Official Title: **A Phase I Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability, and Immunogenicity of an Influenza H1 Stabilized Stem Ferritin Vaccine, VRCFLUNPF099-00-VP, in Healthy Adults**

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VACCINE RESEARCH CENTER

Protocol VRC 321

(NIH 19-I-0032)

A PHASE I OPEN-LABEL CLINICAL TRIAL TO EVALUATE DOSE, SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF AN INFLUENZA H1 STABILIZED STEM FERRITIN VACCINE, VRC-FLUNPF099-00-VP, IN HEALTHY ADULTS

Vaccine Provided by
National Institute of Allergy and Infectious Diseases (NIAID)
Vaccine Research Center (VRC)
Bethesda, Maryland

Clinical Trial Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)
Vaccine Research Center (VRC)
Bethesda, Maryland

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TABLE OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
AdEDC	Advantage Electronic Data Capture
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AoU	assessment of understanding
BMI	body mass index
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
cGMP	current Good Manufacturing Practices
dbGAP	Database of Genotypes and Phenotypes
DNA	deoxyribonucleic acid
DTM	Department of Transfusion Medicine
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
HA	influenza hemagglutinin protein
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRPP	Human Research Protection Program
IB	Investigator's Brochure
ICS	intracellular cytokine staining
ILI	influenza-like illness
IM	Intramuscular
IND	investigational new drug application
IRB	Institutional Review Board
IUD	intrauterine device
LIMS	Laboratory Information Management System
MedDRA	Medical Dictionary for Regulatory Activities
MIV	Monovalent Inactivated Vaccine
MPA	Medroxyprogesterone acetate
MSD	meso-scale delivery
NA	neuraminidase
NAb	Neutralizing antibody
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH CC	NIH Clinical Center
NSAID	nonsteroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RBS	receptor binding site

Abbreviation	Term
RNA	ribonucleic acid
SAE	serious adverse event
SARS	Severe acute respiratory syndrome
SUSAR	serious and unexpected suspected adverse reaction
TNF	tumor necrosis factors
TIV	trivalent inactivated vaccine
ULN	upper limit of normal
UP	Unanticipated Problem
VCMP	Vaccine Clinical Materials Program
VIP	Vaccine Immunology Program
VRC	Vaccine Research Center
WBC	white blood cell
WHO	World Health Organization

PRÉCIS

Title: VRC 321, A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of an Influenza H1 Stabilized Stem Ferritin Vaccine, VRC-FLUNPF099-00-VP, in Healthy Adults

Study Design: This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of VRC-FLUNPF099-00-VP in two regimens. The hypotheses are that the vaccine is safe and tolerable and will elicit an immune response. The primary objective is to evaluate the safety and tolerability of the investigational vaccine in healthy adults. Secondary objectives are related to immunogenicity of the investigational vaccine and dosing regimen.

Study Products: The investigational vaccine, VRC-FLUNPF099-00-VP (H1ssF_3928), was developed by the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID) and is composed of *Helicobacter pylori* non-heme ferritin assembled with influenza virus H1 haemagglutinin (HA) insert to form a nanoparticle displaying eight HA stabilized stem trimers from A/New Caledonia/20/1999 (H1N1) influenza. The vaccine is supplied in single-use vials at a concentration of 180 mcg/mL. H1ssF_3928 will be administered intramuscularly (IM) in the deltoid muscle via needle and syringe.

Subjects: Healthy adults between the ages of 18-70 years, inclusive.

Study Plan: The study will evaluate the safety, tolerability and immunogenicity of 1 or 2 doses of the H1ssF_3928 vaccine in a dose-escalation design. In Group 1, five subjects will receive a single low dose (20 mcg) of H1ssF_3928 on Day 0. For Group 1, the protocol requires 1 vaccination visit, about 9 follow-up visits, and a telephone contact after vaccination.

If the low dose is assessed as safe and well tolerated, enrollment will begin for Group 2A. Groups 2A, 2B, 2C, and 2D are stratified by age as shown in the vaccination schema. In Group 2A, subjects will receive a higher dose (60 mcg) of H1ssF_3928 on Day 0. If this higher dose is assessed as safe and well tolerated, subjects in Group 2A may receive a second vaccination at week 16 and enrollment can begin for Groups 2B, 2C, and 2D. For Groups 2A, 2B, 2C, and 2D, the protocol requires 2 vaccination visits, about 11 follow-up visits, and a telephone contact after each vaccination.

