Protocol Cover Page

Official Title: Evaluation of Human Immune Responses to Influenza Virus Vaccination in Healthy Volunteers

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Evaluation of Human Immune Responses to Influenza Virus Vaccination in Healthy Volunteers

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Protocol Version 6.0

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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 clinical study 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.

Signed: _____ Date_____
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Signed: _____ Date: _____
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** The protocol should be signed by the local investigator who is responsible for the study implementation at his/her specific site.*

Principal Investigator (Administrative Site):

Signed _____ Date: _____
Walter A. Orenstein, M.D.
Principal Investigator, Emory University, Emory
UGA CEIRS

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List of Abbreviations

ACTSI	Atlanta Clinical & Translational Science Institute
AE	Adverse Event
CDC/VIS	Centers for Disease Control and Prevention/Vaccine Information Statement
CFR	Code of Federal Regulations
CIERS	Center for Influenza Excellence in Research and Surveillance
CoC	Certificate of Confidentiality
CPM	Clinical Project Manager
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
eCRF	Electronic CRF
FDA	Food and Drug Administration
FISMA	Federal Information Security Management Act
GCP	Good Clinical Practice
GCRC	General Clinical Research Center of Emory University
HA	Hemagglutinin
HHS	Health and Human Services
HI	Hemagglutination Inhibition
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IDS	Investigational Drug Services
IMGT	Immunogenetics Information System
IM	Intramuscular
IRB	Institutional Review Board
Ml	Milliliter
MO	Medical Officer
MOP	Manual of Procedures
NA	Neuraminidase
NGS	Next Generation Sequencing
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NSAID	Non-steroidal anti-inflammatory drug
PHI	Protected Health Information
QIV	Quadrivalent inactivated vaccine
RedCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
TIV	Trivalent Inactivated Vaccine
US	United States
VAERS	Vaccine Adverse Event Reporting System
WNL	Within Normal Limits

Title: Evaluation of Human Immune Responses to Influenza Virus Vaccination in Healthy Volunteers

Population: Male and female individuals aged 18-49; target enrollment is up to a total of 70 subjects.

Number of Sites: 1

Study Duration: 6 years

Participant Duration: 180 days (6 months)

Objectives:
Primary Objectives:
To characterize HA-specific plasmablasts and memory B cells after influenza vaccination

Secondary Objective:
To investigate the longevity of humoral immunity to influenza virus in humans

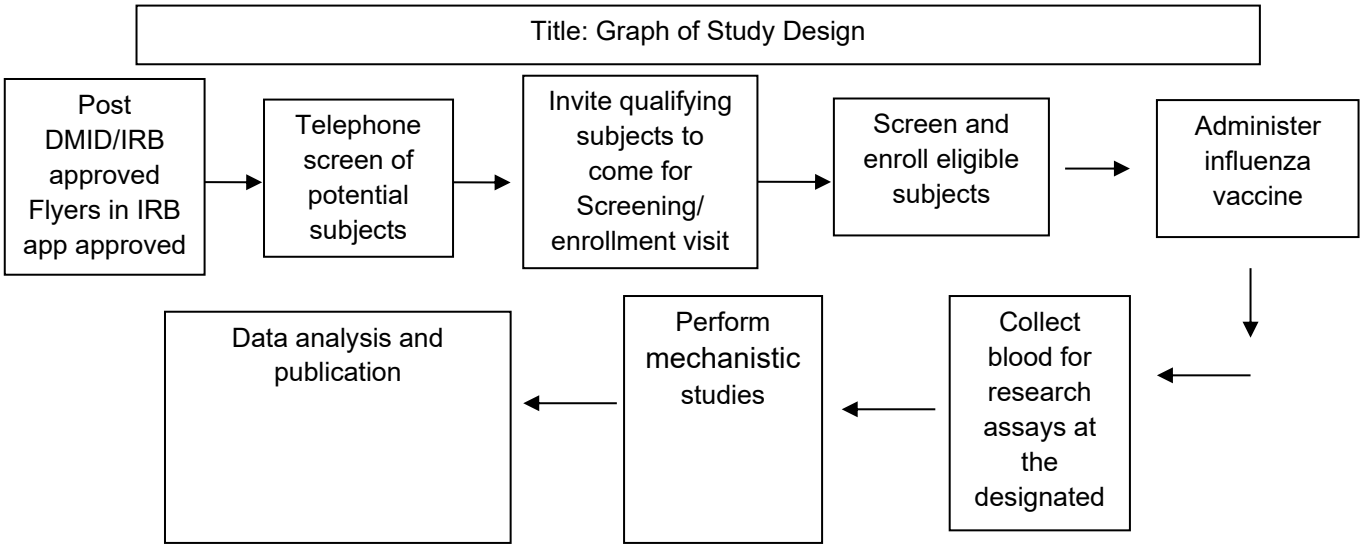
Study Summary:
This is an open label single arm observational study of longitudinal immunologic responses to influenza vaccine in healthy adult subjects. This study will enroll males and non-pregnant females, 18-49 years old. The subjects will be screened at enrollment with a history and physical exam and laboratory testing to ensure they are healthy enough to participate. Qualifying subjects will be vaccinated with an FDA approved seasonal inactivated influenza vaccine (IIV) according to the package insert. Approximately 450 ml of blood will be collected for the research assays during the course of the study. Specifically, 16 ml will be collected for screening; 48ml will be collected at enrollment; 96ml will be collected at visit days 7 and 14; and 64 ml will be collected at 28, 90, and 180 days post vaccination.

Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020. New enrollments were placed on hold for this study. Follow-up visits were also halted, which impacted the timing of participants' subsequent follow-up visits. For this study, there are participants whose Day 180 visits were impacted. We will request an expansion of the study window for the participants whose visits were impacted. Additional details regarding this are detailed further in the protocol. None of the other visits were impacted.

The study will enroll 10 healthy volunteers per vaccination season in years 1, 2, and 4. For years 5 and 6, we anticipate enrolling a maximum of 20 participants for a total enrollment of up to 70

subjects. Individuals who complete the study will be given the option to re-enroll in subsequent years as long as they continue to meet all inclusion/exclusion criteria. Re-enrolling subjects will be re-consented, given new subject identifiers, and counted towards the enrollment number goal for each year of participation.

