59$, or 3.75 mcg

Group 10 subjects will receive two vaccinations, approximately 28 days apart, of one of twelve possible dosages or saline placebo, administered by intramuscular injection:

- A/Anhui/05 vaccine with or without MF59 adjuvant with approximately 50 subjects in each dosage assignment to one of nine possible dosages: 3.75 mcg $\text{MF}59$, 3.75 mcg, 7.5 mcg $\text{MF}59$, 7.5 mcg, 15.0 mcg $\text{MF}59$, 15.0 mcg, 45.0 mcg $\text{MF}59$, 45.0 mcg, 90 mcg, or saline placebo with three groups of 35 subjects each, receiving different volumes of: one injection of 0.5 mL, one injection of 0.75 mL or two injections of 0.75 mL, for comparison.

**Objectives:**

**Primary:**

1. To evaluate booster vaccination with a different variant/clade of A/Anhui/05 vaccine to assess possible prime for a broad immune response by one or two previous doses of A/Vietnam/1203/04.
2. To compare one-dose vs. two-dose priming of A/Vietnam/1203/04 followed by heterologous boosting with A/Anhui/05.
3. To assess the safety of MF59 adjuvanted A/Anhui/05 vaccine in primed subjects.
4. To compare safety and immunogenicity of one and two doses of A/Anhui/05 with or without MF59 adjuvant, or to saline placebo, in a Phase I dose response study in naïve subjects.

**Endpoints**

**Primary endpoints**

- Geometric Mean Titer (GMT), frequency of 4-fold or greater antibody titer increases, and proportion of subjects achieving a serum HAI antibody titer of 1:40 or greater against the two antigens being evaluated, A/Vietnam/1203/04 and A/Anhui/05 H5N1 virus, 1 month and 6 months after last vaccination.
- Local and systemic adverse event (AE) or serious adverse event (SAE) information (solicited in-clinic and via memory aids, concomitant medications, and periodic targeted physical assessments).

**Secondary endpoints**

- Geometric mean titer (GMT), frequency of 4-fold or greater increases and proportion of subjects achieving a titer of 1:40 or greater in neutralizing antibody
titers against the two antigens being evaluated, A/Vietnam/1203/04 and A/Anhui/05 H5N1 virus, 1 month and 6 months after last vaccination.

- GMT at Days 8 and 14 post-vaccination in primed vs. unprimed subjects to A/Vietnam/1203/04 and A/Anhui/05.

Tertiary endpoint
- Antibody titers post dose 2 or 3 to other related H5 antigens, including A/Indonesia.

**Description of Study Design:**

Up to 180 healthy adult subjects aged 18 through 49* who were previously enrolled in DMID 07-0019 and received either one or two priming dose of A/Vietnam/1203/04 H5 influenza vaccine will be enrolled in this study. * Subjects previously enrolled in 07-0019 will be eligible for 08-0013 even if they are older than 49 years of age at the time of enrollment into 08-0013.

Additionally, approximately 555 healthy adult subjects aged 18 through 49 H5 vaccine naïve will be recruited to receive 2 doses, 28 days apart, of A/Anhui/05 vaccine with or without MF59 adjuvant or placebo. Entry criteria for the newly enrolled subjects will be the same as those used in DMID 07-0019. Because extensive safety data were collected for similar H5 vaccines, subjects will not be screened with clinical laboratory tests. Groups 8-9 will have safety serology drawn on Day 0, 8 and 28 to monitor post-vaccination with both unadjuvanted and adjuvanted vaccines. Eligible subjects will have the same schedules as shown in the table below.

The booster for Groups 8-9 will be randomized to one of two possible doses, with approximately 20 subjects per Group (allocation ratio 1:1) in each dose assignment: 3.75 mcg MF59, or 3.75 mcg [note: 100 subjects were originally in this group, but about 60 are expected to re-enroll, see Section 4].

Newly enrolled, vaccine naïve subjects in Group 10 will be randomized to one of nine possible dosages with or without MF59 adjuvant, with approximately 50 subjects in each dosage assignment: 3.75 mcg MF59, 3.75 mcg, 7.5 mcg MF59, 7.5 mcg, 15.0 mcg MF59, 15.0 mcg, 45.0 mcg MF59, 45.0 mcg, 90 mcg, or saline placebo with three groups of 35 subjects each, receiving different volumes of: one injection of 0.5 mL, one injection of 0.75 mL or two injections of 0.75 mL, for comparison. Refer to the following table for randomization and dosing schema.
Randomization will not be stratified, although post hoc analyses may be performed.

Vaccine administration will be performed by an unblinded vaccine administrator, who will not be involved in subsequent assessments. Volunteers will be observed in the clinic for at least 20 minutes after vaccination, and will maintain a Memory Aid to record oral temperature and systemic and local AEs and SAEs for 8 days after vaccination. Volunteers will be contacted by telephone 1 to 3 days after vaccination (approximately Day 2 post dose) to assess for the occurrence of AEs/SAEs, and they will return to the clinic 7 to 9 days after vaccination (approximately Day 8 post dose) for AE/SAE and concomitant medication assessment, a targeted physical

Table 1. Groups/Randomization

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Day 0 Randomization (n)</th>
<th>Day 0 Vaccine and Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 8</td>
<td>Subjects primed with one dose of A/Vietnam/1203/04 (up to 90)*</td>
<td></td>
</tr>
<tr>
<td>8A</td>
<td>Booster with A/Anhui/05 3.75 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>8B</td>
<td>Booster with A/Anhui/05 3.75 mcg</td>
<td></td>
</tr>
<tr>
<td>Group 9</td>
<td>Subjects primed with two doses of A/Vietnam/1203/04 (up to 90)*</td>
<td></td>
</tr>
<tr>
<td>9A</td>
<td>Booster with A/Anhui/05 3.75 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>9B</td>
<td>Booster with A/Anhui/05 3.75 mcg</td>
<td></td>
</tr>
<tr>
<td>Group 10</td>
<td>H5 vaccine naïve Subjects (approx. 555)</td>
<td>Doses on Day 0 and Day 28</td>
</tr>
<tr>
<td>10A (50)</td>
<td>Two Doses with A/Anhui/05 3.75 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10B (50)</td>
<td>Two Doses with A/Anhui/05 3.75 mcg</td>
<td></td>
</tr>
<tr>
<td>10C (50)</td>
<td>Two Doses with A/Anhui/05 7.5 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10D (50)</td>
<td>Two Doses with A/Anhui/05 7.5 mcg</td>
<td></td>
</tr>
<tr>
<td>10E (50)</td>
<td>Two Doses with A/Anhui/05 15.0 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10F (50)</td>
<td>Two Doses with A/Anhui/05 15.0 mcg</td>
<td></td>
</tr>
<tr>
<td>10G (50)</td>
<td>Two Doses with A/Anhui/05 45.0 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10H (50)</td>
<td>Two Doses with A/Anhui/05 45.0 mcg</td>
<td></td>
</tr>
<tr>
<td>10 I (50)</td>
<td>Two Doses with A/Anhui/05 90 mcg *</td>
<td></td>
</tr>
<tr>
<td>10J (35)</td>
<td>Two Doses with Saline Placebo *</td>
<td></td>
</tr>
<tr>
<td>10K (35)</td>
<td>Two Doses with Saline Placebo **</td>
<td></td>
</tr>
<tr>
<td>10L (35)</td>
<td>Two Doses with Saline Placebo ***</td>
<td></td>
</tr>
</tbody>
</table>

*The randomization allocation for Groups 8 and 9 will be 1:1 for as many subjects up to 90 in each of Groups 8 and 9 that can be recruited. (see Section 4)

*Each dose administered as two injections of 0.75 mL 2 cm apart in the same arm

**Each dose administered as one injection of 0.5 mL

***Each dose administered as one injection of 0.75 mL
examination (if indicated), and review of the Memory Aid. Volunteers in Group 10 will return to clinic on Day 28 for review of eligibility criteria to receive Dose #2 of A/Anhui/05 or placebo. Volunteers in this group will then follow the same study schedule as following the first vaccination. All subjects will continue to be followed for 12 months after the last vaccination.

Time to Complete Enrollment

It is anticipated that this study will enroll up to 735 subjects over 10-14 weeks.
1 KEY ROLES

For questions regarding this protocol, contact Sonnie Kim Grossman at the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Department of Health and Human Services (DHHS) at 301-435-2870 or grossmansk@niaid.nih.gov. Complete contact information is as follows:

DMID:

Sonnie Kim Grossman, M.S.
Program Officer, Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
6610 Rockledge Drive, Room 3214
Bethesda, MD 20817
Tel: 301-435-2870
Fax: 301-496-8030
Email: grossmansk@niaid.nih.gov

Soju Chang, M.D., M.P.H.
Medical Monitor
Office of Clinical Research Affairs
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
6610 Rockledge Drive, Room 4508
Bethesda, MD 20817
Tel: 301-402-8176
Fax: 301-480-0728
Email: changsoju@niaid.nih.gov

Tena Knudsen, BSN
Clinical Project Manager
Respiratory Diseases Branch
DMID/NIAID/NIH/DHHS
6610 Rockledge Drive, Room 3221
Bethesda, MD 20817
Tel: 301-451-3747
Fax: 301-496-8030
Email: knudsent@mail.nih.gov
Principal Investigator/ LeadSite:

Robert B. Belshe, MD
Professor of Internal Medicine
Division of Infectious and Immunology
St. Louis University
Edward A. Doisy Research Center
1100 South Grand Blvd.
St. Louis, MO 63104
Tel: 314-977-5500
Fax: 314-771-3816
Email: belsherb@slu.edu
2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Severe disease in humans due to avian influenza viruses of the H5N1 subtype has raised considerable concern regarding the potential emergence of these viruses in pandemic form [1, 2]. Planning for control of such pandemics is of vital importance, and a cornerstone of this planning is the development of effective vaccines for H5N1. However, the relatively low frequencies of immune response seen in previous studies of inactivated H5N1 vaccines in humans [3-5] and the likely need for a two-dose schedule of vaccination pose significant obstacles to vaccine approaches for pandemic control.

In one previous study, serum antibody responses to a two-dose schedule of an H5 vaccine generated from the avian influenza A/Duck/Singapore/97 virus were modest, and required the use of the adjuvant MF59 [3]. However, when these same subjects were revaccinated 16 months later with a single dose of the same vaccine with MF59, brisk responses occurred to titers much higher than those seen after the initial dose series [6]. In addition, subjects who were reimmunized with the A/Duck/Singapore/97 vaccine developed significant levels of antibody against the antigenic variants A/Vietnam/1203/04 and A/Indonesia/05 after vaccination [7]. There were no significant adverse events on revaccination.

Two DMID studies have recently evaluated the potential for boosting doses. In DMID Protocol No. 05-0090, subjects who had initially received two doses of unadjuvanted subvirion rg A/VN/1203/04 vaccine in DMID Protocol No. 04-063 [5] received a third dose at 6 months at the same dose level as received initially. Immunogenicity results suggested that these subjects achieved the same or higher titers of antibody 28 days after Dose 3 of vaccine compared to responses at 28 days after Dose 2, and that this was true at all dose levels evaluated (8). In DMID Protocol No. 05-0043, previous participants in the study evaluating baculovirus-expressed H5 from A/Hong Kong/156/97 [4] returned to receive a single booster dose of the A/Vietnam/1203/04 vaccine. This is a slightly different strategy than that evaluated in DMID Protocol No. 05-0090 because the priming (A/HK/97, clade 3) and boosting (A/VN/1203/04, clade 1) vaccines are significantly different by reciprocal HAI tests [9]. Results of this study [10] suggested that a single 90 mcg dose of A/VN/1203/04 vaccine in heterologously primed subjects resulted in higher titers of HAI and neutralizing antibody than seen after two doses in previously unprimed subjects.

Of interest, a post-hoc subgroup analysis of the data from DMID Protocol No. 05-0043 showed that there was no real relationship between the dose of A/HK/156 H5 vaccine received in 1998 and the subsequent response to the A/VN/1203/04 vaccine in 2006, although there was
a trend towards better responses in those who had also manifested a serum neutralizing response to their previous vaccination.