For all groups, solicited reactogenicity will be evaluated using a 7-day diary card. Assessment of vaccine safety will include clinical observation and monitoring of hematological and chemical parameters at clinical visits throughout the study.

VRC 321 Vaccination Schema				
Group	**Age Cohort**	**Subjects**	**Day 0**	**Week 16**
1	18-40	**5**	20 mcg	
2A	18-40	**12**	60 mcg	60 mcg
2B	41-49	**12**	60 mcg	60 mcg
2C	50-59	**12**	60 mcg	60 mcg
2D	60-70	**12**	60 mcg	60 mcg
Total		**53***	*Enrollment up to 70 is permitted if additional subjects are needed for safety or immunogenicity evaluations.	

Study Duration: Group 1: Subjects will be evaluated for 52 weeks following the vaccine administration and through an influenza season.

Groups 2A, 2B, 2C, 2D: Subjects will be evaluated for 52 weeks following the last vaccine administration and through an influenza season.

1. INTRODUCTION AND RATIONALE

1.1 BACKGROUND

Influenza virus causes seasonal epidemics and pandemics at irregular intervals that result in significant morbidity and mortality. According to the World Health Organization (WHO), the annual global attack rate of influenza is estimated to be 5%–10% in adults and 20%–30% in children; worldwide these annual epidemics result in about 3 to 5 million cases of severe illness and about 250,000 to 500,000 deaths [1]. The US Centers for Disease Control and Prevention (CDC) estimates that a pandemic influenza outbreak costs the United States between $71 billion and $167 billion without counting the financial impact on commerce and society [2].

Influenza is an enveloped, negative single-stranded (-ss) ribonucleic acid (RNA) virus that belongs to the family *Orthomyxoviridae*. Of the five genera of influenza circulating in nature, only influenza A and B are known to cause epidemics in humans [3].

Influenza A viruses consist of 8 RNA gene segments and are classified based on the antigenicity of their surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). There are 18 different HA subtypes (H1 through H18) and 11 NA subtypes known to exist, but only three HA subtypes (H1, H2, and H3) and two NA subtypes (N1 and N2) have caused significant human epidemics [4].

The HA is the predominant viral antigen target for antibody neutralization [5]. HA glycoprotein consists of a globular head domain (which is highly variant in structure between HA subtypes) and a stem domain (which is highly conserved across HA subtypes) [6]. Most of the antibodies produced by the immune system after infection recognize the head domain, whereas the stem domain is recognized by a small population of antibodies [7-9]. The anti-head antibodies are very potent but are strain-specific while the anti-stem antibodies are less potent and are cross-reactive across HA strains [10].

Influenza exhibits genetic flexibility and antigenic variability because of its ability to go through antigenic “drift” (the gradual accumulation of mutations over time) and antigenic “shift” (the replacement of the hemagglutinin gene by reassortment during contemporaneous infection of a host by more than one influenza strain). The emergence of new influenza strains through continuous mutation and reassortment of circulating virus diminishes the effectiveness of annual influenza vaccines [11, 12].

Furthermore, in the United States, the current manufacturing process in egg-based systems can lead to lower yields and significant lag times due to virus strain identification and vaccine production, availability, and distribution [13, 14].

These limitations have raised the need for developing a universal influenza vaccine that can provide durable, cross-strain protection against different influenza viruses, with a rapid manufacturing process in which large vaccine quantities could be produced under well-controlled conditions. A universal influenza vaccine would eliminate the need for annual reformulation and revaccination, and improve pandemic preparedness [15].

With the goal of developing a universal influenza vaccine, the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) has engineered and optimized a stabilized H1 influenza HA stem ferritin vaccine (VRC-FLUNPF099-00-VP) that enhances the presentation of the HA stem to the immune system, potentially improving breadth against group 1 influenza strains.

VRC-FLUNPF099-00-VP (H1ssF_3928) is an investigational vaccine that has not been administered to humans before this study. The H1ssF_3928 vaccine may inform the development of a universal influenza vaccine and play an important role in the planning and preparation for future influenza pandemics [16].

