Evaluation of the effects of age, prior exposure, and previous vaccination on the B cell response to inactivated influenza vaccine in healthy adults and children

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Statement of Compliance

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

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- 21 CFR 312
- ICH GCP E6
- Completion of Human Subjects Protection Training
- NIH Clinical Terms of Award

SIGNATURE PAGE

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

Principal Investigator:

Signed: _____ Date: _____

Angela Branche, MD

Principal Investigator, University of Rochester

CEIRS

AE	Adverse Event/Adverse Experience
ASC	Antibody Secreting Cell
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CO	Contracting Officer
COR	Contracting Officer Representative
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data and Safety Monitoring Board
FWA	Federalwide Assurance
GFPD	Genome Fragment Phage Display
GCP	Good Clinical Practice
HAI	Hemagglutination-Inhibition
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
JAMA	Journal of the American Medical Association
MBC	Memory B Cell
MN	Microneutralization
MOP	Manual of Procedures
N	Number (typically refers to participants)
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SPR	Surface Plasmon Resonance
WHO	World Health Organization

1 PROTOCOL SUMMARY

Title: Evaluation of the effects of age, prior exposure and previous vaccination on the B cell response to inactivated influenza vaccine in healthy adults and children.

Population: 240 Healthy adults and children ages 9 and over

Number of Sites: Single site study

Study Duration: 5 years

Participant Duration: 6 months

Objectives:

Primary Objective

To evaluate the relationship between first influenza A virus exposure (inferred by age), vaccine history, and baseline serum antibody response to seasonal influenza vaccine in healthy adults and children.

Primary Endpoint:

- Measurement of the magnitude and specificity of the serum antibody responses by hemagglutination (HAI) and microneutralization (MN) at day 7 and 28, overall and stratified by age group, vaccine history, and baseline serum antibody level.
-
- Serum antibody response on day 90 after vaccination by HAI and MN overall and stratified by acute antibody response.

Secondary Objectives

Evaluate factors related to failure of vaccine to provide protection against symptomatic influenza and the immune response to infection in vaccinated individuals by prospective surveillance of the vaccine cohort.

Secondary Endpoints

Description of demographic and clinical characteristics and immune response to vaccination among subjects who develop symptomatic influenza (Vaccine Failures) and among subjects who do not.

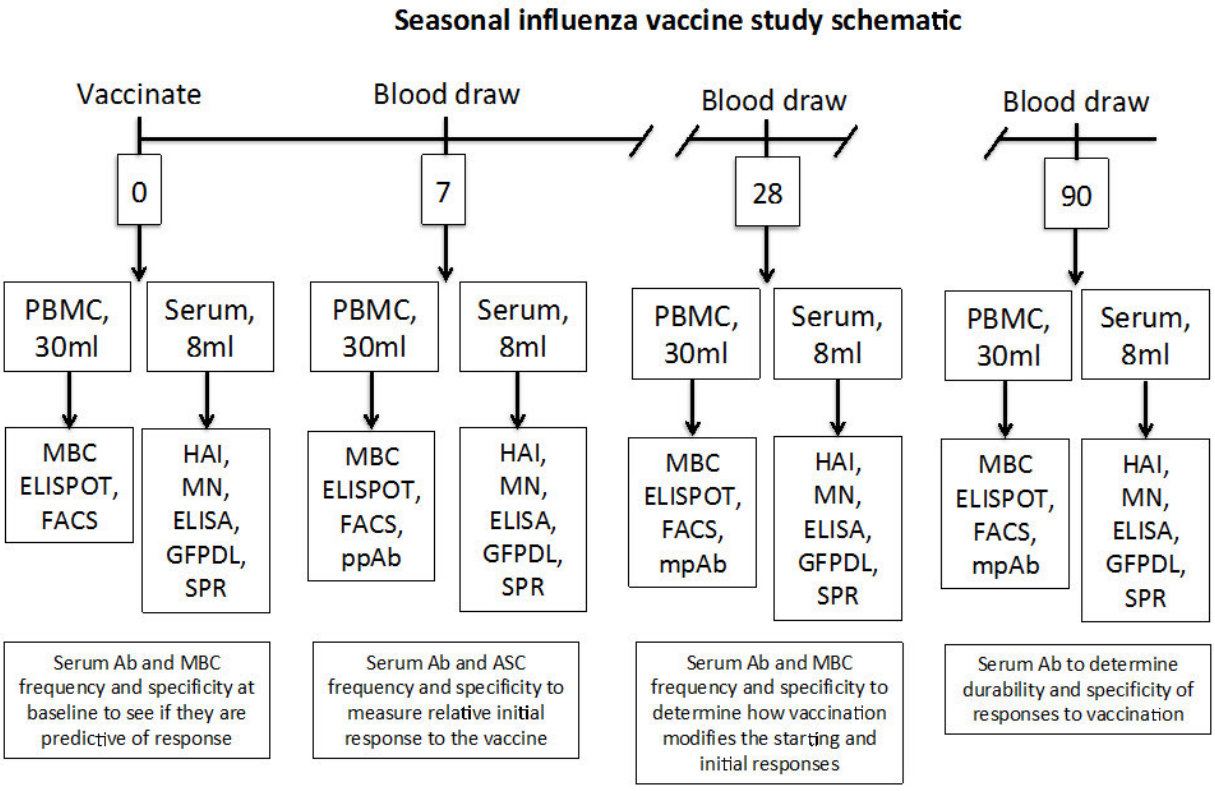
Exploratory Objective

To evaluate the relationship between first influenza A virus exposure (inferred by age), vaccine history, and Memory B cell (MBC) specificity, and the magnitude and breadth of the subsequent B cell response to seasonal influenza vaccine in healthy adults and children.