### Table 2. A/VN/1203/04 responses in 2006

<table>
<thead>
<tr>
<th>Factor</th>
<th>N</th>
<th>Day 28 GMT of HAI (95% CI)</th>
<th>Response* (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose in 1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mcg x 2</td>
<td>12</td>
<td>63.5 (22.9, 175.9)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>45 mcg x 2</td>
<td>7</td>
<td>155.1 (58.6, 410.1)</td>
<td>6 (86%)</td>
</tr>
<tr>
<td>90 mcg x 2</td>
<td>8</td>
<td>42.1 (14.2, 125.0)</td>
<td>5 (63%)</td>
</tr>
<tr>
<td>90 mcg/10 mcg</td>
<td>10</td>
<td>48.7 (19.7, 120.3)</td>
<td>7 (70%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response** in 1998</th>
<th>N</th>
<th>GMT of MN (95% CI)</th>
<th>Response* (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>10</td>
<td>172.5 (69.2, 430.0)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>44.3 (25.4, 77.6)</td>
<td>17 (63%)</td>
</tr>
</tbody>
</table>

* HAI response to A/VN/1203/04 requires a 4-fold increase to a titer of 1:40 or greater, using horse erythrocytes
** MN response to A/HK/97 requires a 4-fold increase to a titer of 1:80 or greater with a positive western blot.

Safety and immunogenicity data are available from 12 clinical trials of the inactivated Monovalent Subvirion Influenza H5N1 Vaccine (rg A/Vietnam/1203/2004 Batch #04-067) licensed for pandemic use and included in the national stockpile. To date, no SAEs associated with Influenza Virus Vaccine, H5N1 (rg A/Vietnam/1203/2004 Batch #04-067) vaccine administration have been reported. One death has been reported, occurring 24 days after receipt of initial dose of Influenza H5N1 Vaccine. The Principal Investigator assessed this event as not related to study vaccine. The NIAID medical monitor assessed this event as related to study vaccine due to lack of an alternative etiology. The independent Safety Monitoring Committee (SMC) convened by NIAID considered this event as highly unlikely to be associated with the vaccine. The Medical Examiner report dated August 15, 2005, attributes the cause of death to chronic alcoholism.

H5 vaccine administration given by intramuscular injection was well-tolerated in adult and elderly subjects. In general, in comparison to placebo reactions, there was an increased frequency of local reactions (mainly pain and tenderness at the injection site) as dose of vaccine increased (3.75 mcg is the lowest dose administered and 90 mcg is the highest dose administered). Reactogenicity to the 3.75 mcg dose was generally equivalent to doses of 7.5 mcg in the same presentations with or without AIOH. There was a similar increasing frequency of local reactions with the addition of AIOH as adjuvant in vaccines administered to adults and

---
elderly. However, systemic reactogenicity in adults and elderly was not increased compared to placebo, either for increasing dose of vaccine or addition of aluminum hydroxide to the vaccine.

Taken together, the results of these studies suggest that previous priming can significantly influence the responses to subsequent booster doses, even if these booster doses represent an antigenic variant. In addition, the results suggest that even priming doses that fail to elicit detectable immune responses initially may serve to prime for effective responses to subsequent boosters. In addition, with the understanding that each study evaluates different strategies and that the sera were not tested concurrently or necessarily in the same laboratory, it appears that both the length of time between priming and revaccination, as well as the antigenic relatedness of the priming and revaccination antigens, may impact the responses. Each of these issues could have an important impact on pre-vaccination strategies prior to the emergence of a pandemic.

The most recent H5 viruses to emerge in birds have belonged to clade 2, and clade 2 viruses have been primarily responsible for human disease in the Middle East and Asia. Compared to clade 1 viruses, clade 2 viruses are more genetically diverse, with multiple subclades, and are more frequently sensitive to the antiviral drug amantadine [11]. In addition, clade 2 viruses are significant antigenic variants, and are generally not neutralized well by sera from clade 1 virus immunized animals. Therefore, the Department of Health and Human Services has contracted for the production of subvirion vaccines generated from prototypic clade 2 virus isolates, A/Indonesia/05/05 and A/Anhui/05 viruses. The A/Anhui/01/2005 vaccine was derived from a reference strain, A/AnhuiX A/PR/8/34 reassortment obtained from the Centers for Disease Control (CDC). A/Anhui/05 H5N1 vaccine is produced by Novartis Vaccines and Diagnostics Limited, Speke, Liverpool, UK (formerly Chiron). Manufacture of this vaccine followed that of the standard licensed Fluvirin. The availability of A/Anhui/05 H5N1 vaccine provides the opportunity to evaluate the following hypotheses:

- Vaccination with different variants/clades of H5 vaccine results in a broader immune response than vaccination and boosting with homologous virus.
- Two doses given at a longer interval (vaccine given on Days 0 and ~180) will have a higher titer antibody response compared with vaccines given at a shorter interval (vaccine given on Days 0 and 28).
- Vaccination with MF59 adjuvant improves the antibody response to H5 antigen relative to unadjuvanted vaccine
- Higher doses of antigen with MF59 stimulate higher and more broad immune responses to H5 antigens.
- Boosting with H5 + MF59 adjuvant after 6 mos in previously primed subjects stimulates higher and more broad antibody than boosting with unadjuvanted H5.
Two priming doses are better than one priming dose when boosted with either H5 alone or H5 with MF59 adjuvant.

- Boosting with higher doses of H5 alone or H5 + MF59 adjuvant are better than boosting with lower doses.
- The safety profile of MF59 adjuvant is satisfactory for a variety of doses and schedules of priming or boosting with H5 antigens.

2.1.1 Public Readiness and Emergency Preparedness Act

This protocol and the vaccine 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, Distributers, Program planners and other Qualified persons who prescribe, administer or dispense the 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/countermeasurescomp/default.htm). 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 vaccine 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, the individual does not have a tort claim that can be filed in a United States Federal or a State court.

### 2.2 Rationale

The preceding study, DMID 07-0019, evaluated immunogenicity with the same or with different H5 variants and the effect of the interval between doses on the subsequent response. In addition, the study evaluated whether the use of a longer duration between doses, or cross-clade priming, will result in enhanced immunogenicity. The licensed administration dose of A/Vietnam/1203/04 is 90 mcg based on previous clinical trials; therefore the trial dose selected was 90 mcg. The following table shows the individual vaccine schedules under evaluation in DMID 07-0019.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day ~180</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (25)</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (25)</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (50)</td>
<td>A/Indonesia/05 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (50)</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (50)</td>
<td>A/Vietnam/1203/04 45 mcg + A/Indonesia/05 45 mcg *</td>
<td>A/Vietnam/1203/04 45 mcg + A/Indonesia/05 45 mcg *</td>
<td>A/Indonesia/05 90 mcg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (50)</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (50)</td>
<td>A/Indonesia/05 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (100)</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td>A/Indonesia/05 90 mcg</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>9 (100)</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td>A/Vietnam/1203/04 90 mcg</td>
<td></td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

**A/Vietnam/1203/04 = inactivated rg A/Vietnam/1203/04, H5N1 (Clade 1)**

**A/Indonesia/05 = inactivated rg A/Indonesia/05/05, H5N1 (Clade 2)**

*To be administered as separate injections in the same arm at both time points

**Booster dose of A/Anhui/05 vaccine with or without MF59 adjuvant is proposed under this protocol. For Groups 8 and 9, all subjects providing consent to participate will be randomized to receive one of 2 possible doses.
Current Study Design

DMID 08-0013 will follow on the schedule above to booster Groups 8 and 9 and add a vaccine naïve Group 10 as indicated in the Table below:

<table>
<thead>
<tr>
<th>Table 4. Groups/ Randomization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td><strong>Group 8</strong></td>
</tr>
<tr>
<td>8A</td>
</tr>
<tr>
<td>8B</td>
</tr>
<tr>
<td><strong>Group 9</strong></td>
</tr>
<tr>
<td>9A</td>
</tr>
<tr>
<td>9B</td>
</tr>
<tr>
<td><strong>Group 10</strong></td>
</tr>
<tr>
<td>10A (50)</td>
</tr>
<tr>
<td>10B (50)</td>
</tr>
<tr>
<td>10C (50)</td>
</tr>
<tr>
<td>10D (50)</td>
</tr>
<tr>
<td>10E (50)</td>
</tr>
<tr>
<td>10F (50)</td>
</tr>
<tr>
<td>10G (50)</td>
</tr>
<tr>
<td>10H (50)</td>
</tr>
<tr>
<td>10I (50)</td>
</tr>
<tr>
<td>10J (35)</td>
</tr>
<tr>
<td>10K (35)</td>
</tr>
<tr>
<td>10L (35)</td>
</tr>
</tbody>
</table>

$^*$The randomization allocation for Groups 8 and 9 will be 1:1 for as many subjects up to 90 in each of Groups 8 and 9 that can be recruited (see Section 4).

$^*$Each dose administered as two injections of 0.75 mL 2cm apart in the same arm

$^{**}$Each dose administered as one injection of 0.5 mL

$^{***}$Each dose administered as one injection of 0.75 mL
This design allows for evaluations of different dose levels of A/Anhui/05, with and without adjuvant of pre-primed A/Vietnam/1203/04 as well as non-primed subjects. Adjuvant is expected to reduce the need for higher doses of the A/Anhui/05 H5N1 vaccine. A/Anhui/05 is not expected to differ in safety profile from the licensed dose of A/Vietnam/1203/04; however saline placebo controls in three sub-groups have been added.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential discomforts of this study include having blood drawn, intramuscular injection of the vaccine, and possible reactions to the vaccine.

Safety data are currently available on over 2,000 adult subjects who have received at least a single dose of the inactivated A/Vietnam/1203/2004 (H5N1) vaccine at dose levels between 3.75 mcg and 90 mcg of HA in both randomized and open-label studies. The vaccine has been well-tolerated, without significant clinical adverse events or changes in clinical laboratory tests. However, vaccination is associated with mild local pain and tenderness, which is dose dependent. At the highest doses, approximately 60% of subjects experience mild pain at the injection site. Although A/Anhui/05 H5N1 vaccine has not been tested in humans prior to this study, this vaccine was produced in a similar process as the licensed seasonal vaccine Fluvirin. It is anticipated that the safety profile of this H5N1 vaccine will be similar to Fluvirin.

Due to the fact that subjects in Groups 8 and 9 were previously vaccinated against one clade (clade 1 A/Vietnam/1203/04 H5N1) and will be boosted with another clade (clade 2 A/Anhui/2005/05 H5N1) with some groups receiving an adjuvant, all subjects in these groups will have safety labs drawn on Day 0 prior to the dose of vaccine, and approximately 8-10 days and approximately 28 days after vaccination, per FDA’s request. Safety labs will include hematology, i.e. WBC, lymphocytes and neutrophils and chemistry/metabolic parameters, i.e. sodium, potassium, creatinine, ALT (SGPT), Total Protein and Albumin. This will add approximately 15 mL of venous blood to be drawn at 3 timepoints, Days 0, 8-10, and 28.

Drawing blood causes transient discomfort and may cause fainting. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure for several minutes. Intramuscular injection also causes transient discomfort. Infection at the site of blood drawing or vaccination is extremely unlikely as alcohol swabbing and sterile equipment will be used.

Occasionally, adult recipients of influenza vaccines may develop flu-like reactions such as fever, body aches, chills, joint pain, fatigue, headache, and/or nausea. These reactions are usually greatest within the first 24 hours after vaccination and last 1 to 2 days. Some subjects may develop reactions at the site of vaccination (ecchymosis, erythema, induration, swelling,
pruritus, pain or tenderness). These reactions typically start within 96 hours of vaccination, are most commonly mild or moderate in severity and are transient in nature. Analgesics (e.g. acetaminophen, ibuprofen) and rest will generally relieve or moderate these symptoms. These reactions should go away in 1 to 4 days and not require additional treatment. From post-marketing surveillance of Fluad® (a vaccine which is registered and marketed in several countries in and outside Europe), adverse events, such as transient thrombocytopenia, transient lymphadenopathy (usually local), allergic reactions, in rare cases leading to shock, angioedema, neuralgia, paresthesia, convulsions, neurological disorders such as encephalomyelitis, neuritis, and Guillain-Barré syndrome, and generalized skin reactions including pruritus, urticaria or non-specific rash and exudative erythema multiforme have been reported.

Guillain-Barré syndrome (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 about 1 per 100,000 vaccine recipients. This has not been seen consistently with other influenza vaccines. Most persons who develop Guillain-Barré Syndrome recover completely. Intensive surveillance of GBS after inactivated influenza vaccines since that time have shown a slight increase in risk following vaccination, typically with onset in the second week after vaccination [12]. Interestingly, although vaccination rates have increased in the last 10 years the numbers of reported cases of vaccine associated GBS have declined [13].

There is a substantial clinical experience with MF59 as adjuvant. MF59 has been evaluated in more than 20,000 subjects and the MF59 adjuvanted inactivated interpandemic influenza vaccine (FLUAD®) has been licensed for commercial sale in several countries in Europe since 1997. These clinical 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. Additionally, post marketing data summaries are submitted to the regulatory authorities in compliance with regional requirements. No causal relationship to the study vaccination was established for any of the deaths. In the clinical database, only two SAE’s, based on the investigator’s judgment, were considered probably and possibly related to immunization respectively. One SAE occurred in a 70-year-old man who was hospitalized with pancreatitis and cholangitis, from which he recovered. The other SAE was abdominal pain in a subject in the age group 65 years or older. Since the subject was lost to follow-up, no further information was available. The fact that only two such events have been reported from a clinical database of nearly 20,000 persons (primarily 65 years or older) immunized with MF59-containing influenza vaccines contributes to the favorable safety profile of MF59.