1.2 RATIONALE FOR DEVELOPMENT OF INFLUENZA H1SSF_3928 VACCINE, VRC-FLUNPF099-00-VP

New vaccine platforms and production technologies directed toward the goal of a universal influenza vaccine include cell-culture-based manufacturing processes, novel live attenuated vaccines, recombinant proteins, recombinant DNA-based vaccines, and nanoparticles [13, 14].

Ferritin is a highly conserved, ubiquitous iron storage protein that is found in various species from Archaea, Eukarya, and Bacteria domains [17]. Ferritin particles can be used in vaccines for antigen presentation of influenza HA [18]. Ferritin self-assembles into a nearly-spherical nanoparticle, composed of 24 subunits organized in an octahedral symmetry with a hollow interior, that mimics the structure of the viral antigen and mediates the interaction with the immune system [18, 19]. The advantages of using ferritin as a vaccine platform to improve antigen presentation and immune stimulation against different strains of influenza viruses rely on the ability to obtain higher levels of protein quaternary structures and the capacity to display heterologous antigens on their surface [18, 20]. Furthermore, as the self-assembly process requires no energy and can be manufactured from simple expression vectors (without relying on egg-based systems), vaccine manufacturing timelines could be potentially shortened, improving the response to an influenza pandemic [18].

Investigators at the VRC, NIAID, NIH, Bethesda, MD, US, have identified a non-haem ferritin from *Helicobacter pylori* (*H. pylori)* as a protein able to display eight trimeric influenza HA spikes that mimic the physiological HA structure of A/New Caledonia/20/1999 (H1N1) influenza.

Kanekiyo, *et al.* genetically fused the ectodomain of A/New Caledonia/20/1999 HA to *H. pylori* ferritin, creating a vaccine that antigenically resembles the native head and stem domains of the HA viral spikes on the surface of the ferritin spherical core [18]. In preclinical immunogenicity studies, this HA ferritin vaccine elicited two types of broadly neutralizing antibodies against the highly conserved HA stem and antibodies against the conserved receptor binding site (RBS) on the head of the viral HA. The HA stem and the RBS are structures of major interest for the development of a universal vaccine against influenza [21].

Yassine, *et al.* genetically fused the ectodomain of A/New Caledonia/20/1999 HA that lacks the immunodominant head domain to *H. pylori* ferritin, creating a synthetic ferritin that antigenically resembles the native stem domain of the HA protein on the surface of the ferritin spherical core [16]. In preclinical immunogenicity studies, this H1 HA stem ferritin vaccine conferred heterosubtypic protection against H5N1 influenza virus challenge in multiple animal models, indicating that vaccine-elicited HA stem–specific antibodies can protect against diverse group 1 influenza strains [16].

Both of these HA ferritin vaccines possess the desired structural properties and have demonstrated the capacity to enhance the breadth of neutralizing antibodies in pre-clinical studies when compared to the current commercial trivalent inactivated vaccine (TIV) containing the same 1999 H1N1 HA [18] [16]. Based on data reported with the HA stem ferritin vaccine in

animal studies (Section 2.3), it is expected that the H1ssF_3928 vaccine will be safe and immunogenic in this Phase I study in humans.

1.3 HUMAN EXPERIENCE WITH VRC-FLUNPF099-00-VP VACCINE

This is the first clinical study to test the VRC-FLUNPF099-00-VP vaccine, and there is no previous human experience with this product. VRC-FLUNPF081-00-VP (HA-F A/Sing) was the first ferritin-based vaccine platform tested in a Phase I clinical trial, therefore human experience with HA-F A/Sing is provided below.

1.3.1 Previous Human Experience with HA Ferritin vaccine (HA-F A/Sing)

The first Phase I clinical trial (VRC 316; NCT03186781) using ferritin particles, HA-F A/Sing ferritin vaccine was opened to accrual in October 2017. The HA-F A/Sing vaccine is composed of *H. pylori* non-haem ferritin genetically fused to the influenza virus H2 HA to form a nanoparticle that antigenically resembles the native head and stem domains of the HA from A/Singapore/1/57 (H2N2) influenza.

As of March 24, 2019, enrollment was complete and all 50 subjects have received at least one injection of the HA-F A/Sing vaccine. All injections with the HA-F A/Sing vaccine have been generally well tolerated.