Exploratory Endpoints

- Frequency and specificity of peripheral blood antibody secreting cells (ASC) on day 7 post vaccination (initial response), overall and stratified by age group, vaccine history, and baseline serum antibody level
- Frequency and specificity of peripheral blood memory B cells (PMBC) on day 28 post vaccination (memory response), overall and stratified by age group, vaccine history, and baseline serum antibody level

Schematic of Study Design:



* Note: For children weighing less than 100 lbs, only 15 mL of blood will be drawn at each time point.and plasma will be used for antibody testing. Subjects will be queried regarding any blood being drawn for other purposes such as clinical testing, and that the total drawn will not exceed the above amounts

In addition, beginning on day 48, subjects will be contacted by phone once every two weeks during the influenza season and asked about influenza symptoms. Subjects who report symptoms will be asked to return for a clinic visit and specimen collection. Influenza season will be defined as when community laboratories detect 4 or more positives in any one week for two weeks in a row.

2 KEY ROLES

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3 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

3.1 Background Information

Inactivated influenza vaccines (IIVs) have been in use in the US and worldwide for the prevention of illness due to seasonal and pandemic influenza since their licensure approximately 60 years ago. Multiple studies over many years have evaluated the immunogenicity of these vaccines in various populations. In general, serum antibody responses to IIV are vigorous in healthy young adults with low levels of pre-vaccination antibody. Nasal antibody responses can also be detected, although they are not as vigorous as those seen after live vaccination.

The serum antibody response to IIV is impacted by several factors, including the age of the subject, the presence of chronic diseases, and the use of immune modulating drugs. In addition, the prior exposure of the subject to influenza vaccine or infection appears to have a substantial impact on both the immune response as well as the protective efficacy of IIV. For seasonal influenza vaccine, the magnitude of the serum antibody response is lower in individuals who begin with higher titers, and highest in those with low prevaccination titers. Consistent with this observation, individuals with prior vaccination, and who therefore have higher baseline antibody titers, tend to have less vigorous antibody responses to revaccination. The impact of prior vaccination on protection is controversial. Some studies have suggested that vaccine efficacy is higher in those who were not vaccinated in the previous season than in those who were [1, 2]. Other studies have not seen this association [3]. The mechanisms by which prior exposure to vaccine or infection might modify the subsequent response to IIV are not known, but would have obvious implications for the design of new influenza vaccines and vaccine strategies.

3.2 Scientific Rationale

This protocol will address the hypothesis that vaccination and infection drive adaptation of the antibody secreting and memory B cell populations, and differ based on the antigenicity of the vaccine strains and immune history of the subject. This protocol will address whether initial influenza priming, most likely occurring during childhood, affects later-in-life immune responses to influenza vaccination. Recent data in the literature [4, 5], and our own preliminary data suggest that initial encounters with influenza have a long lasting effect on circulating antibodies and the specificity of the memory B cell repertoire. This protocol will test whether qualitative and quantitative differences in circulating antibodies and memory B cells can strongly influence subsequent immune responses to seasonal influenza vaccines. This protocol will perform

parallel assessment of antibody, ASC, and memory B cells over the course of an immune response to determine how vaccination modifies the B cell and antibody repertoires. As a measure of the cells responding to the vaccination, our assays will focus on the acutely activated antibody-secreting B cells (ASC, plasmablasts) and antibodies secreted (day 7). Because these ASC can be short lived, later in the response this protocol will also look at in vitro reactivated memory B cells and secreted antibodies at days 0, 28, and 90 as measures of the adapted responses before and after vaccination. Serum antibodies will also be tested for function, specificity, amount, and quality by conventional HAI and MN assays, as well as more sophisticated assays described below (ELISA, SPR, GFPDL).

3.3 Potential Risks and Benefits

3.3.1 Potential Risks

The risks and discomforts of this study include risks associated with the vaccine, administration of the vaccine, the risks associated with study procedures (blood drawing and nasal and throat swabs) and possible loss of confidentiality.

In placebo-controlled trials in adults, inactivated seasonal vaccines are associated with mild local pain at the site of administration. Systemic symptoms like fever and malaise occur at rates equal to placebo. According to the [redacted] package insert, in adults, the most common (≥10%) injection site adverse reaction was pain (36%); the most common systemic adverse events were muscle aches (16%), headache (16%), and fatigue (16%). In children aged 3 through 17 years, the injection site adverse reactions were pain (44%), redness (23%), and swelling (19%). In children aged 3 through 5 years, the most common (≥10%) systemic adverse events were drowsiness (17%), irritability (17%), and loss of appetite (16%); in children aged 6 through 17 years, the most common systemic adverse events were fatigue (20%), muscle aches (18%),headache (16%), arthralgia (10%), and gastrointestinal symptoms (10%).

During the swine influenza vaccine campaign of 1976, about 10 per 1,000,000 vaccine recipients in excess of the background rate developed the paralytic illness called Guillain-Barré Syndrome (GBS). In the subsequent decade, no association between seasonal influenza vaccine and GBS was found. More extensive investigations of this potential association occurring in the 1990s revealed that there was a small but detectable risk of GBS in the 6 weeks following seasonal influenza immunization: an attributable risk of approximately 1 per 1,000,000, adjusted for potential confounders. In the period since the Vaccine Adverse Event Reporting System (VAERS) was established in 1990, the rates of GBS reports following influenza vaccination have declined substantially. The annual reporting rate in that period was highest in the 1993-1994 influenza season (1.7 per 1,000,000 vaccinees) and lowest in the last season analyzed in the report, 2002-2003 (0.4 per 1,000,000 vaccinees) [6]. No cases of GBS have

been reported following receipt of novel H1N1 influenza vaccines to date, but the total number of recipients is only in the thousands. Most persons who develop GBS recover completely.