Schematic of Study Design:



Clinical Group	Numbers	Blood Draws Timepoint (day)						
		Screen	0	7	14	28	90	180
Healthy Controls receiving inactivated influenza vaccine	10/year Years 1, 2, and 4 Maximum of 20/year for Years 5 and 6	16 ml	48ml	96ml	96ml	64ml	64ml	64ml

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Annual influenza virus epidemics by subtype A viruses (H1N1 and H3N2), and/or influenza B viruses have been shown to cause substantial morbidity and mortality, exacting an average of 36,000 deaths and more than 200,000 hospitalizations in the US alone (1).

To combat this important public health threat, two types of inactivated influenza vaccines have been approved by the FDA: 1) a trivalent, inactivated vaccine (TIV); and 2) a quadrivalent inactivated vaccine (QIV). The TIV vaccine contains the hemagglutinin (HA) and neuraminidase (NA) proteins corresponding to the three influenza viruses predicted to be in circulation during the influenza season (two A strains and one B), and the quadrivalent type contains the HA and NA for an additional B strain. These vaccines induce predominantly HA-specific and NA-specific antibodies that are capable of virus neutralization. Antibodies to HA are critical to provide immune protection to the host against influenza virus infection, as this protein plays a key role in virus entry to initiate the replication cycle in the cell. A live attenuated vaccine has also been approved but this study will use only inactivated influenza vaccine.

Currently, the Advisory Committee on Immunization Practices of the CDC recommends that everyone aged 6 months or older in the United States receive the influenza vaccine on an annual basis (2). The recommendation for annual vaccination, regardless of previous vaccination history, is in part due to the constantly changing nature of the influenza strains in circulation. Through the processes of antigenic drift and antigenic shift, the viral HA protein is constantly changing to evade the antibody response. This requires that the formulation of the influenza vaccine must be updated each season. However, when the vaccine formulation remains unchanged from year to year, re-vaccination is still recommended because antibody levels decline over the course of a year (3).

In recent years it has become apparent that a significant fraction of the human antibody response to influenza targets highly conserved neutralizing epitopes on the virus (4). This raises the possibility that an optimally designed vaccine could elicit a broad immunity to the virus. If long lived antibody responses could be generated against these neutralizing epitopes through immunization, it could eliminate the need for yearly re-vaccination. The development of such a vaccine will therefore require an understanding of the factors that lead to the generation of long-lived antibody responses.

2.2 Scientific Rationale

The challenge of developing a broadly protective influenza vaccine stems from three main factors: first, the ever-evolving nature of influenza viruses which allows the virus to overcome pre-existing immunity; second, the fact that the majority of vaccine-elicited neutralizing antibodies are directed against epitopes within the hypervariable region of the HA globular head which facilitates the emergence of antibody-escape viral variants; and third, the apparent short half-life of vaccine-mediated protection, in some instances, even against matched virus strains, raising issues about the longevity of vaccine induced immune responses. Therefore, an ideal influenza vaccine should feature two improvements over the existing ones; it should be able to elicit neutralizing antibody responses against conserved HA epitopes to provide broad protection against diverse influenza virus strains and it should be able to induce long-lived antibody responses.

Generation of immunological memory is the hallmark of adaptive immune responses. Influenza vaccines work by activating antigen-specific B cells to proliferate and differentiate into plasmablasts (PBs) that secrete protective antibodies and memory B cells (MBCs) that can rapidly proliferate and differentiate into PBs upon re-encountering the immunizing antigen. A subset of the plasmablasts migrate to the bone marrow and become long-lived plasma cells (LLPCs) secreting antigen-specific antibodies for protracted periods of time. Memory B cells and long-lived PCs provide a remarkably stable immunological memory that can persist over 50 years after vaccination in humans. It remains unclear what factors direct the differentiation of activated, antigen-specific B cells into either memory B cells or plasmablasts and long-lived plasma cells. Studies in mice have suggested that the B cell repertoire in the memory B and the long-lived plasma cell compartments differ in terms of antigen-binding affinity and breadth. In this study, we will address the fundamental question of what determines the fate (plasmablasts vs. memory B cells) of antigen-specific B cells following vaccination in humans.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The physical risk of drawing blood is local pain and bruising at the site of venipuncture. Qualified phlebotomists or designee will draw blood samples. Care will be taken to obtain these specimens in a safe and hygienic manner. A small number of people experience lightheadedness or fainting. There is a slight risk of infection. Repeated blood drawing may be associated with iron deficiency anemia. To avoid this risk, no more than 432 ml of blood will be obtained over a period of 180 days.

The side effects from inactivated influenza vaccine are generally mild. They include soreness at injection site (10-64% of subjects) that lasts <2 days. When the vaccine is given, the subject may feel a slight pain and burning during the injection. Fever, malaise and myalgia can occur after vaccination with inactivated influenza vaccine. These reactions begin 6-12 hours after vaccination

and can persist for 1-2 days. Allergic reactions rarely occur after influenza vaccination and are generally caused by residual egg proteins. Persons with a history of egg allergy will be excluded from study participation. Associated with the 1976 swine influenza vaccine, there is a slight risk of Guillain-Barre syndrome with the administration of the seasonal inactivated influenza vaccine. Persons with a history of Guillain-Barre syndrome will be excluded from study participation.

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

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

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

During the swine influenza (H1N1) vaccine campaign of 1976, some recipients developed a paralytic illness called Guillain-Barré syndrome (GBS). GBS is an acute inflammatory neuropathy characterized by weakness, hyporeflexia or areflexia, and elevated protein concentrations in cerebrospinal fluid. The rate of GBS was significantly increased in individuals receiving the 1976 swine influenza (H1N1) vaccine at about 1 per 100,000 vaccine recipients. This syndrome has not been seen consistently with other influenza vaccines. Most persons who develop GBS recover completely, although the recovery period may be as little as a few weeks or as long as a few years. About 30% of those with GBS still have residual weakness after 3 years and about 3% may suffer a relapse of muscle weakness and tingling sensations many years after the initial attack. Intensive surveillance of GBS after administration of inactivated influenza vaccines since 1976 has shown a

slight increase in risk over background cases (more than one additional case of GBS per million persons) following vaccination, typically with onset within 6 weeks after vaccination (44). Interestingly, although vaccination rates have increased in the last 10 years the numbers of reported cases of vaccine-associated GBS have declined (45). A recent study in Canada showed that the 2009 H1N1 vaccine was associated with a small but significant risk of GBS in persons 50 years and older (46). An active, population-based surveillance study conducted during the 2009-2010 influenza season found less than 1 excess GBS case per million doses of 2009 H1N1 vaccine administered – a rate similar to that associated with some previously administered annual influenza vaccines (47-49). Another study using the Medicare system showed an elevated risk of GBS with 2009 monovalent H1N1 vaccination (incidence rate ratio = 2.41, 95% confidence interval: 1.14, 5.11; attributable risk = 2.84 per million doses administered, 95% confidence interval: 0.21, 5.48) (50). An international collaboration study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS (51). It is unknown if the administration of the inactivated A/H7N9 vaccine to be used in this study will result in the incidence of GBS that was seen with the 1976 vaccine product as the mechanism leading to this response has not been completely elucidated.