Maximum local reactogenicity in the 7 days after HA-F A/Sing administration was reported as mild local pain/tenderness by 7 of 47 (14.9 %) subjects. No other solicited local symptoms of swelling or redness were reported. No moderate or severe local symptoms have been reported.

Regarding solicited systemic reactogenicity in the 7 days after product administration, 18 of 47 (38.3%) subjects who received HA-F A/Sing had one or more systemic signs or symptoms, the most frequently reported AE was that of mild headache (13/47, 27.7%). Subjects also reported mild malaise (10/47, 21.3%), mild myalgia (8/47, 17.0%), moderate myalgia (1/47, 2.1%), mild chills (2/47, 4.3%), mild nausea (1/47, 2.1) and mild joint pain (2/47, 4.3%).

One subject reported a severe fever that started on Day 4 after the first product administration, resolved within 1 day, and coincided with an influenza-like illness.

Overall for the HA-F A/Sing vaccine groups, 22 of 47 (46.8%) subjects who received at least one administration of the vaccine have had at least one or more unsolicited AE, with maximum severity being Grade 1 (mild) for 12 subjects, Grade 2 (moderate) for 8 subjects, Grade 3 (severe) for 1 subject and Grade 4 (life-threatening) for 1 subject.

The most frequently reported unsolicited AEs were anaemia (8/47, 17.0%), with a maximum severity of Grade 2 for five subjects, and upper respiratory tract infection (5/47, 10.6%), with a maximum severity of Grade 2 for one subject.

A severe AE of viral infection was reported by 1 subject 9 days after the initial study injection. The event was assessed as unrelated to study product and resolved with no residual effects three days after onset.

As of March 24, 2019, 3 mild AEs were assessed as related to HA-F A/Sing vaccine and all resolved with no residual effects: leukocytosis (1/47, 2.1%), blood alkaline phosphatase increased (1/47, 2.1%), and leukopenia (1/47, 2.1%)

A life-threatening SAE of myocardial infarction was reported by 1 subject at 117 days after the HA-F A/Sing study product administration. The event was assessed as unrelated to study product and resolved with sequelae.

1.4 RATIONALE FOR STUDY PRODUCT DOSE

The VRC 316 study results as of March 18, 2018 showed that two dose levels (20 mcg as a single dose and 60 mcg as a repeat dose with a 16-week interval) of HA-F A/Sing were safe and well tolerated. In this study, H1ssF_3928 will be similarly evaluated at 20 mcg as a single dose and 60 mcg as a repeat dose with a 16-week interval. A 16-week interval between first and second doses is based on previous VRC studies suggesting that this interval may be optimal to develop an immune response to the vaccine regimen.

1.5 RATIONALE FOR STUDY POPULATION

To assess safety, tolerability, and immunogenicity of the H1ssF_3928 vaccine, subjects 18-70 years of age will be enrolled. In the higher dose group, subjects will be stratified by age into four groups (Groups 2A-2D) to evaluate how the immune responses to the H1ssF_3928 vaccine vary based on age and the likelihood of exposure to different influenza strains.

1.6 ASSESSMENT OF VACCINE IMMUNOGENICITY

In this study, specimens to evaluate immunogenicity will be collected at baseline and at specified time points. The primary immunogenicity time point is two weeks after the first vaccination and assessed by measuring HA stem-specific antibody by pseudo-neutralization assay. Additional assessment of HA stem-specific antibody measured by pseudo-neutralization assay will be conducted on stored samples obtained throughout the study.

Exploratory evaluations will include measurements of human ferritin antibody and *H. pylori* ferritin antibody. Additional exploratory evaluations may include the detection of antibody by MSD (Meso Scale Delivery) or neutralization assay, and exploratory B and T cell assays.

Research samples for immunogenicity assays will be processed by the Vaccine Immunology Program (VIP) in Gaithersburg, MD, where many of the immunogenicity assays will also be performed. Some immunogenicity assays may be performed by VRC laboratories in Bethesda, MD, or by approved contract laboratories or research collaborators.

Results from this study are expected to provide a foundation for development of a universal influenza vaccine candidate as well as show proof-of-concept for elicitation of antibody responses by influenza HA stem vaccination.