There may be other unknown side effects.
2.3.2 Known Potential Benefits

There are no known benefits to the receipt of these investigational vaccines. It is possible that vaccination with these H5 vaccines will result in some antibody responses against H5 viruses. The duration of any such response is currently unknown.
3 OBJECTIVES

3.1 Study Objectives

This study is designed to gather critical information on the safety, tolerability, and immunogenicity of booster vaccination with influenza A/Anhui/05 with and without the adjuvant MF59 in subjects previously primed with the clade 1 vaccine or unprimed individuals.

3.1.1 Primary Objectives:

1. To evaluate booster vaccination with a different variant/clade of A/Anhui/05 vaccine to assess possible prime for a broad immune response by one or two previous doses of A/Vietnam/1203/04.

2. To compare one-dose vs. two-dose priming of A/Vietnam/1203/04 followed by heterologous boosting with A/Anhui/05.

3. To assess the safety of MF59 adjuvanted A/Anhui/05 vaccine in primed subjects.

4. To compare safety and immunogenicity of one and two doses of A/Anhui/05 with or without MF59 adjuvant, or to saline placebo, in a Phase I dose response study in naïve subjects.

3.2 Endpoints

3.2.1 Primary endpoints

- Geometric Mean Titer (GMT), frequency of 4-fold or greater antibody titer increases, and proportion of subjects achieving a serum HAI antibody titer of 1:40 or greater against the two antigens being evaluated, A/Vietnam/1203/04 and A/Anhui/05 H5N1 virus, 1 month and 6 months after last vaccination.

- Local and systemic adverse event (AE) or serious adverse event (SAE) information (solicited in-clinic and via memory aids, concomitant medications, and periodic targeted physical assessments).

3.2.2 Secondary endpoints

- Geometric mean titer (GMT), frequency of 4-fold or greater increases and proportion of subjects achieving a titer of 1:40 or greater in neutralizing antibody
titers against the two antigens being evaluated, A/Vietnam/1203/04 and A/Anhui/05 H5N1 virus, 1 month and 6 months after last vaccination.

- GMT at Days 8 and 14 post-vaccination in primed vs. unprimed subjects to A/Vietnam/1203/04 and A/Anhui/05.

3.2.3 Tertiary endpoint

- Antibody titers post dose 2 or 3 to other related H5 antigens, including A/Indonesia.
4 STUDY DESIGN

The study will be conducted as a randomized, multi-center trial for Groups 8 and 9 who were previously enrolled in DMID 07-0019 and Group 10 who will be newly recruited. Up to 180 H5 pre-primed, and approximately 555 H5 naïve, healthy adult subjects will be randomized to receive varying doses of inactivated subvirion A/Anhui/05 H5N1 vaccine, or placebo.

Groups 8 and 9 who were previously enrolled in DMID 07-0019 will receive one dose of either 3.75 mcg with or without adjuvant MF59 on Day 0 of this study (DMID 08-0013). The study has given priority to doses of 3.75 mcg with or without MF59 with a minimum goal of 20 subjects per group (a total of 80 subjects, 40 each in groups 8 and 9). This booster dose will be given post Day 365 following the subject's receipt of the first vaccination in DMID 07-0019. As an initial step, site staff conducted a recruitment assessment of subjects previously enrolled in DMID 07-0019. The recruitment assessment was reviewed by an ad hoc committee consisting of Principle Investigator (or designee), a staff representative that conducted the assessment, a DMID representative, and an EMMES study statistician. Groups 8 and 9 were independently assessed. The committee determined that the likely sample size for either was insufficient to obtain meaningful information for doses of 3.75 mcg with and without MF59 and a 90 mcg, thus recommended that randomization be restricted to the two 3.75 mcg groups at a ratio of 1:1.

The H5 naïve subjects enrolled in Group 10 will receive either 3.75 mcg, 7.5 mcg, 15.0 mcg, or 45.0 mcg with adjuvant MF59, or 3.75 mcg, 7.5 mcg, 15.0 mcg, 45.0 mcg or 90.0 mcg without adjuvant MF59, or placebo on Day 0 and Day 28. Within Group 10 there are three sub-groups who will receive placebo in 3 different volumes (one injection of 0.5 mL or 0.75 mL, or two injections of 0.75 mL). Volunteers in Group 10 will return to clinic on Day 28 for review of eligibility criteria to receive Dose #2 of A/Anhui/05 or placebo. Volunteers in this group will then follow the same study schedule as following the first vaccination. All subjects are followed for 12 months after the last vaccination.

Vaccinations will be administered by IM injection. Vaccine preparation and administration will be performed by an unblinded vaccine administrator, who will not be involved in subsequent study procedures. All clinical assessments will be performed by blinded study personnel. [Note: Blinded personnel will be aware of subjects' previous 07-0019 group assignment and those who are newly recruited for Group 10, but will remain blinded as to their product randomization, except for the subjects randomized to receive the 90.0 mcg unadjuvanted dose as 2 injections or saline placebo 0.75 mL as two injections, of whom only Group 10 subjects will be blinded to receipt of vaccine or placebo.]

Subjects will be followed for safety, reactogenicity, and immune responses after vaccination. Subjects will be observed in the clinic for at least 20 minutes after vaccination. Research personnel will assess the subject for any signs or symptoms at the end of this
observation period. Subjects will then be sent home with instructions on how to maintain a Memory Aid for 8 days following vaccination. On the Memory Aid, they will be expected to record their daily oral temperature, and any systemic and local AEs and SAEs that occur within the week following vaccination. Subjects will receive a follow-up telephone call at 1 to 3 days after vaccination (approximately Day 2 post dose) to elicit any AE/SAE information. They will return to the clinic 7 to 9 days after vaccination for review of the Memory Aid, assessment of AEs/SAEs, concomitant medications and a targeted physical examination (if indicated).

During the study, subjects will return to the clinic for blood sample collection and safety follow-up in accordance with the schedule for their assigned group. At Day 180 post the last vaccination, subjects will return to clinic for assessment of SAEs, and have a final research blood sample collection. At Day 365 post the last vaccination study staff will interview subjects by telephone for assessment of any SAEs since the previous visit. Note: Day 365 post vaccination contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 8</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 8 Post Vac. 2</th>
<th>Day 14 Post Vac. 2</th>
<th>Day 21 Post Vac. 2</th>
<th>Day 28 Post Vac. 2</th>
<th>Day 180 Post Last Vac.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>X</td>
<td>V</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>X</td>
<td>V</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10</td>
<td>X</td>
<td>V</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X***</td>
<td>X</td>
</tr>
</tbody>
</table>

*All subjects will be contacted 2 days post vaccination to review Memory Aid information for local and systemic reactogenicity data and AE/SAEs collected.

V = Vaccination  X = Draw blood**

**Serum antibody= 20 mL Days 0, 8, 14, 21, 28, (8, 14, 21, 28 post vac 2, Group 10 only), 180

*** An additional 20mL blood sample to be drawn at visit 9, Group 10 only, if subject consents.

Blood samples will be collected according to the schedule. Sera will be tested in a central laboratory for safety hematology and chemistry (Groups 8 and 9 only). Sera will be tested in a central research laboratory for the levels of HAI and neutralizing antibodies for all Groups.

The Safety Monitoring Committee (SMC) will review adverse events and reactogenicity data and will make recommendations to the Division of Microbiology and Infectious Diseases (DMID) about the safety of the vaccine for individuals, groups, or the entire protocol. They will follow pre-defined halting rules and may advise DMID on other safety issues not specifically
discussed in the halting rules. The SMC will follow a protocol-specific charter, per DMID procedures.

4.1 Substudies

Additional studies of the immune response, such as those to determine the presence and number of memory B and T cells and other responses, may be performed as substudies in a subset of subjects at specific VTEU sites. These substudies will be written and conducted as separate protocols.
5 STUDY POPULATION AND SUBJECT ENROLLMENT AND WITHDRAWAL

Up to 735 subjects aged 18 through 49* will be enrolled in this study. However, subjects previously enrolled in 07-0019 will be eligible for 08-0013 even if they are older than 49 years of age at the time of enrollment into 08-0013. *

Newly recruited subjects for this study will be healthy adults who have no history of exposure to H5 influenza or vaccination. (Note: History of H5 exposure or vaccination for Groups 8 and 9 will be noted at the time of their enrollment in DMID 07-0019).

Groups 8 and 9 will enroll in this study from DMID 07-0019, if all other eligibility continues to be met. Subjects for Group 10 will be recruited by accessing existing study subject databases, word of mouth, and community education efforts and should reflect the community at large at each of the study sites. Information regarding the study may be mailed to potential subjects who have previously participated in vaccine trials conducted at the enrollment sites. The local Institutional Review Boards (IRBs) will approve all materials prior to their use.

5.1 Subject Inclusion Criteria

Subjects must meet all of the following inclusion criteria to participate in this study [for roll-over Groups 8 and 9 allow for DMID 07-0019 as noted above]:

1. Men and women, 18 through 49* years old, who either were previously enrolled and received all scheduled vaccinations in Groups 8 and 9 under DMID 07-0019, or are new subjects who deny exposure to H5 virus or participation in an H5 vaccine study. *

2. In good health, as determined by vital signs (heart rate <100 bpm; blood pressure: systolic ≤140 mm Hg and ≥ 90 mm Hg; diastolic ≤90 mm Hg; oral temperature <100.0°F), medical history to ensure stable medical condition\(^1\) and a targeted physical examination, as indicated, based on medical history.

\(^1\)Stable medical condition – no recent change in prescription medication, dose, or frequency of medication in the last 3 months and health outcomes of the specific disease are considered to be within acceptable limits in the last 6 months. Any change that is due to change of health care provider, insurance company, etc, or is done for financial reasons, as long as in the same class of medication, will not be considered a violation of the inclusion criterion. Any change to prescription medication due to improvement of a disease outcome will not be considered a violation of the inclusion criterion.
3. Women of childbearing potential (not surgically sterile or postmenopausal for ≥1 year) must not be pregnant as indicated by a negative pregnancy test (urine or serum) within 24 hours prior to vaccine administration.

4. Women of childbearing potential who are at risk of becoming pregnant must have a history of practicing adequate contraception (i.e., barrier methods, abstinence, monogamous relationship with vasectomized partner, intrauterine devices, Depo-Provera, Norplant, oral contraceptives, contraceptive patches or other licensed, effective methods) in the 30 days prior to enrollment, and must agree to practice adequate contraception until 30 days following receipt of the last dose of vaccine.

5. Able to understand and comply with planned study procedures.

6. Able to provide informed consent prior to initiation of any study procedures and be available for all study visits.

5.2 Subject Exclusion Criteria

Subjects who meet any of the following exclusion criteria at baseline cannot participate in this study:

1. Has occupational exposure to poultry, to include but is not limited to chicken, turkey, or duck farmer, factory worker in poultry processing plant, veterinary staff that handles poultry; has recreational exposure to poultry, e.g. raising poultry in 4-H club, duck hunter that slaughters/handles the “kill” or history of previous H5N1 vaccination or exposure (other than vaccination in Protocol 07-0019).

2. Has a known allergy to egg proteins (egg or egg products), or other components of the vaccine (including thimerosal, polymyxin, neomycin, beta propiolactone, or nonylphenol ethoxylate).

3. Is female of child-bearing potential who is breastfeeding or intends to become pregnant during the study period up to 30 days following receipt of the last dose of vaccine.

4. Has immunosuppression as a result of an underlying illness or treatment with immunosuppressive or cytotoxic drugs, or use of anticancer chemotherapy or radiation therapy within the preceding 36 months.

5. Has an active* neoplastic disease (excluding non-melanoma skin cancer or prostate cancer that is stable in the absence of therapy) or a history of any hematologic malignancy.

    *Defined as: no neoplastic disease or treatment for neoplastic disease within the past 5 years.

6. Has long-term use (greater than 2 weeks) of oral or parenteral steroids (glucocorticoids), or high-dose inhaled steroids (>800 mcg/day of beclomethasone
diacetate or equivalent) within the preceding 6 months (nasal and topical steroids are allowed).

7. Has a history of receiving immunoglobulin or other blood products within the 3 months prior to enrollment in this study.

8. Has received any other licensed vaccines within 2 weeks (for inactivated vaccines) or 4 weeks (for live vaccines) prior to enrollment in this study, or plans to receive any other licensed vaccines within 2 weeks (for inactivated vaccines) or 4 weeks (for live vaccines) following each study vaccine.