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

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

2.3.2 **Known Potential Benefits**

There is no guarantee of benefit to subjects who will enroll in this protocol. However, seasonal influenza vaccine is considered beneficial to most subjects, as it generally provides protective immunity against the influenza strains within the vaccine. If vaccine is administered after flu season, there is no benefit to the subject. Data from this study may yield a better understanding of the current body of knowledge describing human infections with influenza viruses.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

Primary Objectives:
To characterize HA-specific plasmablasts and memory B cells after influenza vaccination

Secondary Objective:
To investigate the longevity of humoral immunity to influenza virus in humans

3.2 Outcome Measures

3.2.1 Primary Outcome Measures

- Determine the percentage of subjects achieving seroconversion, defined as the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer > 1:40 or a pre-vaccination HI titer > 1:10 and a minimum four-fold rise in post-vaccination HI antibody titer (day 0, 28).

3.2.2 Secondary Outcome Measures

Investigation of the longevity of humoral immunity to influenza virus in humans will be performed by:

- Measuring influenza hemagglutinin stem-specific and head-specific memory B-cells and serum antibody levels at days 0, 28, and 180.
- For serum antibody responses directed against the HA head or stem regions, we will determine the endpoint IgG titers at days 0, 28 and 180 after vaccination.
  For memory B cells, we will determine the frequency of HA head- or stem-specific IgG-secreting memory B cells per total IgG-secreting cells at days 0, 28 and 180 after vaccination.
- Plasmablast responses against either the HA head or stem antigens will be determined by direct ex vivo ELISPOT at day 7 after vaccination.

- Expression of human influenza-specific monoclonal antibodies from Day 7 plasmablasts and assessing their functional by ELISA, HAI, and virus neutralization assays.

3.2.3 **Exploratory Outcome Measures**

Characterization of HA-specific plasmablasts and memory B cells after influenza vaccination will be performed using samples from the following timepoints:

- Sequencing of antibodies from single cell sorted plasmablasts and deep immunoglobulin sequencing of RNA from bulk sorted plasmablasts and plasma cells will be carried out using day 7 samples, and the following assays will be performed:
    - Cloning of human influenza-specific monoclonal antibodies and analysis of strain specificity by ELISA, HAI, and virus neutralization assays.
    - Sequence analysis of influenza-specific antibodies to characterize patterns in VDJ usage, CDR3 region length, and numbers of replacement vs. silent mutations.

- Protein sequencing of serum antibodies which bind to vaccine antigens at different timepoints (day 0, 28, 180) post vaccination.
- Define the gene expression profile of HA-specific plasmablasts and memory B cells (day 7)
- Examine the clonal diversity of HA-specific plasmablasts and memory B cells (day 7)

Investigation of the longevity of humoral immunity to influenza virus in humans will be performed by:

- Identifying which plasmablast clones persist to late time points and examine whether the same plasmablast clones are re-amplified after vaccination.
- Examining associations between specific antibody features (hypermutation level, antigen binding affinity, cross reactivity to previous vaccine strains) and the likelihood of long-term persistence in each compartment.

Using proteomics directed cloning to identify the dominant B-cell clones producing influenza-specific and hemagglutinin stalk-specific antibodies in the serum of vaccine recipients and infected subjects.

4 STUDY DESIGN

Longitudinal assessment of the study participants will be made at every study visit. Assessments will be performed at day 0, 7, 14, 28, 90 and 180 days after immunization as outlined in the Schedule of Events (appendix A). Participants will be monitored by the study nurse for the presence of both expected reactions to vaccination and for unexpected adverse reactions for 15 minutes following vaccination. Subject enrollment will be contingent upon the availability of the seasonal flu vaccine and will end upon expiration of the product.

Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020. New enrollments were placed on hold for this study. Follow-up visits were also halted, which impacted the timing of participants' subsequent follow-up visits. For this study, there are participants whose Day 180 visits were impacted. We will request an expansion of the study window for the participants whose Day 180 visits were impacted. Additional details regarding this are detailed further in the protocol. None of the other visits were impacted.

The clinical study is designed to characterize the specific immunologic response to influenza vaccination of humans using commercially available influenza vaccine. This study will compare and contrast the response of individual components of the immune system using licensed seasonal inactivated influenza vaccine. This vaccine is FDA approved for protection against influenza infection but may have different effects on different components of the immune system. The enrollment goal is up to a total of 70 subjects. The subjects will have blood drawn at designated time points to have *ex-vivo* analysis of specific immunologic responses measured. Blood will be drawn at screening, day 0, 7, 14, 28, 90 and 180 study visits.

Due to the COVID-19 pandemic, there are participants whose Day 180 visits were impacted. We are requesting that the window for the day Day 180 visit be expanded by 90 days. Day 180 is the final visit/study endpoint for this study.

5 STUDY POPULATION

The study will enroll 10 healthy volunteers per vaccination season in years 1, 2, and 4, to analyze the immune response to influenza vaccination in healthy individuals. For years 5 and 6, we anticipate enrolling a maximum of 20 participants, to analyze the immune response to influenza vaccination in healthy individuals.
Total enrollment goal is up to a total of 70 subjects. Individuals who complete the study will be given the option to re-enroll in subsequent years as long as they continue to meet all inclusion/exclusion criteria. Re-enrolling subjects will be re-consented, given new subject identifiers, and counted towards the enrollment number goal for each year of participation.

- Males and non-pregnant females confirmed to be non-pregnant immediately prior to vaccination, aged 18 - 49, inclusive.

- Study subjects will be drawn from existing populations of students, staff, faculty, or volunteers at Emory University or other eligible healthy adults who may hear of the study by word of mouth.