2. STUDY PRODUCTS

The study products are manufactured under current Good Manufacturing Practice (cGMP) by the Vaccine Clinical Materials Program (VCMP) operated under contract by Leidos Biomedical Research, Inc., Frederick, MD.

2.1 VRC-FLUNPF099-00-VP

The VRC-FLUNPF099-00-VP (H1ssF_3928) vaccine is composed of the HA stem domain from Influenza A/New Caledonia/20/1999 (H1N1) genetically fused to the ferritin protein from *H. pylori*. Purified H1ssF_3928 displays eight well-formed HA trimers that antigenically resemble the native H1 stem viral spikes.

VRC-FLUNPF099-00-VP is a sterile, aqueous buffered solution. Product is aseptically filled to a volume of 0.7 ± 0.1 mL into 3 mL single dose vials at a concentration of 180 mcg/mL. The

buffer consists of 20mM Sodium Phosphate, 100mM Sodium Chloride, 5% Sucrose and 0.01% PF-68 at pH 7.2.

More details related to vaccine formulation, preparation, and preclinical studies performed with H1ssF_3928 can be found in the Investigator's Brochure (IB).

2.2 VRC-PBSPLA043-00-VP, PHOSPHATE BUFFERED SALINE (DILUENT)

VRC-PBSPLA043-00-VP will be the diluent for H1ssF_3928 and consists of sterile phosphate buffered saline (PBS) at pH 7.2, aseptically filled to 1.2 mL in a 3 mL glass vial for single use.

2.3 PRECLINICAL STUDIES WITH VRC-FLUNPF099-00-VP

Non-clinical immunogenicity studies in mice and ferrets demonstrated that VRC-FLUNPF099-00-VP administered IM was immunogenic as detected by ELISA and neutralization assay. A dose toxicity study of VRC-FLUNPF099-00-VP was performed in New Zealand white rabbits to evaluate safety. More details on this study and additional pre-clinical studies with VRC-FLUNPF099-00-VP can be found in the IB.

3. STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVES

- To evaluate the safety and tolerability of the VRC-FLUNPF099-00-VP vaccine, administered as a single dose at 20 mcg IM via needle and syringe on Day 0 to healthy adults.
- To evaluate the safety and tolerability of the VRC-FLUNPF099-00-VP vaccine, administered at 60 mcg IM via needle and syringe to healthy adults by repeat dosing on Day 0 and Week 16 for a total of 2 injections.

3.2 SECONDARY OBJECTIVES

- To evaluate the antibody response to the VRC-FLUNPF099-00-VP vaccine administered as a single dose at 20 mcg IM via needle and syringe at two weeks after injection.
- To evaluate the antibody response to the VRC-FLUNPF099-00-VP vaccine administered as repeat dosing at 60 mcg IM via needle and syringe at two weeks after each injection.

3.3 EXPLORATORY OBJECTIVES

- To evaluate the specificity and functionality of vaccine-induced antibodies and the immune response at various timepoints throughout the study.
- To evaluate the frequency, magnitude, and specificity of B-cell and/or T-cell responses at various time points throughout the study.

4. STUDY DESIGN AND CLINICAL PROCEDURES

This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of the VRC-FLUNPF099-00-VP vaccine in two dose regimens in healthy adults. The study schema is shown in **Table 1**. The hypotheses are that the vaccine is safe, well-tolerated, and will induce an antibody response to the stem of influenza virus subtype H1. The

study will be conducted by the VRC Clinical Trials Program at a single site in the NIH Clinical Center (NIH CC), Bethesda, MD.

Table 1: Study schema:

VRC 321 Vaccination Schema				
Group	**Age**	**Subjects**	**Day 0**	**Week 16**
1	18-40	**5**	20 mcg	
2A	18-40	**12**	60 mcg	60 mcg
2B	41-49	**12**	60 mcg	60 mcg
2C	50-59	**12**	60 mcg	60 mcg
2D	60-70	**12**	60 mcg	60 mcg
Total		**53***	*Enrollment up to 70 is permitted if additional subjects are needed for safety or immunogenicity evaluations.	

4.1 STUDY POPULATION

All inclusion and exclusion criteria must be evaluated for eligibility.