9. Has an acute or chronic medical condition that would render vaccination unsafe or would interfere with the evaluation of responses. This includes, but is not limited to: solicited reactogenicity symptoms, known chronic liver disease, significant renal disease, unstable or progressive neurological disorders, diabetes mellitus, and transplant recipients.

10. Has a history of severe reactions following vaccination with contemporary influenza virus vaccines.

11. Has an acute illness or has an oral temperature greater than 99.9°F (37.7°C) within 3 days prior to enrollment.

12. Has received an experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 1 month prior to enrollment in this study, or expects to receive an experimental agent during the study period.

13. Has any condition that would, in the opinion of the site principal investigator place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

14. Has a diagnosis of schizophrenia, bipolar disease or other severe (disabling) chronic psychiatric diagnosis.

15. Has been hospitalized for psychiatric illness, history of suicide attempt or confinement for danger to self or others.

16. Is receiving psychiatric drugs*. Subjects who are receiving a single antidepressant drug and are stable for at least 3 months prior to enrollment without decompensating are allowed enrollment into the study.

*aripiprazole, clozapine, ziprasidone, haloperidol, molindone, loxapine, thioridazine, thiopropazine, pimozide, fluphenazine, risperidone, mesoridazine, quetiapine, trifluoperazine, trifluopromazine, chlorprothixene, chlorpromazine, perphenazine, olanzapine, carbamazepine, divalproex sodium, lithium carbonate or lithium citrate.

17. Has known human immunodeficiency virus, hepatitis B, or hepatitis C infection.

18. Has a history of alcohol or drug abuse in the 5 years prior to enrollment.

20. Has any condition that the investigator believes may interfere with successful completion of the study.

21. Plans to enroll in another clinical trial (that has a study intervention in the form of drug, biologic or device that could interfere with safety assessment of H5N1 vaccine) at any time during the study period.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Enrollment will be performed online using the enrollment module of The EMMES Corporation’s Internet Data Entry System (IDES). The randomization code will be included in the enrollment module for the trial. The randomization code will link the vial allocation number to the treatment assignment. Each subject enrolled in the trial will be assigned to a vial allocation number and vaccination dose after demographic and eligibility data have been entered into the system. Each clinical site will be provided with a code list for emergency unblinding purposes. The list will be kept in a secure place.

Instructions for use of the enrollment module are included in the IDES User’s Guide. Manual backup 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. Screening records will be kept documenting the reason why an individual was screened but failed trial entry criteria.

5.3.2 Reasons for Withdrawal

A study subject will be discontinued from participation in the study if the following occur.

- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject. However, the subject may continue to be followed for safety purposes.

- Development of any exclusion criteria may be cause for discontinuation. Subjects who develop any exclusion criteria between the first and second immunization should not receive the second dose but may continue in the study.

- Subjects may be removed from the study for failure to make follow-up visits.
- Subjects may be removed from the study if new information becomes available that makes further participation unsafe.
- The subject withdraws consent. Subjects may withdraw their consent for study participation at any time during the study without penalty.
- The subject could be removed from the study at any time due to study termination by DMID.

5.3.3 Termination of Study

The study may be terminated at any time by DMID.
6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 Acquisition

Vaccine will be provided by Novartis Vaccines (previously Chiron) who will ship Investigational Product to the DMID Clinical Agents Repository (CAR), Fisher Bioservices. Fisher Bioservices will ship vaccine to each participating site after all regulatory and essential documents are collected from the site and the site is activated.

6.1.2 Formulation, Packaging, and Labeling

A monovalent inactivated surface antigen influenza A/H5N1 (modified Hemagglutinin and Neuraminidase of A/Anhui/01/2005) vaccine will be used for this study. This vaccine was manufactured by Novartis Vaccines & Diagnostics Ltd, Liverpool, UK. All vaccine is supplied in 5mL multi-dose vials with thimerosal [0.01%(w/v)] as a preservative. At each dose level, both unadjuvanted and MF59C.1 adjuvanted formulations have been manufactured, making a total of eight formulations as follows:

- 7.5, 15, 30, or 60 micrograms (mcg) of H5 Hemagglutinin per milliliter (mL) with MF59C.1, and

- 7.5, 15, 30, or 60 micrograms (mcg) of H5 Hemagglutinin per milliliter (mL) without MF59C.1 adjuvant,

Placebo will be sterile normal saline for injection (0.9% Sodium Chloride Injection, USP Preservative-Free) to be supplied by Fisher Bioservices. See the MOP for further details.

6.1.3 Product Storage and Stability

The vaccine should be stored in the original package to protect from light. The influenza vaccine and saline placebo will be stored in secure, limited-access, temperature monitored refrigerator environments at 2°C to 8°C (35.6°F to 46.4°F) until needed. The temperature of the storage unit must be monitored for the duration of the trial, and documentation of proper dedicated storage will be maintained. Between uses, return the multi-dose vial to the
recommended storage conditions. **Do not freeze.** In the event of accidental deep-freezing or disruption of the cold chain, vaccines must not be administered; the investigator or the responsible person should contact the DMID Clinical Agent Repository at Fisher Bioservices for further instructions.

The vial should be gently shaken and visually inspected for particulate matter and/or discoloration prior to administration. If either condition exists, the vial should not be used. However, it is normal for the vaccine to appear clear to slightly opalescent.

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

The influenza A/H5N1 influenza virus vaccine manufactured by Novartis Vaccines will be packaged in 5 mL multi-dose vials of 7.5, 15, 30, or 60 mcg of HA per 1.0 mL with and without MF59C.1 adjuvant. Vaccine doses of 3.75, 7.5, 15, and 45 mcg will be delivered by one deep intramuscular injection. A vaccine dose of 90 mcg will be delivered by two deep intramuscular injections in the same arm. These two injections will be administered one above the other, approximately 2 centimeters apart. To do this, a vaccine dose of 90 mcg will be delivered as two separate injections of 45 mcg /0.75 mL each. Subjects randomized to receive saline placebo in Groups 10J, 10K, or 10L, will receive deep IM injections of 3 different volumes (one injection of 0.5mL, one injection of 0.75mL, or two injections of 0.75mL. Clinical staff as well as the subjects randomized to receive the 90 mcg dose, or saline placebo, as 2 injections will be partially unblinded as to the volume administered. Because study products may differ in appearance (the vaccine to appear clear to slightly opalescent) and will be administered in different volumes, the subject will be asked to look away when receiving the injection. The unblinded administrator should attempt to conceal the syringe, as much as possible, to limit the ability of the blinded staff and the subject to see which syringe is being used. **See the Dosage/Volume Table to follow.**
<table>
<thead>
<tr>
<th>Dose</th>
<th>Vial Formulation</th>
<th>Volume Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75 mcg (vaccine only)</td>
<td>7.5 mcg/mL A/Anhui/05</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>3.75 mcg (with adjuvant)</td>
<td>7.5 mcg/mL A/Anhui/05 MF59</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>7.5 mcg (vaccine only)</td>
<td>15 mcg/mL A/Anhui/05</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>7.5 mcg (with adjuvant)</td>
<td>15 mcg/mL A/Anhui/05 MF59</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>15 mcg (vaccine only)</td>
<td>30 mcg/mL A/Anhui/05</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>15 mcg (with adjuvant)</td>
<td>30 mcg/mL A/Anhui/05 MF59</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>45 mcg (vaccine only)</td>
<td>60 mcg/mL A/Anhui/05</td>
<td>0.75 mL</td>
</tr>
<tr>
<td>45 mcg (with adjuvant)</td>
<td>60 mcg/mL A/Anhui/05 MF59</td>
<td>0.75 mL</td>
</tr>
<tr>
<td>90 mcg (vaccine only)</td>
<td>60 mcg/mL A/Anhui/05</td>
<td>2 separate injections of 0.75 mL</td>
</tr>
</tbody>
</table>

### 6.3 Modification of Study Intervention/Investigational Product for a Participant

There will be no dose or schedule modification for any subject. Subjects in Group 10 who are not eligible to receive the second dose of vaccine will be encouraged to remain in the study to be followed for safety and blood draws for immunogenicity per protocol.

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

Study vaccine will be supplied by the DMID Clinical Agent Repository at Fisher BioServices and the Repository will supply the participating study sites with vaccine prior to the start of the study. The randomization scheme will be generated by the EMMES Corporation and provided to unblinded study personnel at the participating study sites.

After receipt of the vaccines and placebo, the principal investigator is responsible for distribution of the study products, and has ultimate responsibility for product accountability. The principal investigator may delegate shipment of study product directly to the site research pharmacy storage facility. The site research pharmacist or delegated responsible qualified individual will confirm condition and log-in receipt of study products. Logs of receipt,
temperature, maintenance, and disposal must be maintained in the study files. All vaccine and placebo vials, both used and unused, will be retained until monitored and released for disposition. Study monitors may verify study product and accountability documentation during regular monitor visits, at the end of the vaccination period and at study close-out.

6.5 Concomitant Medications/Treatments

Administration of any medication or therapies considered necessary for the subject’s welfare will be reported on the subject’s data acquisition screen and documented in the subject’s source documentation. Concomitant medications will include all medications taken within 30 days prior to each vaccination through 28 days post vaccination or early termination, whichever occurs first.

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, oral, parenteral and high-dose inhaled steroids, immunosuppressive or cytotoxic drugs, aripiprazole, clozapine, ziprasidone, haloperidol, molindone, loxapine, thioridazine, thiothixene, pimozide, fluphenazine, risperidone, mesoridazine, quetiapine, trifluoperazine, trifluopromazine, chlorprothixene, chlorpromazine, perphenazine, olanzapine, carbamazepine, divalproex sodium, lithium carbonate or lithium citrate.
7 STUDY SCHEDULE

The procedures to be performed at the study visits are described in this section as visit types (V = Day of vaccination; T = telephone call; M = Memory Aid review; B = blood draw; F = final Visit; UV = unscheduled visit). These visits are detailed as they apply for the subjects regardless of group, with exceptions noted as applicable. See the group-specific tables in Appendix A and B for the timing of the visit types (denoted with the letter for visit type as shown in this section).

Eligible subjects will be provided with a verbal description of the study (purpose and study procedures), and will be asked to read and sign the Consent Form (first visit only). The consent form will be signed prior to the performance of any screening procedures. The consent may be obtained on the day prior to the day of vaccination to allow time to confirm negative pregnancy test results available within 24 hours prior to vaccination. However, other screening procedures should occur on the day of vaccination prior to receiving the vaccine.

7.1 Day of Vaccination (V)

- Review of Inclusion/Exclusion criteria.
- Review medical history
- A targeted physical examination will be performed if indicated by the subject’s medical history.
- All concomitant medications will be recorded.
- A pregnancy test (urine or serum) will be performed for all females of childbearing potential. Results must be negative and available within 24 hours prior to vaccination.
- Vital signs will be obtained, including temperature, pulse, and blood pressure prior to vaccination.
- Groups 8-9 only -Safety Serological Studies (A 15 mL blood sample prior to vaccination):
  - Hematology--white blood count (WBC), lymphocyte count, neutrophil count.
  - Chemistry--alanine aminotransferase (ALT), creatinine, sodium, potassium, total protein and albumin
- All subjects, a 20 mL blood sample for HAI and neutralizing antibody titer assays will be collected prior to vaccination.
- Subjects will be enrolled in IDES and randomized to a treatment group (Day 0 vaccination only).
- Subjects will receive the dose of vaccine or placebo via deep IM injection in the deltoid muscle. [Note: Individuals who receive 45 and 90mcg will receive 2 injections of each vaccine administered approximately 2 cm apart in the same arm in order to stimulate the same regional lymph nodes. Both injection sites will be measured for induration and erythema. If erythema and/or induration overlap such that measurements cannot be attributed to the respective injection sites, the entire area will be measured and half the size attributed to one injection site and half the area attributed to the other injection site. Specific details for measuring induration and erythema are included in the Manual of Procedures (MOP).]
- Subjects will be observed in the clinic for at least 20 minutes following vaccination. The vaccination site will be examined and any adverse events will be assessed prior to discharge from the clinic.
- Subjects will be provided with a Memory Aid, thermometer, and ruler for measuring daily oral temperature, injection site reactions, and recording systemic AEs, SAEs and concomitant medications. Subjects will be instructed on how to use the Memory Aid and how to rate any adverse events (on a scale from 0 [not present] to 3 [severe]) prior to discharge from the clinic.
- Subjects will be instructed to notify the study center if they develop any severe reactions following vaccination.