- Study subjects will be recruited via word of mouth and placement of recruitment flyers in key locations on campus. Social media messaging via Facebook and Twitter will also be used in an effort to increase the number of enrollees into this study.

5.1 Inclusion Criteria

For inclusion into the study, a subject must satisfy all of the following criteria:

1. Male or female subjects between 18 and 49 years of age, inclusive
2. Subjects capable of providing written informed consent prior to initiation of any study procedures. Subjects able to understand and comply with planned study procedures and be available for all study visits
3. Screening labs within normal limits per the local laboratory normal ranges or considered to be not clinically significant by the investigator[1]. Normal laboratory ranges are as listed below:
    A. Hematology:
        - Hemoglobin: Male- 12.9-16.1 gm/dL, Female- 11.4-14.4 gm/dL
        - White blood cells (WBC): Male- 4.2-9.2/uL, Female- 4-10/uL
        - Platelet count: 150-400/uL
    B. Chemistries:
        - Kidney function:Glomerular filtration rate (GFR) >/= 60 mL/min/1.73 m^2;

- Liver enzymes: Albumin >/= 3.5 g/dL; ALT <66 U/L; AST <62 U/L

4. Subjects who have not received the seasonal influenza vaccine in the current flu season and are not suspected to have had an influenza infection in the current flu season.
5. Female subjects of child bearing potential must have a negative urine pregnancy test at the screening visit, enrollment visit and all subsequent study visits longer than 14 days since the last pregnancy test.

5.2 Exclusion Criteria

Subjects with any of the following will be excluded from the study:

1. Known infection with HIV, HCV, or HBV. This information will be obtained verbally from the patient.
2. If female, active pregnancy or breast-feeding or plans to become pregnant during study participation.
3. Chronic medical conditions that cause immunodeficiency or that require medications which could alter immune function such as immunosuppressants and immunoenhancers.
4. Have any medical disease or condition that, in the opinion of the site principal investigator or appropriate sub-investigator, is a contraindication to study participation. This includes any chronic medical disease or condition, defined as persisting 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 or the subject's successful completion of this study
5. Have an acute illness, as determined by the site principal investigator or appropriate sub-investigator, within 72 hours prior to study vaccination. An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site principal investigator or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.
6. Persons taking anticoagulants, long-term aspirin therapy, or long-term systemic steroids (greater than 3 months in the past 12 months and any within 30 days).
7. Have known hypersensitivity or allergy to eggs, egg or chicken protein or other components of the study vaccine;
8. Have a known latex allergy;

9. Have a history of severe reactions following previous immunization with licensed influenza virus vaccines
10. Have a history of Guillain-Barre syndrome
11. Subjects who had or are suspected to have had an influenza infection in the current influenza season
12. Subjects who, at screening, have abnormal vital signs and/or physical exam, including a temperature ≥ 38.0 C, Systolic blood pressure ≤90 or≥160 mmHg, pulse ≤ 60 or > 110 beats per minute, new rash, signs of infection.
13. Subjects who have already received the seasonal influenza vaccine in the current influenza vaccination season.

5.3 Handling of Withdrawals

Subjects are free to withdraw from the study at any time. Subjects are provided a revocation form at enrollment with withdrawal procedures clearly explained. Subjects who have developed an adverse event or serious adverse event will be followed for safety purposes. Subjects who develop any exclusion criteria will not continue to have blood drawn. Subjects may be taken off the study without their consent if the study doctor determines that it is in his or her best interest not to continue to participate in the study, if the subject is unable to complete the required study procedures, or if the study is stopped by the Institution, the sponsor, or the Food and Drug Administration (FDA) or other health authorities. If the subject is removed from the study, the Principal Investigator or designee will contact the participant to discuss the study stopping procedures.

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after study enrollment visit will not be replaced.

5.4 Termination of Study

The sponsor reserves the right to terminate the study at any time for clinical or administrative reasons.

6 STUDY INTERVENTION PRODUCT

6.1 Study Product Description

Each flu season, the vaccine used in this study will be a licensed seasonal inactivated influenza vaccine that is approved by the FDA for the current flu season. The vaccine product used will depend upon supply stocked by the Emory University Hospital Investigational Drug Services (IDS). These vaccines are typically provided in pre-filled syringes (clear syringe plunger rod), 0.5 ml. If pre-filled syringes are not available from the manufacturer, the study product will be provided in a latex free vial, 5 ml, one vial per carton.

6.1.1 Acquisition

The licensed vaccine will be provided by the Emory University Hospital Investigational Drug Pharmacy and will be stored in a secure temperature-monitored refrigerator in a secure, limited access storage area.

6.1.2 Formulation, Packaging, and Labeling

The vaccine used in this study is the licensed seasonal inactivated influenza vaccine and will be supplied by Emory University Hospital Investigational Drug Services (IDS) in a pre-filled syringe placed in a clear plastic bag labeled with the DMID protocol number, study ID, and dosing instructions per the prescription signed by Dr. Mehta or a designee.

6.1.3 Product Storage and Stability

Per the package insert from the manufacturer, the seasonal inactivated influenza vaccine will be stored at 2-8 degrees C (35-45 degrees F) in a study specific designated location in a refrigerator at the Emory University Hospital Investigational Drug Services. The refrigerator temperature is monitored via the Temp Trak system, an electronic temperature monitoring system that records temperatures every five minutes 24 hours a day. The refrigerator is set to alarm at 2.5 degrees C or 7.5 degrees C. If there is a deviation in temperature, the Temp Trak system will notify the Emory IDS pharmacist via an alarm system.

6.2 Dosage, Preparation and Administration of Study Intervention Product

Upon receipt of the signed prescription, an Emory IDS pharmacist will prepare the study drug and, per standard operating procedure, will have a 2nd pharmacist double check the study prescription with the study product and label before dispensing the drug to the clinical research nurse for administration. An Emory IDS pharmacist or pharmacy technician will walk over the study product to the clinic procedure area and then deliver it to the research coordinator. The pharmacist or pharmacy technician will hand the subject's medication to the clinical research nurse who will then verify the correct protocol, subject name and study ID, study product, dosing instructions per signed prescription, and study product expiration date using a checklist.

A vaccine dose of 0.5 mL will be administered by qualified personnel at the GCRC intramuscularly (IM) in the subject's preferred arm per the manufacturer's instructions.