4.1.1 Inclusion Criteria

***A subject must meet all of the following criteria**:*

1. Healthy adults between the ages of 18-70 years inclusive
2. Based on history and examination, in good general health and without history of any of the conditions listed in the exclusion criteria
3. Received at least one licensed influenza vaccine from 2014 to the present
4. Able and willing to complete the informed consent process
5. If enrolled in Group 1: Available for clinic visits for 52 weeks after enrollment and through an influenza season
6. If enrolled in Group 2A, 2B, 2C, or 2D: Available for clinic visits for 68 weeks after enrollment and through an influenza season
7. Willing to have blood samples collected, stored indefinitely, and used for research purposes
8. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process
9. Physical examination and laboratory results without clinically significant findings and a Body Mass Index (BMI) ≤ 40 within the 28 days before enrollment

Laboratory Criteria within 28 days before enrollment

10. White blood cells (WBC) and differential either within institutional normal range or accompanied by the site Principal Investigator (PI) or designee approval
11. Total lymphocyte count ≥ 800 cells/mm^3
12. Platelets = 125,000 – 500,000/mm^3
13. Hemoglobin within institutional normal range

14. Serum iron either within institutional normal range or accompanied by the site PI or designee approval
15. Serum ferritin within institutional normal range or accompanied by the site PI or designee approval
16. Alanine aminotransferase (ALT) ≤ 1.25 x institutional upper limit of normal (ULN)
17. Aspartate aminotransferase (AST) ≤ 1.25 x institutional ULN
18. Alkaline phosphatase (ALP) <1.1 x institutional ULN
19. Total bilirubin within institutional normal range
20. Serum creatinine ≤ 1.1 x institutional ULN
21. Negative for HIV infection by an FDA-approved method of detection

Criteria applicable to women of childbearing potential:

22. Negative beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) on the day of enrollment
23. Agrees to use an effective means of birth control from at least 21 days prior to enrollment through the end of the study

4.1.2 Exclusion Criteria

A subject will be excluded if one or more of the following conditions apply:

1. Breast-feeding or planning to become pregnant during the study.

Subject has received any of the following substances:

2. More than 10 days of systemic immunosuppressive medications or cytotoxic medications within the 4 weeks prior to enrollment or any within the 14 days prior to enrollment
3. Blood products within 16 weeks prior to enrollment
4. Live attenuated vaccines within 4 weeks prior to enrollment
5. Inactivated vaccines within 2 weeks prior to enrollment
6. Investigational research agents within 4 weeks prior to enrollment or planning to receive investigational products while on the study
7. Current allergy treatment with allergen immunotherapy with antigen injections, unless on maintenance schedule
8. Current anti-TB prophylaxis or therapy
9. Previous investigational H1 influenza vaccine
10. Previous investigational ferritin-based vaccine
11. Receipt of a licensed influenza vaccine within 6 weeks before trial enrollment

Subject has a history of any of the following clinically significant conditions:

12. Serious reactions to vaccines that preclude receipt of study vaccinations as determined by the investigator
13. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema

14. Asthma that is not well controlled
15. Diabetes mellitus (type I or II), with the exception of gestational diabetes
16. Thyroid disease that is not well controlled
17. Idiopathic urticaria within the past year
18. Autoimmune disease or immunodeficiency
19. Hypertension that is not well controlled (baseline systolic > 140 mmHg or diastolic > 90 mmHg)
20. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws
21. Malignancy that is active or history of malignancy that is likely to recur during the period of the study.
22. Seizure disorder other than 1) febrile seizures, 2) seizures secondary to alcohol withdrawal more than 3 years ago, or 3) seizures that have not required treatment within the last 3 years
23. Asplenia, functional asplenia or any condition resulting in the absence or removal of the spleen
24. Guillain-Barré Syndrome
25. Any medical, psychiatric, social condition, occupational reason or other responsibility that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a subject's ability to give informed consent.

4.2 Clinical Procedures and Evaluations

Evaluation of this vaccine will include laboratory tests, medical history, physical assessment by clinicians, and subject self-assessment recorded on a diary card for 7 days after each injection. Potential adverse reactions will be further evaluated prior to continuing the vaccination schedule. The schedule of study evaluations is described in this section and shown in table format in Appendix I.