Drawing blood causes transient discomfort and may cause fainting. Bruising at the blood draw site may occur but can be prevented or mitigated by applying direct pressure to the draw site for several minutes. The use of alcohol swabbing and sterile equipment will make infection less likely at the site where blood will be drawn. Nasal and throat swabs can be uncomfortable and may cause gagging.

Personal health information of the subjects will be collected to determine eligibility and to evaluate safety and reactogenicity outcomes throughout the study. Research personnel will make every effort to keep this information confidential. Still, a risk of participation is that the confidentiality of this information could be lost.

3.3.2 Known Potential Benefits

Administration of licensed vaccines for seasonal influenza is recommended for all children and adults and would be expected to provide partial protection against influenza illness caused by the strains contained within the vaccine.

4 OBJECTIVES AND OUTCOME MEASURES

4.1 Study Objectives

Primary Objective

To evaluate the relationship between first influenza A virus exposure (inferred by age), vaccine history, and baseline serum antibody response to seasonal influenza vaccine in healthy adults and children

Secondary Objectives

- Evaluate factors related to failure of vaccine to provide protection against symptomatic influenza and the immune response to infection in vaccinated individuals by prospective surveillance of the vaccine cohort.

Exploratory Objectives

Evaluate the relationship between first influenza A virus exposure (inferred by age), vaccine history, and Memobry B cell (MBC) specificity, and the magnitidue and breath of the subsequent B cell response to seasonal influenza vaccine in healthy adults and children

4.2 Outcome Measures

Primary Outcome Measures:

- Measurement of the magnitude and specificity of the serum antibody responses by hemagglutination (HAI) and microneutralization (MN) at day 7 and 28, overall and stratified by age group, vaccine history, and baseline serum antibody level
- Serum antibody response on day 90 after vaccination by HAI and MN and overall and stratified by acute antibody response

Secondary Outcome Measures

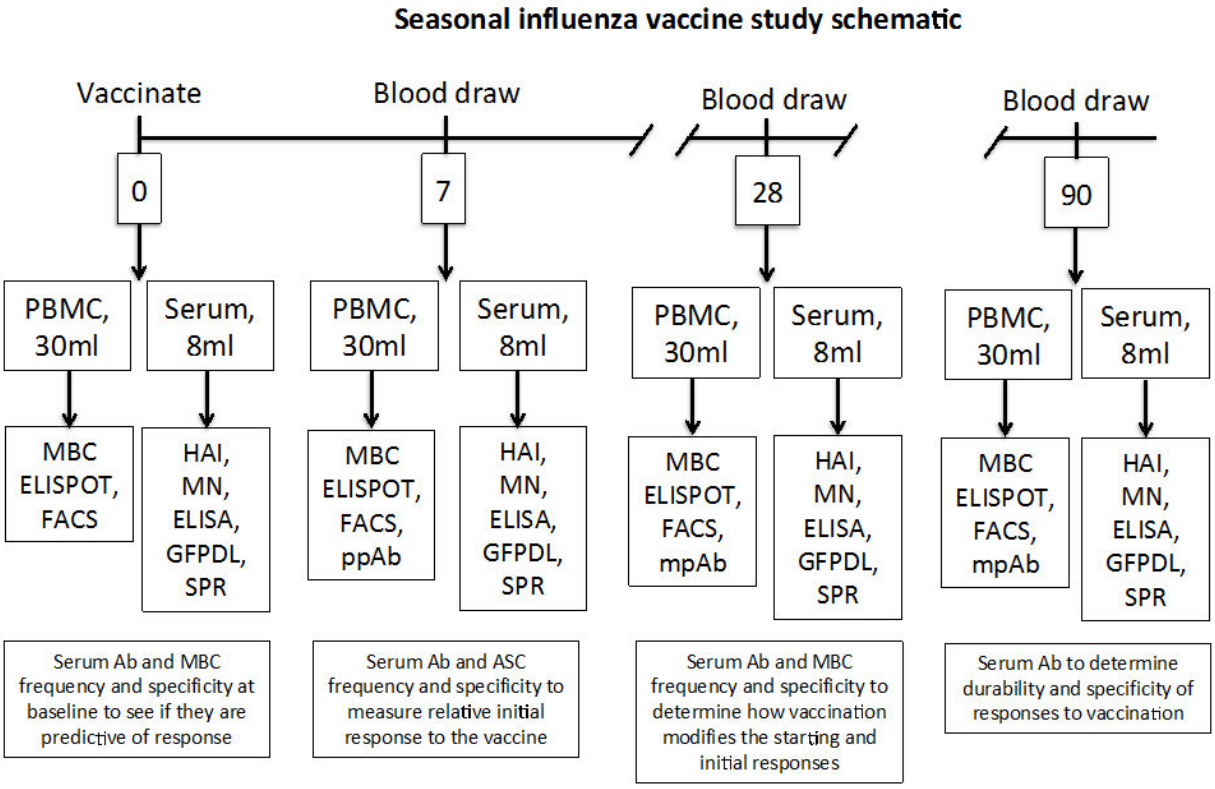
- Description of demographic and clinical characteristics and immune response to vaccination among subjects who develop symptomatic influenza (Vaccine Failures) and among subjects who do not.