6.3 Accountability Procedures for the Study Intervention Product(s)

The Emory Investigational Drug Service will maintain and monitor the inventory of the influenza vaccine per their standard operating procedures (SOPs).

6.4 Assessment of Participant Compliance with Study Intervention Product

Each participant will be assessed for compliance with the influenza vaccination via his or her receipt of 0.5ml of vaccine without immediate reaction.

6.5 Concomitant Medications/Treatments

Subjects with chronic medical conditions or taking medications that are prohibited according to Section 5.2 Exclusion Criteria will be excluded from study participation. The medication history will be reviewed and updated at every study visit to ensure that subjects are not taking the medications prohibited for study participation. Subjects will be allowed to take anti-pyretics (e.g. acetaminophen) and/or non-steroidal anti-inflammatories (NSAIDs; e.g. ibuprofen) after vaccination if desired by subject.

7 STUDY SCHEDULE

7.1 Screening (Day -1 to day -30)

Potential participants will be screened for eligibility within 30 days prior to the administration of the study product. Screening period begins at screening visit and continues through enrollment visit. Qualified subjects will be scheduled for enrollment visit 1-day post screening visit through 30 days post screening visit. At screening, the following activities will be performed:

- Participants will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures, including any screening procedures.
- Complete medical history will be obtained by interview of participants to assure eligibility, including history of prior influenza vaccinations.
- All concomitant medications will be recorded on the appropriate data collection form.
- Demographic information will be collected from the participant.
- Eligibility criteria will be reviewed.
- A urine pregnancy test will be performed on all female participants of childbearing potential and must be negative.
- Approximately 16 ml (about 1 tablespoon) of venous blood will be collected for chemistry and hematology performed by the site laboratory. The results will be provided by the site laboratory and be reviewed by the principal investigator.
- Targeted physical exam, including height, weight and vital signs (oral or axillary temperature, pulse, blood pressure.).

7.2 Enrollment/Baseline

Day 0 – Baseline and vaccination

- Reconfirm participant's willingness to take part in the study prior to performing any study procedures.
- Eligibility criteria, including results of all clinical screening laboratory evaluations, will be reviewed to assure continued eligibility prior to vaccination.
- All concomitant medications taken since the screening visit will be recorded on the appropriate data collection form.
- Vital signs, including oral or axillary temperature, pulse, and blood pressure, will be obtained to assure eligibility.
- In the event that the enrollment visit exceeds 14 days from screening visit, a urine pregnancy test will be performed for all females of childbearing potential, to confirm non-pregnant status.

- Approximately 48 ml of blood (3 tbsp) will be drawn from the subject prior to vaccination
- A seasonal inactivated influenza vaccine will be administered by an intramuscular injection into the deltoid muscle of the subject's preferred arm.
- The subject will be monitored for 15 minutes after vaccination to ensure that no acute reactions occur.

- Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020. New enrollments were placed on hold for this study.

7.3 Follow-up and Final Visits

Follow-up visits: Days 7, 14, 28, 90, 180.

- Study participants will be asked to return for five follow up visits, with the respective window periods, per the table below:

Time point	Day	7	14	28	90	180
	Window	+/- 1	+/- 2	+/- 3	+/- 10	+/- 14 (a 90 day window expansion is being requested)
Blood Volume		96mL	96mL	64mL	64mL	64mL

- Medical history will be reviewed and updated as appropriate.
- All concomitant medications taken since the previous 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.
- Approximately the amount of venous blood, as indicated by the table above, will be collected for research assays.
- Targeted physical exam will be done only as dictated by a change in medical history.
- A urine pregnancy test will be performed on all female participants of childbearing potential

for any visit longer than 14 days from the last pregnancy test..

- Follow-up visits were also halted, which impacted several participants' Day 180 follow-up visit.
- We are requesting that the windows for the Day 180 visit be extended by 90 days.
- Extending the window for the Day 180 follow-up visits would allow samples from participants whose visits were halted at this time point to be collected once the Emory restrictions regarding COVID-19 are lifted and staff can reasonably collect samples, dependent upon when the University and applicable Unit and School allow. The scientists feel that the samples, even if a few months late, will greatly help evaluate the longevity of the responses to flu vaccines.
- Study activities will not be changed, just the timing of the Day 180 visit.

7.4 **Early Termination Visit**

There are no special or routine evaluations to be done at an early termination visit; subjects will not be asked to come in for an additional visit if they decide to end their participation in the study or the study is terminated. If the study is terminated early, the subject will be informed via telephone of the reason for early termination.

7.5 **Unscheduled Visit**

Unscheduled visits are not anticipated with this study, but may occur at any time during the study. Any of the following activities may be performed:

- Medical history will be reviewed 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.
- Vital signs, including oral or axillary temperature, pulse, blood pressure and will be obtained.
- A targeted physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Approximately 10 mL of venous blood will be collected for chemistry and/or hematology performed by the local or site laboratory if deemed necessary by Dr. Mehta or a designee.

- A urine pregnancy test will be performed on all female participants of childbearing potential.

8 STUDY PROCEDURES AND EVALUATIONS

8.1 Clinical Evaluations

- Clinical interview of subject at screening to include:
    - Demographics: age, date of birth, gender, ethnicity, race
    - History of allergies: food, drug, environmental, specifically solicit if any allergy to vaccines or eggs
    - History of influenza vaccination, and previous reactions, if any
    - History of Guillain-Barre syndrome
    - History of medications taken within the past 90 days to include prescription and over the counter medications, specifically solicit if any blood thinners or immunosuppressive agents have been used
    - History of any vaccinations within the past year
    - Acute or chronic medical conditions as reported by subject
- Vital signs (blood pressure, pulse, temperature, height, weight)
- Targeted physical exam
- Collection of blood for clinical laboratory tests at screening visit (16 ml)
- Collection of research blood samples at study visits: day 0 (48 ml), 7, 14 (96 ml), 28, 90 and 180 (64 ml).
- Administration of licensed seasonal inactivated influenza vaccine by the clinical research nurse at Day 0
- Clinical health assessment at follow up study visit to include inquiry regarding any illness, any change in medication, and any overall health changes since last study visit.
- A urine pregnancy test will be performed on all female participants of childbearing potential and must be negative.