4.2.1 Screening

Screening for this study will be completed through the VRC's screening protocol, VRC 500 (NIH 11-I-0164, NCT01375530). Subjects will be recruited through Institutional Review Board (IRB)-approved advertising. The evaluations and sample collections that will be included in screening are a medical history, physical exam, laboratory tests needed to confirm eligibility, and pregnancy test for females of reproductive potential.

If screening evaluations suggest a current concerning health condition or infection, such as hepatitis, then appropriate laboratory tests may be conducted to evaluate these conditions. Additional assessments of health may be conducted at screening based on clinical judgment. Screening evaluations for specific eligibility criteria (Section 4.1) must be completed within the time interval specified prior to enrollment for the given parameter and may be repeated, as needed, to confirm eligibility. Research blood samples will be collected during screening;

although generally collected in the 28 days prior to enrollment; a particular interval of time prior to enrollment for collection of these samples is not required.

The informed consent form (ICF) will be reviewed and counseling related to pregnancy prevention will be provided.

Subjects who are not up to date on standard vaccinations may receive these, if available, during their participation in the screening protocol or at a later date during study participation. As part of the informed consent process, an Assessment of Understanding (AoU) is completed on the day the subject is scheduled to enroll prior to signing the VRC 321 protocol ICF. Incorrect answers will be explained to the subject, who will sign the ICF only after the study clinician is satisfied with the subject's understanding of the study.

4.2.2 Study Schedule

The Schedule of Evaluations in Appendix I provides details on the study schedule, the permitted windows for completing the visits, and the evaluations to be performed at each visit. The visit schedule is based on intervals of time after each study injection (Appendix I). The clinicians will discuss the target dates and timing of the study vaccination(s) and sample collections with each subject before completing enrollment to help ensure that subjects can comply with the projected schedule.

After enrollment, deviations from the visit windows are discouraged and will be recorded as protocol deviations but are permitted at the discretion of the PI (or designee) in the interest of completing the vaccination schedule and obtaining subject safety and immunogenicity evaluations.

4.2.3 Enrollment

The study has staged enrollment with required interim safety reviews, as described in Section 4.3, for dose escalation, continuing enrollment, and second product administration. The first three subjects of Groups 1 and 2A will be enrolled with no more than one subject per day.

Subjects will be stratified by age into Group 2A, 2B, 2C, and 2D.

The pharmacy database will be set up prior to opening the study to accrual. The group assignment is known to the staff and subject before completing the electronic enrollment into the study on Day 0. Any subject who receives at least one study injection will be expected to continue with follow-up through the end of the study.

4.2.4 Acceptable and Effective Methods of Birth Control

Women of reproductive potential are required to agree to use an acceptable and effective method of birth control beginning 21 days prior to enrollment and continuing through end of study.

Acceptable and effective methods of birth control for women of reproductive potential in this study include: abstinence (no sex) with male partners, birth control pills or patch, condoms, Medroxyprogesterone acetate (MPA) injection, diaphragm or cervical cap, intrauterine device (IUD), Implant (Nexplanon®), NuvaRing®, partner has vasectomy, or use of spermicides.

4.2.5 Vaccine Administration

All study injections will be completed according to the assigned group and will be administered IM in the deltoid muscle. Scheduled blood collection must be completed before vaccinations.

On the injection day (prior to injection), study subjects will be clinically evaluated and samples will be collected as per Schedule of Evaluations (Appendix I). A subject who arrives at the clinic with fever or evidence of an acute illness that precludes administration of the vaccine may be rescheduled within the allowed study visit window.

Pregnancy test results for women of reproductive potential must be obtained on each injection day prior to the study injection and the results must be negative to proceed.

When choosing an arm for injection, clinicians should consider whether there is an arm injury, local skin problem or significant tattoo that precludes administering the injection or will interfere with evaluating the arm after injection.

Subjects enrolled in Group 1will be observed for a minimum of 1 hour following the injection. Vital signs (temperature, blood pressure, pulse and respiratory rate) will be collected at least 1 hour after the injection, prior to subject departure from the clinic.

Subjects enrolled in Group 2A, 2B, 2C, and 2D will be observed for a minimum of 30 minutes following each injection; vital signs will be collected after at least 30 minutes.

For all subjects, the injection site will be inspected for evidence of local reaction following the observation period.

In keeping with the NIH CC policy and good medical practice, acute medical care will be provided to subjects for any immediate allergic reactions or other injury resulting from participation in this research study.