7.2 Telephone Calls: Day 2 post each vaccination (T), Window Day 1-3

- Using the study script, study staff will interview subjects by telephone to solicit any AE/SAE information. Subjects will be reminded to record all information on the memory aid and to return to clinic for the next study visit. Note: Day 2 post vaccination contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.
7.3 Memory Aid Review Visit: Day 8 post each vaccination (M, B), Window Day 7-9

- Study personnel will review the Memory Aid with the subject and record symptoms in the appropriate CRF.
- Assessment of any vaccine reactions and/or AEs/SAEs since vaccination.
- A targeted physical examination will be performed if indicated by the subject’s medical history.
- All concomitant medications will be recorded.
- Groups 8-9 only -Safety Serological Studies (A total 15 mL blood sample):
  - Hematology--white blood count (WBC), lymphocyte count, neutrophil count.
  - Chemistry--alanine aminotransferase (ALT), creatinine, sodium, potassium, total protein and albumin.
- A 20 mL blood sample for HAI and neutralizing antibody titer assays will be collected.


- Subjects will return to the clinic for assessment of any vaccine reactions and/or AEs/SAEs since vaccination.
- A targeted physical examination will be performed if indicated by the subject’s medical history or at clinician’s discretion.
- Concomitant medications will be reviewed, if visit is prior to Day 32 post last vaccination.
- Day 28 visit , Groups 8-9 only -Safety Serological Studies (A 15 mL blood sample):
  - Hematology-- white blood count (WBC), lymphocyte count, neutrophil count.
  - Chemistry--alanine aminotransferase (ALT), creatinine, sodium, potassium, total protein and albumin
- All subjects, each visit, will have a 20 mL blood sample for HAI and neutralizing antibody titer assays collected.
In addition, subjects from Group 10 only will be asked within the consent to allow an additional blood sample of 20 mL to be drawn at approximately Day 28 post second vaccination to provide reference sera for future research.

7.5 Final Study Visit: Telephone Call: Day 365 post last vaccination (F), Window Day 358-372

- Study staff will interview subjects by telephone* for assessment of any SAEs since last vaccination.
- Study staff will interview subjects by telephone* to solicit any SAE information.
  * Note: Day 365 post vaccination contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.

Unscheduled Visit (UV)

- Subjects may be asked to return to the clinic for assessment of reported AEs in the judgment of investigators as needed.
- A targeted physical examination will be performed if indicated by the subject’s medical history or at clinician’s discretion.

7.6 Early Termination Visit

Subjects who withdraw from the study will be encouraged to continue follow-up for AEs/SAEs and to donate scheduled blood samples, if possible.
Tables outlining the above procedures for each study group are described below.

### Table 7. Groups 8 and 9 Visit Schedule

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>Study Day</th>
<th>Day relative to Vaccination</th>
<th>Visit Window</th>
<th>Visit Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Dose 1)</td>
<td>0</td>
<td>Day 0</td>
<td>N/A</td>
<td>V</td>
</tr>
<tr>
<td>Telephone Call*</td>
<td>2</td>
<td>Day 2</td>
<td>Day 1-3</td>
<td>T</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Day 8 post Dose 1</td>
<td>Day 7-9 post Dose 1</td>
<td>M, B</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>Day 14 post Dose 1</td>
<td>Day 13-15 post Dose 1</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>Day 21 post Dose 1</td>
<td>Day 20-22 post Dose 1</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>Day 28 post Dose 1</td>
<td>Day 26-32 post Dose 1</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>Day 180 post Dose 1</td>
<td>Day 173-187 post Dose 1</td>
<td>B</td>
</tr>
<tr>
<td>7 Telephone Call*</td>
<td>365</td>
<td>Day 365 post Dose 1</td>
<td>Day 358-372 post Dose 1</td>
<td>F, T</td>
</tr>
</tbody>
</table>

**Key:**

- **V** Day of vaccination.
- **T** Telephone call. *Day 2 and Day 365 post vaccination telephone contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.
- **M** Memory Aid Review Visit.
- **B** Blood Draw Visit.
- **F** Final Visit.

### Table 8. Group 10 Visit Schedule

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>Study Day</th>
<th>Day relative to Vaccination</th>
<th>Visit Window</th>
<th>Visit Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Dose 1)</td>
<td>0</td>
<td>Day 0</td>
<td>N/A</td>
<td>V</td>
</tr>
<tr>
<td>Telephone Call*</td>
<td>2</td>
<td>Day 2</td>
<td>Day 1-3</td>
<td>T</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Day 8 post Dose 1</td>
<td>Day 7-9 post Dose 1</td>
<td>M, B</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>Day 14 post Dose 1</td>
<td>Day 13-15 post Dose 1</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>Day 21 post Dose 1</td>
<td>Day 20-22 post Dose 1</td>
<td>B</td>
</tr>
<tr>
<td>5 (Dose 2)</td>
<td>28</td>
<td>Day 28 post Dose 1</td>
<td>Day 26-32 post Dose 1</td>
<td>V</td>
</tr>
<tr>
<td>Telephone Call*</td>
<td>30</td>
<td>Day 2 post Dose 2</td>
<td>Day 1-3 post Dose 2</td>
<td>T</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>Day 8 post Dose 2</td>
<td>Day 7-9 post Dose 2</td>
<td>M, B</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>Day 14 post Dose 2</td>
<td>Day 13-15 post Dose 2</td>
<td>B</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>Day 21 post Dose 2</td>
<td>Day 20-22 post Dose 2</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>56</td>
<td>Day 28 post Dose 2</td>
<td>Day 26-32 post Dose 2</td>
<td>B</td>
</tr>
<tr>
<td>10</td>
<td>208</td>
<td>Day 180 post Dose 2</td>
<td>Day 173-187 post Dose 2</td>
<td>B</td>
</tr>
<tr>
<td>11 Telephone Call*</td>
<td>365</td>
<td>Day 365 post Dose 2</td>
<td>Day 358-372 post Dose 2</td>
<td>F, T</td>
</tr>
</tbody>
</table>

**Key:**

- **V** Day of vaccination.
- **T** Telephone call. *Day 2 and Day 365 post vaccination telephone contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.
- **M** Memory Aid Review Visit.
- **B** Blood Draw Visit.
- **F** Final Visit.
8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Medical History: Medical history will be obtained by interview of the subjects. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, GI tract, liver, pancreas, kidney, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. Medical history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. History of method of adequate contraception practice for 30 days prior to enrollment and until 30 days following receipt of the last dose of vaccine will be documented for women of childbearing potential.

Medications History: All current medications (prescription and over-the-counter drugs) taken within 30 days prior to each vaccination through 28 days post vaccination will be included. Assessment of eligibility will include a review of permitted and prohibited medications.

Targeted Physical Examination: A physical exam may be conducted if indicated based on medical history. Oral temperature, blood pressure and pulse will be determined before each vaccination.

Reactogenicity Assessments: Will include brief history for assessment of AEs. Solicited AEs will include pain, tenderness, redness, swelling, feverishness, myalgias, malaise, headache, pruritus, and nausea. The severity of solicited AEs will be graded according to the severity scales in section 9.2.1.2.

Memory Aids: Will be reviewed for AEs/SAEs and concomitant medications.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

The safety of the subvirion A/Vietnam/1203/04 H5 vaccine manufactured by Sanofi Vaccines which has now been licensed for use in healthy adults at high risk for exposure to H5N1, has been evaluated in several other studies conducted by NIAID. The safety profile of the closely related, but not licensed, subvirion A/Anhui/05 vaccine is very similar to Novartis’ licensed flu vaccine, Fluvirin, and other hemagglutinin egg based vaccines for other influenzas including seasonal influenza vaccine. Beyond Groups 8 and 9 which will have safety serology and chemistry oversight on Day 0, 8 and 28, no other screening or follow-up clinical laboratory
evaluations are planned for this study, with the exception of pregnancy testing prior to vaccination in females of childbearing potential. Safety Serology will consist of hematology (white blood count [WBC], lymphocyte count and neutrophil count), and chemistry (alanine aminotransferase [ALT], creatinine, sodium, potassium, total protein and albumin) performed by a central lab. Pregnancy tests (urine or serum) will be performed within 24 hours prior to vaccination on women who are of childbearing potential performed at local sites. Post-safety laboratory results, any abnormal laboratory values that are Grade 1 will be followed to resolution or stabilization at the discretion of the principal investigator (PI). Any abnormal laboratory values that are Grade 2 or greater will be followed until they are less than Grade 2 or followed further at the discretion of the PI.

8.2.2 Serum Antibody to H5 Viruses

Serum Hemagglutination Inhibition (HAI): Serum HAI assay will be performed using horse erythrocytes and standard techniques as previously described [14] at the Southern Research Institute (SRI) in Birmingham, AL.

Serum Neutralizing Antibody by Microneutralization (MN): Serum neutralizing antibody will be assessed by microtiter neutralization [15] at SRI.

Following the above HAI and MN assay analyses, reference sera collected from Group 10 subjects will be separated according to those subjects who showed a low titer or high titer response to A/Anhui/05. These extra samples drawn at approximately 28 days post second vaccination will be de-identified and pooled according to low or high titer. These two pools of collected reference sera will be stored in a repository for future research.

8.2.3 Specimen Preparation, Handling, and Shipping

Instructions for specimen preparation, handling, storage and shipment are found in the manual of procedures (MOP).
9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency, incidence and severity of AEs and SAEs solicited in-clinic and via memory aids, concomitant medications and periodic physical evaluations for each dose group.

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

9.2.1 Adverse Events, Reactogenicity, Serious Adverse Events

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product. Vaccine reactions will be assessed for at least 20 minutes after vaccination. Subjects will be asked to record both solicited vaccine reactions and any unsolicited AEs/SAEs on a Memory Aid for 8 days following vaccination. Study personnel will contact subjects by telephone at 1 to 3 days after vaccination (approximately Day 2 post dose) to review any AEs and SAEs.

Adverse events will be collected through 28 days following each vaccination for all subjects. Serious adverse events will be collected throughout the study until final visits are completed.

Solicited systemic AEs will include the following: Feverishness, malaise, body aches (exclusive of the injection site), nausea, headache and pruritus.

Solicited injection site AEs will include the following: Pain, tenderness, redness, and swelling.

9.2.1.1 Definition of Adverse Event

Adverse Event: International Conference on Harmonization (ICH) guideline 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 medicinal (investigational) product. The occurrence of an AE may come to the attention of study
personnel during study visits and interviews or by a vaccine recipient presenting for medical care.

All AEs must be graded for severity and relationship to study product (see below). Severity can be assessed by a licensed clinician (i.e., physician, nurse, nurse practitioner, physicians assistant). Relationship to study product can only be assessed by a clinician licensed to make medical diagnoses (i.e. physician, nurse practitioner, physicians assistant) listed on the Form FDA 1572.

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 source document and eCRF.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity:

- **Mild**: events require minimal or no treatment and do not interfere with the patient’s daily activities.

- **Moderate**: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Severe**: events interrupt a patient’s usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

- **Life threatening**: any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Relationship to study products/vaccines: The investigator’s assessment of the relationship of an AE to study drug/vaccine 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. All AEs must have their possible relationship to study vaccine assessed using the following terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Associated**: The event is temporally related to the administration of the study product and no other etiology explains the event.
• **Not Associated** – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

### 9.2.1.2 Reactogenicity

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

<table>
<thead>
<tr>
<th>Table 9. Toxicity Grading Scale - Local Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Reaction</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Pain</td>
</tr>
<tr>
<td>Tenderness</td>
</tr>
<tr>
<td>Erythema/Redness</td>
</tr>
<tr>
<td>Erythema/Redness (mm)*</td>
</tr>
<tr>
<td>Induration/Swelling</td>
</tr>
<tr>
<td>Induration/Swelling (mm)*</td>
</tr>
</tbody>
</table>

* Size of reaction will not be used as a criterion for stopping the study (halting rules). In addition, grading of the measured values will be performed during analysis and separate from the functional grading assessment.
<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</td>
<td>No interference with daily activity</td>
<td>Some interference with daily activity</td>
<td>Significant interference, prevents daily activity</td>
</tr>
<tr>
<td>Fatigue/Malaise</td>
<td>No interference with daily activity</td>
<td>Some interference with daily activity</td>
<td>Significant interference, prevents daily activity</td>
</tr>
<tr>
<td>Myalgia/Body Ache</td>
<td>No interference with daily activity</td>
<td>Some interference with daily activity</td>
<td>Significant interference, prevents daily activity</td>
</tr>
<tr>
<td>Headache</td>
<td>No interference with daily activity</td>
<td>Some interference with daily activity</td>
<td>Significant interference, prevents daily activity</td>
</tr>
<tr>
<td>Nausea</td>
<td>No interference with daily activity</td>
<td>Some interference with daily activity</td>
<td>Significant interference, prevents daily activity</td>
</tr>
<tr>
<td>Pruritus</td>
<td>No interference with daily activity</td>
<td>Some interference with daily activity</td>
<td>Significant interference, prevents daily activity</td>
</tr>
</tbody>
</table>