8.2 Laboratory Evaluations/Assays

The participating laboratories are Emory Medical Laboratories.
The following clinical laboratory evaluations will be performed in the study:

- Urine Pregnancy Test for women of childbearing potential

- Hematology: hemoglobin, white blood cells (WBC), platelet count.[Acceptable ranges: WBC: Male- 4.2-9.1/uL, Female- 4-10/uL; Hemoglobin: Male- 12.9-16.1/dL, Female- 11.4-14.4/dL; Platelet 150-400/uL]
- Chemistries: creatinine, liver enzymes [Acceptable ranges: calculated Glomerular filtration rate (GFR) >/= 60 mL/min/1.73 m^2; Albumin >/= 3.5 gm/DL; ALT <66 u/L; AST <62 u/L]

Research Laboratory Assays:

- Innate responses in the blood and mucosal sites: The serum analyses of cytokines and chemokines associated with innate immunity will be determined by multiplex analysis,, including IP-10, Th1 cytokines (IFN-g, TNF, IL-2), Th2 (IL-4, IL-5, IL-13) and regulatory (IL-10) cytokines. Flow cytometric analysis of innate cell populations (dendritic cell subsets, monocyte subsets, NK cell subsets and innate lymphoid cells) will be performed using antibody panels routinely in use in the research laboratory
- B cell and CD4 T cell responses: The following influenza virus-specific responses will be analyzed in the blood after vaccination:
  - Serum antibody*:* We will measure the HAI titers, neutralizing antibody responses, and the influenza-specific serum antibody levels (IgG, IgA, and IgM) using ELISA at all time-points.
  - Plasmablasts: The influenza-specific plasmablast response will be quantitated using both ELISPOT and flow cytometry at day 7 post-vaccination.
  - Memory B cells: The kinetics of the IgG memory B cell influenza-specific response will be quantitated using an established assay.
  - CD4 T cells: The HA-specific CD4 T cell responses will be quantitated at each time point by ex vivo stimulation using overlapping HA peptides or HA protein of the different influenza strains present in the vaccine. The following cytokines will be analyzed using ICS or ELISPOT: Th1 cytokines (IFNg, TNF-a, and IL-2); Th2 cytokines (IL-4 and IL-10); and T_{FH} cytokines (IL-21 and IL-4). We will put a specific focus on analyzing CD4 T follicular helper (T_{FH}) responses since these cells specialize in B cell help and are critical for the formation and maintenance of germinal centers. The germinal center reaction, in turn, is necessary for the generation of high affinity antibody responses and memory B cells. In addition, T_{FH} cells secrete the cytokine IL-21 that is essential for generating long-lived plasma cells and sustaining antibody responses. Therefore the generation of long-term humoral immunity is critically dependent on CD4 T_{FH} cells.

8.2.1 **Specimen Collection, Preparation, Handling and Shipping**

Samples will be drawn, labeled and logged by a skilled clinical research nurse or phlebotomist, under the supervision of the principal investigator and in accordance with site specific SOPs. Checklists will be utilized to verify appropriate sample collection, transportation, processing, and storage.

Subjects consented to participate in this study will also be asked to consent to storage of any unused blood for future assays. If new studies are identified, the de-identified samples of subjects who have consented to storage of their unused blood will be utilized.

8.3 Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

For subjects with values in the clinically significant range, the lab abnormalities will be explained to the study participant by the principal investigator or a designee. The designee may be clinical study personnel (Clinical Research Coordinator, Study Coordinator or Research Nurse) as indicated on the Delegation of Authority log. The subject will be given a copy of his or her abnormal lab report and instructed to follow up with his or her primary health care provider or health clinic. Abnormal clinical values obtained from screening labs, which are done prior to treatment, will be considered to render the subject a screen failure and will not considered to be adverse events. Any subject with Lab values outside of clinical range will be informed of the value and referred to their PCP for follow up.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Only 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.

For the purposes of this study, reportable adverse events are limited to any occurrence or worsening of an undesirable or unintended sign, symptom or disease that is specifically associated with a study procedure that is not part of the normal standard of care for the participant. As previously mentioned, there are minimal risks associated with venipuncture for obtaining research related labs. Foreseeable adverse events associated with venipuncture are: mild, temporary discomfort at the venipuncture site, bruising, and phlebitis. Very rarely, subjects may experience "vaso-vagal syndrome" during phlebotomy. Vaso-vagal reactions may include diaphoresis, nausea, syncope and rarely loss of consciousness.

Anticipated adverse events related to administration of the inactivated influenza vaccine include: localized erythema, induration, swelling, itching and/or pain at the injection site, mild fever, myalgia, headache and malaise following vaccination. Since immunization with flu vaccine annually is offered as standard of care to healthy adults to prevent influenza infection, these anticipated reactions to the vaccine will not be considered reportable adverse events. Illness requiring hospitalization as deemed related to vaccination will be reported as an adverse event.

Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine will be considered an adverse event for the study.

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

9.2.1 Adverse Events

Any adverse events occurring as a result of obtaining blood samples will require clinical review, monitoring and treatment as needed until resolution or stabilization, and assessment for severity and relatedness. Subjects will be asked about final resolution of symptoms upon completion of each visit.

Adverse Event:

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation participant 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 product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to stabilization or resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it will be recorded as an AE.

All AEs will be graded for severity and relationship to study product.

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

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

- Mild: events require minimal or no treatment and do not interfere with the subject'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 subject'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 subject 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.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent will require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician will assess an AE's relationship to influenza vaccine as part of the documentation process, but it is not a factor in determining what is or is not reported reported. All AEs will have their relationship to study product assessed using the terms: related or not related using the following guidelines:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

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

Adverse events will be followed until resolved or considered stable. Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine will be considered an adverse event for the study. Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination found using the following link http://vaers.hhs.gov/resources/VAERS_Table_of_Reportable_Events_Following_Vaccination.pdf that occurs within the specified time period after vaccination or, if in the judgment of the PI, any unexpected reaction to the vaccine will be reported to VAERS with a copy faxed or emailed to the DMID CPM and MO at time of VAERS reporting. These reports will be made using the timelines outlined at https://vaers.hhs.gov/resources/VAERS_Table_of_Reportable_Events_Following_Vaccination.pdf.