Exploratory Outcome Measures

- Frequency and specificity of peripheral blood antibody secreting cells (ASC) on day 7 post vaccination (initial response), overall and stratified by age group, vaccine history, and baseline serum antibody level
- Frequency and specificity of peripheral blood memory B cells (PMBC) on day 28 post vaccination (memory response), overall and stratified by age group, vaccine history, and baseline serum antibody level

5 STUDY DESIGN

The study will be designed as a prospective surveillance of the immune response to seasonal vaccination in healthy adults of varying ages. A schematic of the study design is shown below:



* Note for children weighing less than 100 lbs, only 15 mL of blood will be drawn at each time point, and plasma will be used for antibody testing. Subjects will be queried regarding any blood being drawn for other purposes such as clinical testing, and that the total drawn will not exceed the above amounts

Influenza season will be defined as when community laboratories detect 4 or more positives in any one week for two weeks in a row. If influenza season has begun subjects will be contacted by phone once every two weeks starting on day 48 and asked about influenza symptoms. Subjects who report symptoms will be asked to return for a clinic visit and specimen collection.

6 STUDY POPULATION

The study population will consist of up to 240 medically stable adults and children ages 9 and older enrolled over 5 years. Recruitment will be stratified in an attempt to achieve an even distribution of ages with a goal of 30 subjects in each of 8 age strata (9-19, 20-29…80+)

6.1 Inclusion Criteria

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

1. Aged equal to or greater than 9 years of age and weigh at least 50 pounds.

2. The subject must be in good health, as determined by: medical history; and targeted physical examination, when necessary, based on medical history. Stable medical or psychiatric condition is defined as: no recent increase 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.

3. The subject is able to understand and comply with the planned study procedures, including being available for all study visits.

4. The subject/parent has provided informed consent/assent prior to any study procedures.

5. Subjects who have not received seasonal flu vaccine for the current year.

6.2 Exclusion Criteria

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

1. Subject report of known hypersensitivity to allergy to components of the study vaccine or other components of the study vaccine.

2. Subject report of known latex allergy

3. Subject report of a history of severe reactions following previous immunization with licensed or unlicensed influenza virus vaccines.

4. Subject report of a history of Guillain-Barre syndrome within 6 weeks of receipt of a previous influenza vaccine.

5. The subject is a female of childbearing potential who is currently pregnant or breastfeeding or intends to become pregnant during the study period between enrollment and 90 days following receipt of vaccine. Pregnancy will be determined by subject interview. Pregnancy testing is not done in this study since there is no increased risk in pregnancy.

6. The subject is immunosuppressed 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.

7. The subject 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 hematological malignancy. For this criterion, “active” is defined as having received treatment within the past 5 years.

8. Have taken high-dose inhaled corticosteroids within 30 days prior to study vaccination.
High-dose defined as per age as using inhaled high dose per reference chart *https://www.nhlbi.nih.gov/health-pro/guidelines/current/asthma-guidelines/quick-reference-html#estimated-comparative-daily-doses*

9. The subject received immunoglobulin or another blood product within the 3 months prior to enrollment in this study.

10. The subject has received an inactivated vaccine within the 2 weeks or a live vaccine within the 4 weeks prior to enrollment in this study or plans to receive another vaccine within the next 28 days after vaccination.

11. The subject has an acute or chronic medical condition that, in the opinion of the investigator or appropriate sub-investigator, would render vaccination unsafe or would interfere with the evaluation of responses. These conditions include any acute or chronic medical disease or conditions defined as persisting for 3 months (defined as 90 days) or longer, that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses of the subject’s successful completion of the study.

12. Subjects with an active infection or that has an acute illness or an oral temperature greater than 99.9F (37.7C) within 3 days prior to enrollment or vaccination. Subjects who had an acute illness that was treated symptoms resolved are eligible to enroll as long as treatment is completed and symptoms resolved > 3 days prior to enrollment.

13. The subject is currently participating or plans to participate in a study that involves an experimental agent (vaccine, drug, biologic, device, blood product, or medication) or has received an experimental agent within 1 month prior to enrollment in this study, or expects to receive another experimental agent during participation in this study, or intends to donate blood during the study period.

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

15. The subject has a history of alcohol or drug abuse in the 5 years prior to enrollment.

16. The subject has a known human immunodeficiency virus, hepatitis B, or hepatitis C infection.

17. Subject weighs less than 50 lbs.

18. Subject expects to have a medical procedure during the upcoming 8 weeks that estimates blood loss to exceed 400 cc for adults or for children would exceed 3 ml/kg.

6.3 Handling of Withdrawals

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after receiving the assigned vaccine will not be replaced. Participants who consented to the study but did not receive the influenza vaccine will be replaced. These subjects will be considered as screen failures.

6.4 Termination of Study

Although the study sponsor has every intention of completing the study, the sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to internal safety review and recommendation, or at the discretion of DMID.

7 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

7.1 Study Product Description

[redacted] Quadrivalent (IIV-4) split virion licensed vaccine produced in embryonated hen's eggs and administered intramuscularly at a dose of not less than 15 ug of HA (as determined by SRID) of the following 4 influenza virus strains (2 A strains and 2 B strains): A/Singapore/GP1908/2015 (H1N1) IVR-180 (an A/Michigan/45/2015 [H1N1] pdm09-like virus), A/Singapore/INFIMH-16-0019/2016 (H3N2) NIB-104, B/Maryland/15/2016 NYMC BX-69A (a B/Colorado/06/2017-like virus), and B/Phuket/3073/2013.