An oral temperature of 37.8°C (100.0°F) is considered fever in adults. Fever severity will be graded as follows:

<table>
<thead>
<tr>
<th>Systemic (Objective)</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (°C)</td>
<td>≥37.8 - &lt;38</td>
<td>≥38 - &lt;39</td>
<td>≥39</td>
</tr>
<tr>
<td></td>
<td>≥100 - &lt; 100.4° F</td>
<td>≥ 100.4 - &lt; 102° F</td>
<td>≥ 102° F</td>
</tr>
<tr>
<td>Tachycardia, beats per minute †</td>
<td>101 - 115</td>
<td>116 - 130</td>
<td>≥131</td>
</tr>
<tr>
<td>Bradycardia, beats per minute ‡</td>
<td>54 – 50</td>
<td>49 - 40</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Hypertension (systolic), mm Hg</td>
<td>141 – 155</td>
<td>156 - 165</td>
<td>≥166</td>
</tr>
<tr>
<td>Hypertension (diastolic), mm Hg</td>
<td>91 – 95</td>
<td>96 - 100</td>
<td>≥101</td>
</tr>
<tr>
<td>Hypotension (systolic), mm Hg</td>
<td>89 – 85</td>
<td>84 - 80</td>
<td>≤79</td>
</tr>
</tbody>
</table>

*Oral temperature, no recent hot or cold beverages or smoking. [Note: A fever can be considered not product-related if an alternative etiology can be documented and it is confirmed to be not product-related by the Independent Safety Monitor.]
† subject at rest.
‡ not considered an AE if baseline heart rate is 50 - 54 beats per minute.
### Table 12. Severity Grading for Laboratory Abnormalities (Hematology)

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
<th>Life Threatening (Grade 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (Increase) cell/mm³</td>
<td>10,801 – 15,000</td>
<td>15,001 – 20,000</td>
<td>20,001 – 25,000</td>
<td>&gt;25,000</td>
</tr>
<tr>
<td>WBC (Decrease) cell/mm³</td>
<td>2,500 – 3,799</td>
<td>1,500 – 2,499</td>
<td>1,000 – 1,499</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td>Lymphocytes (Increase) cell/mm³</td>
<td>4,101 – 5,250</td>
<td>5,251 – 7,000</td>
<td>7,001 – 8,750</td>
<td>&gt;8,750</td>
</tr>
<tr>
<td>Lymphocytes (Decrease) cell/mm³</td>
<td>750 – 849</td>
<td>500 – 749</td>
<td>250 – 499</td>
<td>&lt;250</td>
</tr>
<tr>
<td>Neutrophils (Increase) cell/mm³</td>
<td>8,001 – 9,000</td>
<td>9,001 – 12,000</td>
<td>12,001 – 15,000</td>
<td>&gt;15,000</td>
</tr>
<tr>
<td>Neutrophils (Decrease) cell/mm³</td>
<td>1,500 – 1,799</td>
<td>1,000 – 1,499</td>
<td>500 – 999</td>
<td>&lt;500</td>
</tr>
</tbody>
</table>

### Table 13. Severity Grading for Laboratory Abnormalities*(Chemistries/Metabolic)

<table>
<thead>
<tr>
<th>Chemistries/Metabolic</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
<th>Life Threatening (Grade 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Hyponatremia) mEq/L</td>
<td>132 – 134</td>
<td>130 – 131</td>
<td>125 – 129</td>
<td>&lt;125</td>
</tr>
<tr>
<td>Sodium (Hypernatremia) mEq/L</td>
<td>147 – 148</td>
<td>149 – 150</td>
<td>151 – 152</td>
<td>&gt;152</td>
</tr>
<tr>
<td>Potassium (Hyperkalemia) mEq/L</td>
<td>5.4 – 5.5</td>
<td>5.6 – 5.7</td>
<td>5.8 – 5.9</td>
<td>&gt;5.9</td>
</tr>
<tr>
<td>Potassium (Hypokalemia) mEq/L</td>
<td>3.3 – 3.4</td>
<td>3.1 – 3.2</td>
<td>2.9 – 3.0</td>
<td>&lt;2.9</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.5 – 1.7</td>
<td>1.8 – 2.0</td>
<td>2.1 – 2.5</td>
<td>&gt;2.5 or requires dialysis</td>
</tr>
<tr>
<td>ALT, SGPT (increase by factor)</td>
<td>1.1 – 2.5 X ULN</td>
<td>&gt;2.5 – 5.0 X ULN</td>
<td>&gt;5.0 – 10 X ULN</td>
<td>&gt;10 X ULN</td>
</tr>
<tr>
<td>Albumin (Hypo) g/dL</td>
<td>2.8 – 3.1</td>
<td>2.5 – 2.7</td>
<td>&lt;2.5</td>
<td>---</td>
</tr>
<tr>
<td>Total Protein (Hypo) g/dL</td>
<td>18-64y: 5.5 – 5.9</td>
<td>18-64y: 5.0 – 5.4</td>
<td>18-64y: &lt;5.0</td>
<td>---</td>
</tr>
</tbody>
</table>

*ULN – Upper Limit of Normal
9.2.1.3 Serious Adverse Event

A SAE is defined as an AE meeting one of the following conditions:

- Results in death during the period of protocol defined surveillance.
- Is life-threatening (defined as a subject at immediate risk of death at the time of the event).
- Requires inpatient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance.
- Results in congenital anomaly or birth defect.
- Results in a persistent or significant disability/incapacity.
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. 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.

9.3 Reporting Procedures

Adverse events including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, date of onset, Investigator assessment of severity, date of resolution of the event, seriousness, and outcome. The intensity of nonserious AEs can be assessed by a licensed clinician (i.e., physician, nurse, nurse practitioner, physicians assistant) listed on the FDA Form 1572. Investigator assessment of relationship to study product (causality) will be determined for all unsolicited adverse events. Causality of nonserious AEs can be assessed only by a clinician licensed to make medical diagnoses (i.e., physician, nurse practitioner, physician’s assistant) listed on the FDA Form 1572. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or until considered stable.
Any medical condition that is present at screening will be considered as baseline and will not be reported as an AE. If the severity of any pre-existing medical condition increases during the study period, then it will be recorded as an AE.

### 9.3.1 Serious Adverse Event Detection and Reporting

All SAEs will be:

- Assessed for intensity and causality by a licensed clinician listed on the FDA Form 1572 as the principal investigator or subinvestigator.
- Recorded on the appropriate SAE report form.
- Followed through resolution by a study physician.
- Reviewed by an Independent Safety Monitor, the SMC, DMID, and the IRB.

Any AE considered serious by the principal investigator or subinvestigator or that meets the aforementioned criteria must be submitted on an SAE form to PPD, NIAID’s pharmacovigilance contractor, at the following address:

**Medical Affairs/Pharmacovigilance**
PPD
929 North Front Street
Wilmington, NC 28401-3331
SAE Fax line: 888-488-9697

Questions about SAE reporting can be referred to SAE Hotline at: 800-201-8725.

In addition to the SAE form, selected SAE data fields must also be entered into IDES. (Please see the Manual of Procedures for details regarding this procedure). Timelines for submission of an SAE form are as follows:

- All deaths and life-threatening events regardless of relationship will be recorded on the SAE form and sent by fax within 24 hours of site awareness of the death or life-threatening event.
- All other SAEs, regardless of relationship, will be reported via fax by the site within 72 hours of becoming aware of the event.
Other supporting documentation of the event may be requested by the pharmacovigilance contractor and should be provided as soon as possible.

All SAEs will be followed until satisfactory resolution or until the principal investigator or subinvestigator deems the event to be chronic or the subject to be stable.

9.3.2 Regulatory Reporting for Trials Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report events that are both serious and unexpected that are related to study product(s) to the Food and Drug Administration (FDA) within the required timelines as specified in 21 Code of Federal Regulations (CFR) Part 312 Section 312.32: fatal and life-threatening events within 7 calendar days (by telephone or fax). All written reports will be sent within 15 calendar days. 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 to IDES. No further vaccinations will be administered to pregnant subjects, but all study-mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Pregnancies will be followed to outcome pending the subject’s permission.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Adverse events will be followed until resolved or considered stable.

9.5 Halting Rules

The SMC will be provided safety data in order to advise DMID on matters of safety, including halting the trial, or halting an arm or arms of the trial. The safety data will consist of immediate reactogenicity events (in the 20 minutes after vaccination), reactogenicity events recorded on the memory aid (each day for the week after vaccination), AEs, and SAEs. The data will be entered by the sites, collated by EMMES, and provided to the SMC for review. The presentation of the data will include whether any events contributing towards a halting rule have been met. The frequency of SMC review will be determined by charter.

The study will be halted for further evaluation if during the 24 hours following product administration the following occurs:
• Any laryngospasm, bronchospasm, or anaphylaxis associated with product administration.

The study will be halted for further evaluation if during the 8 days after vaccination any of the following occur across all dose groups:

• 15% or more (minimum of 2 if less than 20 subjects have been vaccinated) of the subjects enrolled to date experience a severe (Grade 3) vaccine-related local reaction. Size of erythema and induration (measured in millimeters) will be recorded but will not be graded or used as a criterion for halting the study.

• 15% or more (minimum of 2 if less than 20 subjects have been vaccinated) of the subjects enrolled to date experience a severe (Grade 3) vaccine-related quantitative systemic reaction.

• 15% or more (minimum of 2 if less than 20 subjects have been vaccinated) of the subjects enrolled to date experience a severe (Grade 3) vaccine-related subjective systemic reaction corroborated by study personnel.

• 2 or more events of urticaria occur associated with product administration.

• Any ulceration, abscess, or necrosis associated with product administration.

• Any subject experiences a vaccine-related SAE.

If any of the halting rules are met, the study will not proceed with the remaining enrollment or vaccinations without a review by and recommendation from the SMC to proceed.

9.6 Safety Oversight (ISM plus SMC)

Safety oversight will be under the direction of an Independent Safety Monitor (ISM) and a Safety Monitoring Committee (SMC). The SMC and ISM will be approved by DMID in accordance with DMID guidelines. Serious and severe adverse events will be reported to DMID, the ISM and the SMC. The DMID Medical Monitor is empowered to stop study enrollment and vaccinations if adverse events that meet the halting criteria are reported. The study may not resume until available safety data are reviewed by the SMC and the SMC recommends to DMID that the study proceed. The ISM, investigators, or DMID may convene the SMC for any safety concerns. The SMC will meet according to the schedule set forth by the protocol and stated in the charter.
9.6.1 Safety Monitoring Committee (SMC)

A Safety Monitoring Committee (SMC), comprised of the physician independent safety monitors at each site to advise DMID, will be established by DMID. The primary responsibilities of the SMC are to:

- Periodically review and evaluate the accumulated study safety data in a blinded fashion for participant safety, study conduct and progress, and
- Make recommendations to DMID concerning the continuation, modification, or termination of the trial.

The operating rules of the SMC will be established in conjunction with DMID guidelines and stated in the charter.

9.6.2 Independent Safety Monitor (ISM)

An independent safety monitor (ISM) at each site will review serious and severe adverse events in a timely fashion and ensure that appropriate management is initiated and completed at the site. The ISM will have direct contact with the principal investigator and follow all events on an ongoing basis.
10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site Monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs. DMID, the sponsoring agency, or its designee will conduct site monitoring visits as detailed in the monitoring plan or in the Manual of Procedures.

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, case report forms, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.
11 STATISTICAL CONSIDERATIONS

This is a Phase I randomized study of the safety and immunogenicity of the A/Anhui/05 clade 2 vaccine and will evaluate priming and boosting strategies for H5N1 influenza using unadjuvanted, inactivated, subvirion clade 1 vaccine in H5 naïve healthy adults as the priming dose and inactivated subvirion clade 2 vaccine with or without adjuvant as the boosting dose. Up to 180 healthy adult subjects aged 18 through 49 who participated in the previous 07-0019 H5 vaccine study will be randomized to receive a single dose of A/Anhui/05 clade 2 vaccine, and approximately 555 healthy adult subjects aged 18 through 49 who have no history of prior H5 influenza exposure or vaccination will be enrolled in this study to receive two doses of vaccine. Eligible subjects will be randomized to receive one of twelve possible dose levels of A/Anhui/05 vaccine with or without the adjuvant MF59 or placebo, as shown in the table below. Randomization will not be stratified, although post hoc analyses may be performed.