All adverse events that meet the reporting requirements of the Institutional Review Board will be reported to the IRB within 10 days of the investigator learning of the event as delineated by the requirements listed on the Emory IRB website at: http://www.irb.emory.edu/documents/AE-PD-UPchart.pdf. These events will also be faxed or emailed to the Clinical Project Manager at time of IRB reporting. Per these guidelines, these adverse events would include those that are unanticipated, related to the study intervention and involve risk to the study participant

All Adverse events not meeting the reporting requirements of the Emory IRB will be reported to the clinical project manager periodically in monthly clinical reports and to the IRB periodically at annual continuing reviews.

9.2.2 Serious Adverse Events

Serious Adverse Event (SAE): Serious Adverse Events will be collected from the time the participant begins the study until study completion, or until the participant withdraws from the study. SAE's will be documented on the Adverse Event form and reported to the DMID CPM and MO.

- All deaths and life-threatening events, regardless of relationship will be recorded on the SAE form and sent by fax to DMID CPM and MO within 24 hours of site awareness of the death or life-threatening event.
- All other SAEs, regardless of relationship will be recorded on the SAE form and sent by fax within 72 hours of becoming aware of the event.

A Serious Adverse Event is defined as an AE meeting one of the following conditions:

- Death during the period of protocol defined surveillance
- Life Threatening Event (defined as a subject at immediate risk of death at the time of the event)

- An event requiring 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 a serious adverse experience 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.

Events **not** considered to be serious adverse events are hospitalizations for the:

- routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen
- treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission

All SAEs will be followed until satisfactory resolution or until the PI or co-investigator deems the event to be chronic or the subject to be stable. All SAEs will be reported to the Vaccine Adverse Event Reporting System (VAERS).

The DMID Medical Officer will review and assess the SAE for regulatory reporting and potential impact on the study subject safety and protocol conduct.

9.2.3 Other Adverse Events (If applicable)

Not Applicable

9.3 Reporting Procedures and Safety Oversight

This is a minimal risk study. Due to its nature very few, if any, serious adverse events are likely to be chronologically or causally related to study procedures, and any adverse events that do occur are likely to be mild, transient, and self-limiting. Therefore, adverse events, including serious adverse events, will be collected and reported to the IRB/IEC and DMID except for anticipated reactions noted in Section 9.2.1, an Independent Safety Monitor will not be required, and a formal Safety Monitoring Committee will not be constituted. All Adverse events will be communicated to the DMID and to the IRB as delineated by Emory Internal Review Board reporting requirements stated in section 9.2.1 and 9.2.2.

9.3.1 Reporting of Pregnancy

A negative urine pregnancy testing is required for all women of childbearing potential prior to participating in the study. A urine pregnancy test will be performed at the screening visit and subsequently at any visit if the visit occurs +14 days after the last pregnancy test visit. If a subject becomes pregnant after enrollment in the study or reports it to the research team, the pregnancy will be reported as stated in section 9.3. Pregnant subjects will be withdrawn from the study and pregnancy will be followed for safety monitoring monthly to the point of pregnancy outcome.

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

Clinical studies at Emory University are compliant with ICH E6 guidelines for Good Clinical Practice.

Adverse events will be documented and reported according to the DMID and Emory IRB policies (as described in section 9.2.1 and 9.3). All AEs and SAEs will be followed until resolved, return to baseline or stable as determined by the study team. The occurrence of AEs and the status of existing AEs will be assessed at each study visit and events are recorded in the regulatory binder. Subjects will be encouraged to contact study staff of any health changes that may occur between study visits. An unscheduled visit or telephone follow-up may occur depending on the adverse event.

9.5 Halting Rules

This is a minimal risk study. It is not anticipated that there will be a need to halt the study due to SAEs. The study will be halted if the following occurs:

- One or more participants have laryngospasm, bronchospasm, or anaphylaxis associated with study procedure and occurring within 72 hours post study procedure;
- Any SAE associated with study product or procedure;
- Two or more participants experience the same or similar severe or life-threatening AE judged to be associated with study procedure.

9.6 Safety Oversight

Serious and severe adverse events will be reported to DMID. DMID has the authority to stop study enrollment, vaccinations and procedures if AEs are reported that meet halting criteria.

9.7 Independent Safety Monitor (ISM)

Not Applicable.

10 CLINICAL MONITORING

Purpose: 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.

10.1 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 which provides details for site monitoring. 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. Emory University IRB and other regulatory agencies may conduct study monitoring visits.

11 STATISTICAL CONSIDERATIONS

For this study we will assess immunologic responses in the periphery (blood). These responses contain a variety of immunologic assays with different types of response output. Hence our primary data analytic strategy will be the use of longitudinal data analysis methods to determine the temporal course of a given response measurement and factors associated with heterogeneity in that temporal course. Study outcomes will be measured using numerical data from laboratory assays including flow cytometry and ELISPOT assays. A detailed data management system will utilize general linear mixed models for continuous responses to assess differences among time points.

11.1 Study Hypothesis

To investigate the longevity of humoral immunity to influenza virus in humans. Our overarching hypothesis is that an understanding of how long-term humoral immunity to influenza virus is generated and maintained is essential for the development of a “universal” vaccine against influenza virus. This aim will be accomplished by measuring the total influenza-specific, memory B-cells and serum antibody levels over extended periods following vaccination.

11.2 Sample Size Considerations

In our preliminary studies, we found that vaccine-elicited plasmablasts that are specific to the stalk region of HA (the broadly protective region) were induced in 20% of subjects after receiving the annual inactivated trivalent influenza vaccine. This analysis was done on only 20 subjects and we would like to test the consistency of our observation with a larger sample size of 50.

11.3 Planned Interim Analysis

Not Applicable

11.4 Final Analysis Plan

We will be utilizing the paired Student’s *t*-test to determine whether the observed increase in serum antibody titers from baseline to 4 weeks after vaccination is significant or not. We do not anticipate any major change in our analysis strategy. For all of this analysis we will work the Emory/UGA CEIRS Data Management Team headed by Dr. Hertzberg using the approved Data Management Plan for this contract.