[redacted] Quadrivalent (IIV-4) split virion licensed vaccine produced in embryonated hen's eggs and administered intramuscularly at a dose of not less than 15 ug of HA (as determined by SRID) of the following 4 influenza virus strains (2 A strains and 2 B strains): A/Singapore/GP1908/2015 (H1N1) IVR-180 (an A/Michigan/45/2015 [H1N1] pdm09-like virus), A/Singapore/INFIMH-16-0019/2016 (H3N2) IVR-186, B/Maryland/15/2016 NYMC BX-69A, (a B/Colorado/06/2017-like virus), and B/Phuket/3073/2013.

7.1.1 Acquisition

Vaccine will be ordered for each influenza season from the University of Rochester Medical Center hospital pharmacy which will acquire the vaccine through distributors.

7.1.2 Formulation, Packaging, and Labeling

[redacted]

7.1.3 Product Storage and Stability

Influenza vaccine will be stored according to manufacturer's directions in secure, limited-access temperature monitored refrigerator environment at 2°C to 8°C (36°F to 46°F) until needed. DO NOT FREEZE. The temperature of the storage unit will be monitored during the duration of the trial, and documentation of proper dedicated storage will be maintained. In the event of accidental deep-freezing or disruption of the cold chain, vaccines will not be administered; and the PI or the responsible person will contact the sponsor for further instructions.

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

Vaccine will be formulated in single dose vials or syringes and will not require additional formulation prior to administration. A vaccine dose of 0.5mL will be administered by a clinical research nurse intramuscularly (IM) in the subject's preferred deltoid (upper arm) per the manufacturer's instructions.

7.3 Modification of Study Intervention/Investigational Product for a Participant

As there is only a single dose administered, there will be no dose or schedule modifications for any subject.

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

Records of vaccine receipt and dispensation to the study subject as well as storage and destruction of the vaccine will be maintained according to existing standard operating procedures (SOPs)

7.5 Assessment of Participant Compliance with Study Intervention/Investigational Product

Not applicable, single intervention within the vaccine clinic only.

7.6 Concomitant Medications/Treatments

Administration of any medication or therapies considered necessary for the subject's welfare will be recorded and documented in the subject's source documentation. Concomitant medications will include all medications taken within 30 days prior to enrollment through 62 days post vaccination or early termination, whichever occurs first.

The following criteria will be reviewed with the subject's during each follow up visit. If any of these become applicable during the study, it will be noted in the subject's record.

1. Use of any investigational drug or investigational vaccine other than the study article.
2. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs (topical and nasal steroids are allowed).
3. Receipt of a licensed vaccine.
4. Receipt of immunoglobulins and/or any blood products.

8 STUDY SCHEDULE

8.1 Vaccination/Baseline (Defined as Day 0)

At the baseline visit, subjects will be enrolled in the study and if they meet the entry criteria will receive influenza vaccine. The following procedures will be performed at the baseline visit.

- The study will be explained to the subject/parent, and the informed consent document will be signed. Subjects/Parents will keep a signed copy of the informed consent for their information. Subjects 9 to 12 will be read an Assent Script and subjects 13-17 will sign an Assent Form.
- A medical history will be obtained including history of prior Influenza vaccinations, and details recorded in the case report form.
- Concomitant medications will be reviewed and recorded.
- A targeted physical examination will be performed, if indicated by the medical history, directed at the relevant portions of the exam.
- The entry criteria will be reviewed and it will be verified that the subject meets all entry criteria. If the subject does not meet the entry criteria, the subject will be discontinued from the study, or can be rescreened in the case of entry criteria mandating temporary delays.

Subjects will be considered enrolled if they have signed the consent form. However, only subjects who meet the entry criteria will be vaccinated and continue in the study. The reasons for screen failure will be recorded for all subjects who do not meet the entry criteria. If the entry criteria are met:

- 30 mL of blood will be obtained from an arm vein (3 x 10 mL green top tubes) for isolation of Peripheral Blood Mononuclear Cells (PBMC).
- 8 mL of blood will be obtained from and arm vein (1 x 8 mL red top tube) for serum.
- For children who weigh less than 100 lbs, only 15 mL of blood will be drawn
- Subjects will receive vaccine by intramuscular injection in open label fashion.

- Subjects will be observed in the clinic for 30 minutes post vaccination for development of acute adverse events

8.2 Follow-up and Final Visits

8.2.1 Day 7 visit (+/- 1 day)

Subjects will return one week after vaccination for the day 7 visit. Because the timing of assessment of the cellular response to vaccination is critical, every effort will be made to ensure that subjects return on day 7. Visits that occur out of window will still be evaluated but will not be included in the primary analysis. On the day 7 visit, the following procedures will be done:

- Interval medical history will be obtained including concomitant medications, reviewing any new events since the last visit.
- The site of vaccination will be examined. If indicated by medical history, a directed physical exam will also be performed.
- Any adverse events (AE) will be recorded and appropriate corrective actions taken if indicated.
- 30 mL of blood will be obtained from an arm vein (3 x 10 mL green top tubes) for isolation of Peripheral Blood Mononuclear Cells (PBMC).
- 8 mL of blood will be obtained from and arm vein (1 x 8 mL red top tube) for serum.
- For children who weigh less than 100 lbs, only 15 mL of blood will be drawn

8.2.2 Day 28 visit (+/- 3 days)

Subjects will return for follow up approximately 28 days after vaccination for assessment of the immune response. At that visit, the following procedures will be performed:

- Interval medical history will be obtained including concomitant medications, reviewing any new events since the last visit.
- If indicated by medical history, a directed physical exam will also be performed.