The sample sizes for Groups 8 and 9 were based on a recruitment assessment of available subjects willing to return for this protocol. As discussed in Section 4, an initial assessment was made of the potential sample size for each of these groups. The ad hoc committee consisting of the PI, site staff, DMID representative and the study statistician determined that the likely sample size for either was insufficient to obtain meaningful information for doses of 3.75 mcg with and without MF59 and a 90 mcg, thus recommended that randomization be restricted to the two 3.75 mcg groups at a ratio of 1:1.
<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Day 0 Randomization (n)</th>
<th>Day 0 Vaccine and Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 8 (up to 90)#</td>
<td>Subjects primed with one dose of A/Vietnam/1203/04</td>
<td></td>
</tr>
<tr>
<td>8A</td>
<td></td>
<td>Booster with A/Anhui/05 3.75 mcg MF59</td>
</tr>
<tr>
<td>8B</td>
<td></td>
<td>Booster with A/Anhui/05 3.75 mcg</td>
</tr>
<tr>
<td>Group 9 (up to 90)#</td>
<td>Subjects primed with two doses of A/Vietnam/1203/04</td>
<td></td>
</tr>
<tr>
<td>9A</td>
<td></td>
<td>Booster with A/Anhui/05 3.75 mcg MF59</td>
</tr>
<tr>
<td>9B</td>
<td></td>
<td>Booster with A/Anhui/05 3.75 mcg</td>
</tr>
<tr>
<td>Group 10 (approx. 555)</td>
<td>H5 vaccine naïve Subjects</td>
<td>Doses on Day 0 and Day 28</td>
</tr>
<tr>
<td>10A (50)</td>
<td>Two Doses with A/Anhui/05 3.75 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10B (50)</td>
<td>Two Doses with A/Anhui/05 3.75 mcg</td>
<td></td>
</tr>
<tr>
<td>10C (50)</td>
<td>Two Doses with A/Anhui/05 7.5 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10D (50)</td>
<td>Two Doses with A/Anhui/05 7.5 mcg</td>
<td></td>
</tr>
<tr>
<td>10E (50)</td>
<td>Two Doses with A/Anhui/05/15.0 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10F (50)</td>
<td>Two Doses with A/Anhui/05 15.0 mcg</td>
<td></td>
</tr>
<tr>
<td>10G (50)</td>
<td>Two Doses with A/Anhui/05 45.0 mcg MF59</td>
<td></td>
</tr>
<tr>
<td>10H (50)</td>
<td>Two Doses with A/Anhui/05 45.0 mcg</td>
<td></td>
</tr>
<tr>
<td>10 I (50)</td>
<td>Two Doses with A/Anhui/05 90 mcg *</td>
<td></td>
</tr>
<tr>
<td>10J (35)</td>
<td>Two Doses with Saline Placebo *</td>
<td></td>
</tr>
<tr>
<td>10K (35)</td>
<td>Two Doses with Saline Placebo **</td>
<td></td>
</tr>
<tr>
<td>10L (35)</td>
<td>Two Doses with Saline Placebo ***</td>
<td></td>
</tr>
</tbody>
</table>

*The randomization allocation for Groups 8 and 9 will be 1:1 for as many subjects up to 90 in each of Groups 8 and 9 that can be recruited (see Section 4),
*Each dose administered as two injections of 0.75 mL 2 cm apart in the same arm
**Each dose administered as one injection of 0.5 mL
***Each dose administered as one injection of 0.75 mL
Vaccine administration will be performed by an unblinded vaccine administrator, who will not be involved in subsequent assessments. Subjects will be observed in the clinic for at least 20 minutes after vaccination, and will maintain a Memory Aid to record oral temperature and systemic and local AEs and SAEs for 8 days after vaccination. Subjects will be contacted by telephone approximately 1 to 3 days after vaccination (approximately Day 2 post dose) to assess for the occurrence of AEs/SAEs, and they will return to the clinic at approximately 7 to 9 days after each vaccination for AE/SAE and concomitant medication assessment, a targeted physical examination (if indicated), and review of the Memory Aid. Volunteers in Group 10 will return to clinic on Day 28 for review of eligibility criteria to receive Dose #2 of A/Anhui/05 or placebo. Volunteers in this group will then follow the same study schedule as following the first vaccination. All subjects are followed for 12 months after the last vaccination.

11.1 Overview and Study Objectives

This study is designed to gather critical information on the safety and immunogenicity of the A/Anhui/05 clade 2 vaccine, and the schedule of prime-boost strategies using subvirion inactivated influenza vaccines belonging to different clades. The safety of the subvirion A/Vietnam/1203/04 H5 vaccine in healthy adults has been evaluated in other studies conducted by NIAID and is now licensed for use in healthy adults, and the safety profile of the closely related, but not licensed, subvirion A/Anhui/05 H5N1 vaccine is very similar to Novartis' licensed flu vaccine, Fluvirin, and other hemagglutinin egg based vaccines for other influenzas including seasonal influenza vaccine. Therefore, no screening or follow-up clinical laboratory evaluations are planned for this study, with the exception of pregnancy testing prior to vaccination in females of childbearing potential. Pregnancy tests (urine or serum) will be performed within 24 hours prior to vaccination on women who are of childbearing potential.
11.1.1 Primary Outcome Measures

The Primary Objectives:
1. To evaluate booster vaccination with a different variant/clade of A/Anhui/05 vaccine to assess possible prime for a broad immune response by one or two previous doses of A/Vietnam/1203/04.
2. To compare one-dose vs. two-dose priming of A/Vietnam/1203/04 followed by heterologous boosting with A/Anhui/05.
3. To assess the safety of MF59 adjuvanted A/Anhui/05 vaccine in prime subjects.
4. To compare safety and immunogenicity of one and two doses of A/Anhui/05 with or without MF59 adjuvant, or to saline placebo, in a Phase I dose response study in naïve subjects.

The Primary Endpoints:
- Geometric Mean Titer (GMT), frequency of 4-fold or greater antibody titer increases, and proportion of subjects achieving a serum HAI antibody titer of 1:40 or greater against the two antigens being evaluated, A/Vietnam/1203/04 and A/Anhui/05 H5N1 virus, 1 month and 6 months after last vaccination.
- Local and systemic adverse event (AE) or serious adverse event (SAE) information (solicited in-clinic and via memory aids, concomitant medications, and periodic targeted physical assessments).

11.1.2 Secondary Outcome Measures
- Geometric mean titer (GMT), frequency of 4-fold or greater increases and proportion of subjects achieving a titer of 1:40 or greater in neutralizing antibody titers against the two antigens being evaluated, A/Vietnam/1203/04 and A/Anhui/05 H5N1 virus, 1 month and 6 months after last vaccination.
- GMT at Days 8 and 14 post-vaccination in primed vs. unprimed subjects to A/Vietnam/1203/04 and A/Anhui/05.
11.1.3 Tertiary Outcome Measures

- Antibody titers post dose 2 or 3 to other related H5 antigens, including A/Indonesia.

11.2 Statistical Methods

This is a Phase I randomized clinical trial and is not designed to test a formal null hypothesis. Statistical analyses will rely primarily on descriptive methods. Some exploratory modeling will be conducted, for example, testing the impact of dose, adjuvant and number of priming doses on immune response. However, the presentation of results will rely on standard descriptive statistics and their 95% confidence intervals. Parameters to be analyzed along with some additional details on how they will be treated are contained in Sections 11.3, 11.4 and 11.5.

11.3 Demography and Baseline Characteristics

Demography and baseline characteristics, including age, gender, ethnicity, medical history and physical exam, will be presented descriptively. No inferential statistics will be presented.

11.4 Immunogenicity Analysis

Immunogenicity responses will be measured by serum HAI and neutralizing antibody titers against the A/Vietnam/1203/04 and A/Anhui/05 H5N1 viruses. The geometric mean of duplicate results for each specified time point will be used for all immunogenicity calculations. Exact confidence intervals will be reported for all proportional endpoints. Geometric mean titers of antibody and their confidence intervals will be computed by transforming results to a logarithmic scale, assuming asymptotic normality conditions were satisfied on this scale and converting back to the original scale.

For both serum HAI and neutralizing antibody titers, the proportion of subjects with a post-vaccination titer of 1:40 or greater, the proportion of subjects with a four-fold or greater increase from pre-vaccination, and the geometric mean titer (GMT) will be calculated, with 95% confidence intervals, for each dose group and follow-up blood draw. Between group comparisons will be made based on these three endpoints for serum HAI, evaluated at 28 days post last vaccination for all subjects.

For the proportion of subjects with a post-vaccination serum HAI of 1:40 or greater and the proportion of subjects with a four-fold or greater increase in HAI from pre-vaccination, the group comparisons will be made by calculating the odds ratio between groups with 95% confidence.
intervals. For the GMT, the geometric mean ratios (GM Ratios) will be calculated with 95% confidence intervals.

11.5 Safety Analysis

Rates of local and systemic solicited and unsolicited adverse events will be tabulated by level of severity. All rates will be determined with 95% confidence intervals.

AEs will be coded by the Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class. The rate of AEs in aggregate, and by MedDRA® categories will be computed. No formal hypothesis testing will be carried out.

AEs will be presented for each treatment group. Descriptive statistics and graphical summaries will be presented.

In the tabulations, counting will be performed by subject and event separately. For counts by subject, subjects reporting the same event more than once will have that event counted only once within each body system and once within each preferred term.

A data listing of all information relating to adverse events will be provided. This data listing will include all events a subject experiences along with the severity level and relationship to the vaccination. The following will also be provided:

- number and percentage of subjects with at least one adverse event;
- number and percentage of subjects with serious adverse events;
- number and percentage of subjects with vaccine-related adverse events; and
- total number of adverse events.

A data listing of the serious adverse events will be provided. The following breakdown of adverse events will be provided on a patient and an event basis:

- by body system, preferred term and severity;
- by body system, preferred term and relationship to study drug; and
- by body system, preferred term and time of onset.
### 11.6 Sample size

Up to 180 healthy adult subjects aged 18 through 49* who participated in the previous 07-0019 H5 vaccine study will be enrolled to receive a single dose of vaccine, and approximately 555 healthy adult subjects aged 18 through 49 who have no history of prior H5 influenza exposure or vaccination will be enrolled in this study to receive two doses of vaccine (See Study Design Scheme). * Subjects previously enrolled in 07-0019 will be eligible for 08-0013 even if they are older than 49 years of age at the time of enrollment into 08-0013.

The sample size for each dose group in this Phase I study is for the purpose of gathering information on the safety, dose and schedule of prime-boost strategies using subvirion inactivated influenza vaccines belonging to different H5N1 clades. The sample size is not based on testing a formal hypothesis. No formal hypothesis tests will be carried out. As such, no corrections are made to control for multiple comparisons.

In an attempt to further understand the implications of the sample size for varying cells within this study, the Hemagglutination inhibition (HAI) Geometric Mean Titers (GMTs) and their variability in DMID 04-063 (the study that led to licensure of A/Vietnam/1203/04) was reviewed. One possible comparison for two groups is the ratio of the post-vaccination GMTs. Based on the 04-063 results, the standard deviation for the Log2 (HAI titer) is approximately from 1.3 to 2.2. The following table illustrates the required ratio of, true but unknown, post-vaccination GMTs to have 80% or 90% power to declare a statistically significant difference.

<table>
<thead>
<tr>
<th>Power</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>3.94</td>
</tr>
<tr>
<td>90%</td>
<td>4.68</td>
</tr>
</tbody>
</table>

For example, assuming a standard deviation of 1.3 on the Log2 scale, comparing 20 volunteers in each group assumes a ratio of at least 3.94 for Power=80% (line 3 in the table below) and 4.68 for Power=90% (last line in table). A more conservative view (standard deviation of 2.2) would increase these values to 6.29 and 7.54, respectively.

<table>
<thead>
<tr>
<th>Power</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>6.29</td>
</tr>
<tr>
<td>90%</td>
<td>7.54</td>
</tr>
</tbody>
</table>
### Table 13-A. Sample Size Calculations - GMT Ratios

<table>
<thead>
<tr>
<th>N for Group 1</th>
<th>Power</th>
<th>N for Comparator Group</th>
<th>Range for Minimum Post-Vaccination GMT Ratio Required to Declare Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Std Dev =1.3*</td>
</tr>
<tr>
<td>20</td>
<td>80%</td>
<td>20</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>3.94</td>
</tr>
<tr>
<td>20</td>
<td>90%</td>
<td>20</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>4.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>4.38</td>
</tr>
</tbody>
</table>

*Log2 scale

The same approach is taken for a comparison of the three control groups (10 J, K and L) combined (N=\(3\times35=105\)) as seen in the following table.