12 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 serve as source documents. Laboratory reports will serve as source documents for lab results required at screening. All study documents will be secured by key and/or password protection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

A Clinical Quality Management Plan (CQMP) has been approved by DMID, for the overall Center and for this specific protocol. The site will conduct the study, generate data and maintain documentation in compliance with the protocol, the CQMP, ICH-GCP and all applicable regulatory requirements. All source documents will be reviewed by the study team. Adverse events will be assessed for severity and graded in accordance with that scale (mild, moderate, severe, life-threatening) and causality and reviewed by the PI or designee. Study staff will be trained to use the clinical data collection program RedCap which includes tools for QA/QC. The clinical PIs will supervise and oversee the clinical staff on this study, and the center PI will supervise the administrative personnel. During the study, the site will maintain complete and accurate documentation for the study. The Investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data submitted. Research data resulting from this study will be reported to the CEIRS Data Processing and Coordinating Center (DPCC) as defined in the contract agreement.

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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 in real time during study procedures to ensure and document the site's work processes by study staff, to ensure that accepted procedures are followed.

Clinical Quality Management assessment will be will be approached as an ongoing, interactive tool that will address the current version of the protocol.

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 Emory University IRB will review and provide approval of this protocol and the associated informed consent documents, recruitment materials and procedures as required by OHRP and local requirements before subject enrollment. The Emory University IRB currently holds and will maintain a US FWA issued by OHRP for the entirety of the study. Any amendments to the protocol or consent materials will be approved before they are placed in use. An independent ethics committee may review the study at any time.

14.3 Informed Consent Process

Informed consent will be initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects. Consent forms describe in detail the study procedures and risks, and written documentation of informed consent is required prior to enrollment in the study. Consent forms are IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the research nurse will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. They will have ample opportunity to discuss the study or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. 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 is not adversely affected if they decline to participate in, or withdraw from, this study.

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

Not applicable

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

Not applicable

14.5 Participant Confidentiality

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 Emory IRB may also have access to the subject's record.

To protect privacy, the NIH has provided a Certificate of Confidentiality, CoC. With this CoC, the participating sites cannot be forced to release information that may identify the participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The participating sites will use the CoC to resist any demands for information that would identify the participant, except as explained below.

The CoC cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study or local laws, such as for reporting of communicable diseases.

A CoC does not prevent the participant from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive study information, then the participating sites may not use the CoC to withhold that information.

The CoC does not prevent the participating sites from reporting without the participant's consent, information that would identify the subject as a participant in the study regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.6 Protocol Deviations

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

All protocol deviations, as defined above, must be addressed in study subject source documents. A completed copy of the Protocol Deviation Form must be maintained in the Regulatory File as well as in the subject's chart.

It is the responsibility of the principal investigator and other study personnel to use continuous vigilance to identify and report deviations. The principal investigators and other study personnel are responsible for knowing and adhering to their IRB/IEC requirements. Only protocol deviations that are related to subject safety and/or eligibility will be reported to the local IRB/IEC per its

guidelines. Line listings of protocol deviations that are reported to the IRB/IEC will be submitted to DMID on a quarterly basis.

14.7 **Study Discontinuation**

If the study is discontinued, participants will be contacted via telephone and in writing. Due to the nature of this study, subjects are not required to come in for a final visit in the event of study closure and it is not anticipated that an enrolled study participant will require continued follow-up after study closure.

14.8 **Future Use of Stored Specimens**

Subjects who consent to participate in the study will be asked, as part of the informed consent process, to agree to or refuse his/her specimen to be used for future research as noted in the protocol and consent form. This decision can be changed at any time by the participant without penalty.

15 DATA HANDLING AND RECORD KEEPING

The 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.

15.1 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. Research data resulting from this study will be reported to the CEIRS Data Processing and Coordinating Center (DPCC) as defined in the contract agreement.

15.2 Data Capture Methods

Both paper and electronic recording of data will be used in this study. Paper data will be scanned and electronically uploaded to a central clinical database (RedCap) that is password-protected, HIPAA compliant and FISMA compliant.

Exposure data will be captured on CRFs after interviewing the participants. Laboratory data will be recorded on CRFs upon completion of the laboratory analysis. All CRFs will be used as source documents and all data will be entered into one clinical database, RedCap. CRFs may be used as source documents if they represent data collected for the study and are where data were initially recorded.

If data are obtained at a later date (i.e., after the study visit) and are recorded on the CRF as source documentation, it will be signed/initialed and dated.

If data are transcribed from another source onto the CRF, the CRF is not considered to be the original source document and it will not be used as source documentation.

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

- Basic research data: provided to Data Processing Coordinating Center within 2 months post publication

15.3 Types of Data

Data for this study will include safety assessments and research laboratory assays data.

15.4 Timing/Reports

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

15.5 Study Records Retention

Records and documents pertaining to the conduct of this clinical study, including CRFs, source documents, and consent forms will 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 will be retained for a longer period, however, if required by local regulations.

16 PUBLICATION POLICY

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

17 LITERATURE REFERENCES (in section 2.1)

(1) Thompson, WW; Shay, DK; Weintraub, E; Brammer, L; Cox, N, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA.* 2003;289:179–86.3.

(2) Centers for Disease Control and Prevention (CDC). Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices--United States, 2013-2014.*MMWR Recomm Rep.* 2013 Sep 20;62(RR-07):1-43.

(3) Künzel W, Glathe H, Engelmann H, Van Hoecke C. Kinetics of humoral antibody response to trivalent inactivated split influenza vaccine in subjects previously vaccinated or vaccinated for the first time. Vaccine. 1996 Aug;14(12):1108-10

(4) Pica N, Palese P. Toward a universal influenza virus vaccine: prospects and challenges. Annu Rev Med. 2013;64:189-202..

18 SUPPLEMENT/APPENDICES

Appendix A: Schedule of Events

Procedures	Screening (day -30 thru -1)	Day 0	Day 7	Day 14	Day 28	Day 90	Day 180	Unsch. Visit
Study Consent	X							
Demographics	X							
Medical HX/ Medication HX	X							
Height/Weight	X							
Targeted Physical Exam	X							X
Vital Signs	X	X	X	X	X	X	X	X
Clinical/Baseline labs: Hgb, Hct, Plts, GFR, LFT's	X							X
AE/SAE Assessment			X	X	X	X	X	X
Conmeds		X	X	X	X	X	X	X
Pregnancy Testing	X	X		X	X	X	X	X
Enrollment		X						
Peripheral blood draw for research immunity testing		X	X	X	X	X	X	
Administration of Influenza Vaccine		X						

* For oral temperature, participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking temperature

** for women of child-bearing potential for whom enrollment visit occurs >14 days after screening visit

Appendix B: Informed Consent Form (See attached)

Appendix C: Study Flow Chart