- Any adverse events (AE) will be recorded and appropriate corrective actions taken if indicated.
- 30 mL of blood will be obtained from an arm vein (3 x 10 mL green top tubes) for isolation of Peripheral Blood Mononuclear Cells (PBMC).
- 8 mL of blood will be obtained from and arm vein (1 x 8 mL red top tube) for serum.
- For children who weigh less than 100 lbs, only 15 mL of blood will be drawn

8.2.3 Day 90 visit (+/- 7 days)

Subjects will return approximately 90 days after vaccination for a final follow-up visit. At this visit the following procedures will take place:

- Interval medical history will be obtained, including concomitant medications, reviewing any new events since the last visit.
- If indicated by medical history, a directed physical exam will also be performed.
- Any adverse events (AE) will be recorded and appropriate corrective actions taken if indicated.
- 30 mL of blood will be obtained from an arm vein (3 x 10 mL green top tubes) for isolation of Peripheral Blood Mononuclear Cells (PBMC).
- 8 mL of blood will be obtained from and arm vein (1 x 8 mL red top tube) for serum.
- For children who weigh less than 100 lbs, only 15 mL of blood will be drawn

8.2.4 Blood Volumes in Children

The blood draws in this protocol will impose an upper limit of 3 mL/kg over any 8-week period for all pediatric patients. This volume remains well below the allowed upper limit of 10% of total blood volume in an 8-week period as minimal risk. From healthy subjects who weigh at least 100 pounds, the total amount of blood drawn in any 8 week period is 38 x 3, or 114 mL. For subjects who weigh less than 100 lbs, the total blood drawn will be 45 mL. Neither of these amounts will exceed the limit of 3 mL/kg

8.2.5 Surveillance for acute influenza

Influenza season will be defined as when community laboratories detect 4 or more positives in any one week for two weeks in a row, and will end when influenza is no longer detected. During influenza season, subjects will be contacted by phone every other week to enquire about influenza like symptoms. Subjects who report symptoms meeting the case definition of influenza (fever or feverishness plus either cough, rhinitis, or sore throat on the same or consecutive days) will be asked to return for an acute illness visit. At this visit the following procedures will take place

- Interval medical history will be obtained, including concomitant medications, reviewing any new events since the last visit.
- The presence and severity of respiratory symptoms will be assessed and recorded using a visual analog scale.
- A combined nasal and throat swab will be obtained for rtRT-PCR detection of influenza virus.
- If positive, the subject will be invited to participate in the Acute Flu study (DMID 14-0101) to assess the immune response to infection

8.3 Early Termination Visit

If subjects discontinue from the study, they will be asked to make an early termination visit. At the time of the early termination visit, the reason for early termination will be recorded, current health status since the last visit will be reviewed, and all concomitant medications will be recorded. A targeted physical examination may be performed, as indicated, and information regarding AEs will be solicited. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established.

Subjects will be encouraged to permit continued follow-up of AEs and to donate scheduled blood samples, if possible.

8.4 Unscheduled Visit

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

- Medical history will be reviewed including concomitant medications, and updated as appropriate.
- All concomitant medications taken since the study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- Study personnel will discuss with participants and assess and record all AE/SAEs. Previously recorded AE/SAEs will be updated as appropriate.
- Depending on the reason for the unscheduled visit, vital signs, including oral or axillary temperature, pulse, and blood pressure, may be obtained.
- If indicated by medical history, a directed physical examination relevant to the interval medical history may be performed.
- Additional laboratory tests may be obtained depending on the nature of the unscheduled visit.

9 STUDY PROCEDURES AND EVALUATIONS

9.1 Clinical Evaluations

Medical History: Study personnel will take the medical history of all subjects. This history will include significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. It will also include a history of allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease. Patients will also be queried regarding any previous influenza vaccinations, the date and source of vaccine, and the type of vaccine administered. We will use personal history as the primary indicator but will attempt to verify vaccination status where possible.

Medication History: Study personnel will record all medications, including prescription and over-the-counter drugs (such as vitamins, minerals, supplements, homeopathic preparations and/or therapies), taken by the subject in the 30 days prior to enrollment.

Targeted Physical Examination: Licensed study clinicians (i.e., physician, physician's assistant, nurse practitioner) may conduct a targeted physical examination, if necessary to assist in

determining eligibility and height and weight will be collected. All subjects will have vital signs (blood pressure, pulse, and oral temperature) measured prior to vaccination.

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9.2 Laboratory Evaluations/Assays

9.2.1 Immunogenicity Evaluations

Serum hemagglutination-inhibition (HAI). HAI will be performed in microtiter format using turkey RBCs and egg-grown, betapropriolactone-inactivated vaccine viruses as antigen. The titer of antibody will be defined as the highest dilution resulting in complete inhibition of hemagglutination. Sera will be treated with receptor-destroying enzyme and heat inactivated prior to testing at an initial starting dilution of 1:4. Sera with no detectable HAI titer will be assigned a titer of 1:2 for calculation purposes.

Microneutralization (MN) assay: Sera will be tested by microtiter technique for neutralization of the vaccine viruses in MDCK cells. Viral growth will be determined by ELISA of the cells following fixation with methanol using a combination of M- and NP-specific monoclonal antibodies. The titer of antibody will be defined as the highest titer resulting in 50% inhibition of antigen signal compared to un-neutralized control wells. Sera will be treated with RDE and heat inactivated prior to testing at an initial starting dilution of 1:10. Sera with no neutralizing titer will be assigned a value of 1:5 for calculation purposes.