### Table 13-B. Sample Size Calculations for Control Groups Combined - GMT Ratios

<table>
<thead>
<tr>
<th>N for Three Control Groups Combined</th>
<th>Power</th>
<th>N for Comparator Group</th>
<th>Range for Minimum Post-Vaccination GMT Ratio Required to Declare Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Std Dev =1.3*</td>
</tr>
<tr>
<td>105</td>
<td>80%</td>
<td>20</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>20</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>3.31</td>
</tr>
</tbody>
</table>

*Log2 scale
The following table summarizes the probability of detecting a Serious Adverse Event, occurring at a range of hypothesized rates, for sample sizes of 20 (size of dose/adjuvant arms for Groups 8 and 9), 50 (size of dose/adjuvant arms for Group 10) or 320 (total number of subjects receiving vaccine adjuvanted with MF59).

<table>
<thead>
<tr>
<th>Assumed “True” Rate</th>
<th>N=20</th>
<th>N=50</th>
<th>N=320</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 or more Event</td>
<td>2 or more Events</td>
<td>1 or more Event</td>
</tr>
<tr>
<td>0.33%</td>
<td>6.46%</td>
<td>0.20%</td>
<td>15.38%</td>
</tr>
<tr>
<td>1.00%</td>
<td>18.21%</td>
<td>1.69%</td>
<td>39.50%</td>
</tr>
<tr>
<td>3.00%</td>
<td>45.62%</td>
<td>11.98%</td>
<td>78.19%</td>
</tr>
<tr>
<td>5.00%</td>
<td>64.15%</td>
<td>26.42%</td>
<td>92.31%</td>
</tr>
<tr>
<td>7.50%</td>
<td>78.97%</td>
<td>44.87%</td>
<td>97.97%</td>
</tr>
<tr>
<td>10.0%</td>
<td>87.84%</td>
<td>60.83%</td>
<td>99.48%</td>
</tr>
</tbody>
</table>

11.7 Interim Analysis

There are no planned interim analyses. As noted in section 9.6, if safety concerns arise, the safety data may be reviewed by the Safety Monitoring Committee. This review of the data would be for safety purposes only and no hypotheses would be tested.
12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The sites will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits 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, subjects’ memory aids or evaluation, 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. Forms for use as source documents will be derived from the electronic case report forms (eCRFs) and provided by the Data Coordinating Center (DCC).
13 QUALITY CONTROL AND QUALITY ASSURANCE

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

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The Data Coordinating Center (DCC) 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 investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

The institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use unless proceeding with a change is in the best interest of the subjects’ safety. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate. Refer to: http://ohrp.osophs.dhhs.gov.

Prior to enrollment of subjects into this trial, the DMID protocol and the informed consent form will be reviewed and approved by the appropriate IRB and submitted to the FDA. Any amendments to the protocol or consent materials will also be reviewed and approved by DMID and the appropriate IRB.

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. Notification of the IRB’s composition, or the IRB’s MPA or FWA number, will be provided to DMID. The site will submit to DMID a copy of the IRB letter of approval of the amendment.

Should amendments to the protocol be required, the amendments will be written by the study team and submitted to the FDA. Amendments will be provided to all participating sites for submission to the local IRB.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual’s agreeing to participate in the study and continues throughout the individual’s study participation. Extensive
discussion of risks and possible benefits of this intervention will be provided to the subjects. Consent forms will describe in detail the study interventions, products, study procedures, and risks to the subject and written documentation of informed consent is required prior to starting intervention or administering study product. Consent forms will be approved by the IRB and the subject will be asked to read and review the document. Upon reviewing the document, the investigator or designee will explain the research study to the subject and answer any questions that may arise. The subjects should have the opportunity to discuss the study with their family and/or friends, and think about it prior to agreeing to participate. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The investigator will choose subjects in accordance with the eligibility criteria detailed in section 5. The investigator will not exercise selectivity so that bias is prevented. All subjects will sign an informed consent form that complies with the requirements of both 21 CFR Part 50 and HIPAA before entering the trial. Or, a consent form that complies with the requirements of 21 CFR Part 50 and a separate HIPAA compliant authorization form for the use and disclosure of the subject’s protected health information (PHI) may be used per institutional standard operating procedures.

Prior to participation in the trial, subjects will receive a comprehensive explanation of the proposed treatment including the nature and risks of the trial, alternate therapies, any known adverse events, 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. The consent form must not include any exculpatory statements.

DMID will provide the investigator, in writing, any new information that significantly bears on the subjects' risk to receive the investigational product. This new information will be communicated by the 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 asked to again provide consent, if necessary, due to changes.

Site staff may employ recruitment efforts prior to the subject consenting; however, before any protocol-specific procedures are performed to determine protocol eligibility, an informed consent form must be signed. Subjects will be given a copy of all consent forms that they sign.

By signing the informed consent form, the subject agrees to complete all evaluations required by the trial, unless the subject withdraws voluntarily or is terminated from the trial for any reason.
14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This study will be inclusive of all healthy adults who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background.

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 investigators, their staff, the sponsor(s), and their agents. This confidentiality extends to genetic and biological sample tests, 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 study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator. This includes, but is not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study. Clinical study sites will permit access to such records.

14.6 Study Discontinuation

If the study is discontinued, enrolled subjects will continue to be followed for safety assessments. No further doses of vaccine will be administered.

14.7 Subject Compensation

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

14.8 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining specimen for possible use in future research studies, such as testing for antibodies against other viruses or bacteria. Some samples will be stored at the local site and some at a central clinical storage facility. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples prior to obtaining new, written permission (consent)
from the subject. 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 can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject’s decision can be changed at any time prior to the end of the study by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used.
15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse Events must be graded, assessed for severity and causality and reviewed by the site Principal Investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Principal Investigator. During the study, the Investigator must maintain complete and accurate documentation for the study. The Investigator is responsible to ensure the accuracy, completeness, legibility and timeliness of the data reported.

The EMMES Corporation will serve as the Statistical and Data Coordinating Center for this study, and will be responsible for data management, quality review, analysis and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs, concomitant medications, and reactogenicity data) will be entered into a 21CFR11-compliant Internet Data Entry System provided by The EMMES Corporation. 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 source documents.

15.2.1 Source Documents and Electronic Case Report Forms

Forms derived from the electronic case report forms (eCRFs) will be provided by the DCC for use as source documents and maintained for recording data for each subject enrolled in the study. All source documents 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.

Date reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies explained.
The sponsor will provide guidance to Investigators on making corrections to the source documents and eCRFs.

15.3 Types of Data

Data for this study will include safety and immunologic outcome measures (e.g., reactogenicity and immunogenicity).

15.4 Timing/Reports

Safety Reports: The SMC will meet to review safety and reactogenicity data as outlined in the SMC charter for this study. Immunogenicity reports blinded by group and dose will be provided to the sponsor and investigators following preliminary analysis by the DCC.

15.5 Study Records Retention

An investigator shall retain records required to be maintained (including, but not limited to, CRFs, source documents, consent forms, laboratory test results and medication inventory records) under this part (21 CFR 312.57(c)) for a period of 2 years following the date a marketing application is approved for the vaccine for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified, or until DMID authorizes transfer or destruction of study records. Clinical investigators are directed to contact ORA as well as the responsible DMID Program/Project Officer for authorization to destroy records. The final authorization for destruction of documents will be provided through the DMID Program/Project Officer after concurrence by ORA. No study records shall be destroyed without prior authorization from DMID; however, these documents may be retained for a longer period if required by local regulations.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. 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 to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via The EMMES Corporation's IDES.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (IDES form) must be maintained in the regulatory file, as well as in the subject’s source document. Protocol deviations must be sent to the local IRB/Independent Ethics Committee per their guidelines. The site principal investigator/study staff is responsible for knowing and adhering to their IRB/Independent Ethics Committee requirements.
16 PUBLICATION POLICY

Following completion of the study, the investigator may 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, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. This trial will be registered in NLM in accordance with the new NLM requirements under the FDAAA. Trials initiated after 9/27/2007, or trials that are ongoing as of 12/26/2007 must be registered in full by the later of 12/26/2007 or 21 days after the first patient is enrolled.

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 1 trials), would be exempt from this policy. This study is of great public health interest. As a result, this study will be registered in the NLM registry, ClinicalTrials.gov.

*Journal Citation:
17 LITERATURE REFERENCES


18 SUPPLEMENTS/APPENDICES

Schedule of Event Tables:

Groups 8 and 9, Appendix A

Group 10, Appendix B
<table>
<thead>
<tr>
<th>GROUPS 8 and 9</th>
<th>Appendix A: Schedule of Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Day</td>
<td>0 V, B</td>
</tr>
<tr>
<td>Day Relative to Vaccination</td>
<td>Day 0</td>
</tr>
<tr>
<td>Visit Number</td>
<td>Dose 1</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>X</td>
</tr>
<tr>
<td>Review Eligibility</td>
<td>X</td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
</tr>
<tr>
<td>Targeted Exam if indicated</td>
<td>X</td>
</tr>
<tr>
<td>Vital Signs (temp, HR and BP)</td>
<td>X</td>
</tr>
<tr>
<td>Pregnancy Test</td>
<td>X</td>
</tr>
<tr>
<td>Concomitant Meds</td>
<td>X</td>
</tr>
<tr>
<td>Randomization</td>
<td>X</td>
</tr>
<tr>
<td>Safety Serology</td>
<td>X</td>
</tr>
<tr>
<td>Research Blood Draw</td>
<td>X</td>
</tr>
<tr>
<td>IM injection</td>
<td>X</td>
</tr>
<tr>
<td>Distribute memory aid</td>
<td>X</td>
</tr>
<tr>
<td>Review memory aid</td>
<td>X</td>
</tr>
<tr>
<td>AE assessment</td>
<td>X</td>
</tr>
<tr>
<td>SAE assessment</td>
<td>X</td>
</tr>
<tr>
<td>Telephone call</td>
<td>X</td>
</tr>
<tr>
<td><strong>Day of vaccination.</strong>&lt;br&gt;Telephone call. Day 2 and Day 365 post vaccination telephone contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.</td>
<td></td>
</tr>
<tr>
<td><strong>Memory Aid Review Visit.</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Safety Serology:</strong> 5mL purple top, 10mL red top. Sera will be tested in a central laboratory for safety serology and chemistry.</td>
<td></td>
</tr>
<tr>
<td><strong>Research Blood Draw:</strong> 20 mL blood sample for HAI and neutralizing antibody titer assays to be tested in a central research laboratory.</td>
<td></td>
</tr>
<tr>
<td>See MOP for details.</td>
<td></td>
</tr>
<tr>
<td><strong>Final Visit.</strong></td>
<td></td>
</tr>
</tbody>
</table>
## GROUP 10

### Appendix B: Schedule of Events

<table>
<thead>
<tr>
<th>Study Day</th>
<th>0&lt;sup&gt;V,B&lt;/sup&gt;</th>
<th>2&lt;sup&gt;T&lt;/sup&gt;</th>
<th>7&lt;sup&gt;M,B&lt;/sup&gt;</th>
<th>14&lt;sup&gt;B&lt;/sup&gt;</th>
<th>21&lt;sup&gt;B&lt;/sup&gt;</th>
<th>28&lt;sup&gt;V,B&lt;/sup&gt;</th>
<th>30&lt;sup&gt;T&lt;/sup&gt;</th>
<th>36&lt;sup&gt;M,B&lt;/sup&gt;</th>
<th>42&lt;sup&gt;B&lt;/sup&gt;</th>
<th>49&lt;sup&gt;B&lt;/sup&gt;</th>
<th>56&lt;sup&gt;B&lt;/sup&gt;</th>
<th>208&lt;sup&gt;B&lt;/sup&gt;</th>
<th>365&lt;sup&gt;F,T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day Relative to Vaccination</td>
<td>Day 0</td>
<td>Day 2</td>
<td>Day 8</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 28</td>
<td>Day 2 post Dose 2</td>
<td>Day 8 post Dose 2</td>
<td>Day 14 post Dose 2</td>
<td>Day 21 post Dose 2</td>
<td>Day 28 post Dose 2</td>
<td>Day 180 post Dose 2</td>
<td>Day 365 post Dose 2</td>
</tr>
<tr>
<td>Visit Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review Eligibility</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted Exam if indicated</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Vital Signs (temp, HR and BP)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy Test</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concomitant Meds</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Randomization</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research Blood Draw&lt;sup&gt;B&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IM injection&lt;sup&gt;V&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribute memory aid</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review memory aid&lt;sup&gt;B&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE assessment</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SAE assessment</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Telephone call&lt;sup&gt;T&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of vaccination.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day and Day 365 post vaccination telephone contact for safety may alternatively be made by e-mails, text messaging, or in-clinic visits as appropriate.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory Aid Review Visit.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Research Blood Draw; 20 mL blood sample (40 mL total at visit 9 if the subject has consented) for HAI and neutralizing antibody titer assays.
| Final Visit. |
| Post second vaccination |