B cell responses: Our previous studies have shown that the antibodies secreted by activated B cells (plasmablasts) and re-activated memory B cells have different specificities and affinities that those that accumulate in the serum. In addition, there is evidence from analyzing secreted antibodies that vaccination affects the specificities and improves affinities. Therefore, the studies of antibody-secreting (ASC) and memory B cells require the collection of Peripheral Blood Mononuclear Cells (PBMC) at different times before and after vaccination for analysis. PBMC will be obtained before and on days 0, 7, 28, and 90 after vaccine and evaluated for B-cell responses. First, antibody secreting cells stimulated by the vaccination, which peak 7 days after vaccination in the blood, will be analyzed by performing B cell ELISPOT assays on plates coated with recombinant HA proteins that match the influenza strains in that year's vaccine. In addition, supernatants will be collected from cultured plasmablasts for analysis by HAI, MN, and ELISA as described above. Memory B cell responses will be assessed similarly at all the time points by reactivating the B cells in culture [7], performing B cell ELISPOT assays and collecting culture supernatants for analysis as described above. The analysis of the ASC and

memory B cells provides a measure of how the vaccine modifies the B cell repertoire, as well as how well someone is responding to the vaccine.

9.2.2 Specimen Collection, Preparation, Handling and Shipping

Instructions for specimen preparation, handling, and storage are included in the Manual of Procedures (MOP).

10 ASSESSMENT OF SAFETY

10.1 Specification of Safety Parameters

An FDA approved, licensed seasonal inactivated influenza vaccine will be administered in this protocol as standard of care during influenza vaccine season, following the manufacturers' instructions and safety precautions.

[redacted] is a licensed vaccine, NIAID does not expect that any new vaccine related safety signal will be detected in this trial.

The National Childhood Vaccine Injury Act (NCVIA) **requires** healthcare providers to report to VAERS:

- Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine; and/or
- Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination that occurs within the specified time period after vaccination (see below).
    - Anaphylaxis or anaphylactic shock (7 days) (see halting criteria)
    - Shoulder Injury Related to Vaccine Administration (7 days)
    - Vasovagal syncope (7 days)
    - Guillain-Barré Syndrome (42 days)
    - Any acute complication or sequelae (including death) of above events (interval - not applicable)

In addition, CDC encourages reporting any serious, unexpected (not listed in product label or Investigator's Brochure), suspected adverse reaction to VAERS and provide a copy of the VAERS report to DMID and local IRB as required.

11 CLINICAL MONITORING

The purpose of clinical monitoring is to protect the rights and well-being of human subjects in this study; to ensure that data are accurate, complete and verifiable from source documents; to ensure that conduct is in compliance with the currently approved protocol/amendments, with Good Clinical Practice, and with regulatory requirements.

11.1 Site Monitoring Plan

Site monitoring will be conducted using the DMID tools provided to ensure that human subject protection, study procedures, laboratory procedures, 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 standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as defined in the CQMP.

A protocol-specific Clinical Quality Management Plan (CQMP) has been approved for this study by DMID. The Quality Assurance (QA) plan will be implemented by a weekly review of source documents by the CRC to determine adherence to protocol requirements. The Quality Control (QC) plan will be implemented by daily observation and documentation of the site's work processes by study staff, to ensure that accepted procedures are followed.

Site visits may 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, sample tracking log, CRFs, 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. The University of Rochester's IRB and other regulatory agencies may conduct study monitoring visits.

12 STATISTICAL CONSIDERATIONS

12.1 Study Hypothesis

The primary hypothesis being tested in this study is that there will be differences in the specificity and magnitude of the HA-specific B cell response depending on the age, previous vaccine history and baseline B cell reactivity.

12.2 Sample Size Considerations

Because the degree of variability in many of the measurements in this study are unknown, estimating the precision of the correlation of patient characteristics (age and prior vaccination history) with outcomes (antibody quality and quantity and B cell response) is not possible. The

study will evaluate these outcomes and if trends towards a relationship are discovered subsequent studies will evaluate these in more detail.

However, the precision of the estimate of the proportion of subjects who achieve a pre-specified number of memory B cells specific for any antigen on day 28 would be determined by the number of subjects. If there were 30 subjects in any particular age strata, then the half-width of the 95% confidence interval for the estimate of the the proportion achieving any level of mBC in that strata is shown below

No. of subjects	Proportion achieving mBC frequency	Half-width of the 95% CI for estimate of proportion
30	10%	10.7%
30	20	14.3
30	30	16.4
30	40	17.5
30	50	17.9

12.3 Planned Interim Analyses (if applicable)

No formal interim analysis is planned. However, assay results will be reviewed as they become available

12.3.1 Safety Review

NA, no interim analysis of safety is planned.

12.3.2 Immunogenicity or Efficacy Review

NA, no interim analysis of safety is planned.

12.4 Final Analysis Plan

Data will be analyzed at the end of the year of enrollment.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this study in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical 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, pharmacy dispensing records, recorded data from automated instruments, x-rays, and subject files kept at the laboratories involved in the study. CRFs will consist of paper document collection forms which will then be entered into the database, and will serve as source documents. All electronic study documents will be secured by key and/or password protection.

14 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance.

The Principal Investigators will provide direct access to all study-related field site, source documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The Principal Investigators will ensure all study staff are appropriately trained and current documentations are maintained on site.

DMID-designated clinical monitors will verify that the clinical study is conducted and data generated, recorded, and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The site staff will implement QC procedures with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be clarified and resolved by going back to the source documents and checking with the clinical team that collected the data.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 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).

15.2 Institutional Review Board

The University of Rochester will provide this protocol and the associated informed consent documents for the review and approval of by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless it is in the best interest of the subjects' safety to implement changes prior to approval. The UR IRB operates under U.S. Federal-Wide Assurance (FWA)

Prior to enrollment of subjects into this trial, the approved protocol and the informed consent form will be reviewed and approved by the appropriate IRB. Any amendments to the protocol or consent materials will also be reviewed and approved by the appropriate IRB and submitted to the sponsor. Notification of the IRB's composition, or the IRB's Federal-wide Assurance number, will be provided.

Should amendments to the protocol be required, the amendments will be written by the PI for submission to the IRB and also submitted to the sponsor. The site will submit to the sponsor a copy of the IRB letter of approval of the amendment.

15.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. At the time the study staff will seek informed consent, the study worker will ask the eligible participant (or the participant's representative) if the participant is literate. If the eligible participant reports he or she is not literate, then the study staff will request that a witness be present while the study worker reads and explains the study and what participation will entail. If the eligible subject accepts to take part in the study, he or she will make a mark on the signature line of the consent form. The witness will also sign and date the form, if the witness is confident that the participant

has understood the explanation and is participating willingly. In addition, the witness will complete the date line for the participant.

Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants should have the opportunity to discuss the study with their families or think about it prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants 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.

15.3.1 Informed Consent/Assent Process (in Case of a Minor)

Appropriate consent will be obtained from all subjects prior to any study related procedures. Adults will sign informed consent, while adolescents ages 13 to 17 will sign an informed assent document, and consent will be obtained from a parent or legal guardian. In rare cases where a subject turns 18 during the course of the study, informed consent will be obtained at the next study visit. Consent from a parent or legal guardian will be obtained for children 9 to 12 years of age. Children 9-12 years of age will give verbal assent.

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

Because pregnancy could alter the immune response to vaccination, known pregnancy is an exclusion to participation. Otherwise there are no exclusions of special populations.

15.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological specimens in addition to the clinical information relating to participants.

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, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. A representative from the University of Rochester IRB may also have access to the subject's record.

15.6 Study Discontinuation

If the study is discontinued, enrolled subjects will continue to be followed for safety assessments.

15.7 Future Use of Stored Specimens

Subjects must give permission to keep any remaining specimen for possible use in future research studies, such as testing for antibodies against other viruses or bacteria, in order to participate. Samples will be stored under this protocol (16-0101) at the local site and will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality. Such testing may be performed by collaborating laboratories located at other sites.

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, but subject's samples may be kept with the study records or in other secure areas.

16 DATA HANDLING AND RECORD KEEPING

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data using black ink to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed out with a single line, initialed and dated. The original text will not be erased, overwritten, or altered with correction fluid or tape on the original.

16.1 Data Management Responsibilities

All source documents and laboratory reports will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete.

Data collection is the responsibility of the clinical study staff at the site under the supervision of the clinical PIs. During the study, the investigators will maintain complete and accurate documentation for the study.

16.2 Data Capture Methods

Clinical data will be initially recorded on paper source documents, and then transferred to electronic case report forms (eCRF) within BLISS. Source documents will be retained for monitoring purposes in a secure location. Laboratory data will be directly entered into BLISS from laboratory notebooks

As detailed in the CEIRS contract, overall CEIRS data sharing will adhere to the following schedule:

- Sequence data: provided to Data Processing Coordinating Center within 45 days
- Surveillance data: provided to Data Processing Coordinating Center within 12 months
- Virus phenotypic data: provided to Data Processing Coordinating Center within 12 months
- Basic research data: provided to Data Processing Coordinating Center within 2 months post publication.

16.3 Types of Data

Data for this study will include subject demographics, clinical data, safety assessments, and research laboratory results including antibody responses and measurements of B cell responses.

16.4 Timing/Reports

The final report will include a comprehensive analysis of the data.

16.5 Study Records Retention

Records and documents pertaining to the conduct of this clinical study, including CRFs, source documents, and consent forms must be retained by the investigator for at least 2 years following the date of completion of the study. No study records will be destroyed without prior authorization by DMID. These documents should be retained for a longer period, however, if required by local regulations.

16.6 Protocol Deviations

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

These practices are consistent with ICH E6:

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

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. A line listing of deviations will be reported to DMID on a monthly basis.

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

17 PUBLICATION POLICY

Following completion of the study, the research investigators will share data as defined in the contract and as directed by the CO and COR.

18 LITERATURE REFERENCES

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19 SUPPLEMENT/APPENDICES

Table of procedures by visit and study day								
	V1	V2	V3		V4			
Procedure	D0	D7	D28	*D48, D62, D76*	D90	*D104*	*D118, D132, D146, D160, D174, D188*	Flu** Illness
Informed Consent	X							
Medical History	X							
Interim History		X	X		X			X
Concomitant Meds	X	X	X		X			X
Targeted physical exam*	X	X	X		X			X
Review Enrollment Criteria	X							
Peripheral blood (mL)***	38	38	38		38			
Administer Vaccine	X							
30 minute observation	X							
Review Adverse Events	X	X	X					
Flu surveillance phone call				*X*		*X*	*X*	
NP swab for flu PCR								X
*As indicated by symptoms or history Visits in *italics* occur only during influenza season (see protocol for definition) ** Subjects reporting flu-like symptoms will be asked to make an influenza visit *** Blood drawing in children will not exceed 3 mL/kg in any 8 week period								