4.2.6 7-Day Solicited Reactogenicity and Follow-up

Subjects will be given a "Diary Card" to use as a memory aid, on which they can record temperature and symptoms daily for 7 days after each injection. Subjects will be trained and encouraged to use the secure electronic database but will have the option to complete a paper diary card. When the diary card parameters are recorded directly by the subject in the electronic database, the subject's electronic record will be the source for these data. When collected on paper, the paper diary card will be the source document. When neither paper nor electronic diary is available from the subject, the study clinician will document the source of reactogenicity information recorded in the study database.

The solicited signs and symptoms on the diary card will include the following parameters: unusually tired/feeling unwell, muscles aches (other than at injection site), headache, chills, nausea, joint pain, and pain/tenderness, redness, swelling, skin lesions at injection site. Subjects will also record the day's highest measured temperature and measurement of largest diameter for redness and swelling at injection site.

Follow-up on subject well-being will be performed by telephone on the first day following all injections and by clinic visits as shown in the Schedule of Evaluations (Appendix I).

Events following any study injection that may require clinical evaluation include rash, urticaria, fever of 38.5˚C (Grade 2) or higher lasting greater than 24 hours, or significant impairment in the activities of daily living. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

4.2.7 Follow-Up through End of Study

Group 1: Study follow-up will continue via clinical visits through 52 weeks after the study injection and through an influenza season.

Groups 2A, 2B, 2C, and 2D: Study follow-up will continue via clinical visits through 52 weeks after the last study injection and through an influenza season.

4.2.8 Mucosal Sample Collection

Throughout the study, nasopharyngeal swabs for diagnostic purposes will be requested from subjects who meet criteria for influenza-like illness (ILI) as defined in Section 5.1.

4.2.9 Blood Sample Collection

At intervals throughout the study, blood will be drawn for safety and immunologic assays. Blood will be drawn from the arm veins of subjects by standard phlebotomy procedures. Total blood volume drawn from each subject will not exceed the NIH CC guidelines.

4.2.10 Apheresis

Group 2 subjects will be offered apheresis as an optional procedure at Visit 09 in order to collect blood cells of special interest for research. The apheresis procedure will be carried out by trained Department of Transfusion Medicine (DTM) medical staff using automated cell separator devices.

Apheresis performed for this protocol will be performed solely for research purposes. All study subjects will be treated according to standard DTM whole blood and apheresis donation policies and procedures. Prior to the scheduling apheresis, the subject must have a venous assessment performed by the DTM staff.

In order to undergo apheresis procedures, a subject must meet the apheresis eligibility criteria as described in Section 4.2.11 and have no medical contraindications, as determined by the DTM staff. A VRC study clinician will complete a checklist for apheresis eligibility before referring a subject for the procedure.

Prior to beginning the apheresis procedure, a study clinician may request in advance that other laboratory samples be collected as needed to monitor the well-being of the subject or if needed by a research laboratory. In addition, for women of reproductive potential, a pregnancy test by blood or urine will be performed by a VRC study clinician within 72 hours prior to the apheresis procedure. Results must be negative to proceed with apheresis.

The Dowling Apheresis Clinic staff at the NIH CC routinely performs a hemoglobin test prior to initiating apheresis, per DTM Apheresis Clinic standard policies. If a subject is found to have a hemoglobin value less than permitted by the Apheresis Clinic, then the apheresis will not be initiated, and the ordering provider will be notified.

In this study, the procedure will require two antecubital venous access sites and will involve processing 1 to 4 liters of whole blood. The expected mononuclear cell yield is approximately 0.5 to 1.0 x 10^9 cells per liter processed, and the apheresis device can process about 2 to 3 liters per hour. Thus, 1 to 2 hours are required to process 1 to 4 liters of blood and obtain about 1 to 4 x 10^9 leukocytes. The packed red cell loss during the procedure is the equivalent of a 6 mL blood draw; this is the volume that will be used for the purposes of calculating cumulative blood draw when apheresis is performed.

During or following an apheresis visit, if there is any concern about the well-being of the subject, the DTM clinic may conduct appropriate medical evaluations by history-taking, physical examination, laboratory tests, and/or other testing.

Research blood samples will be processed and stored at VIP or a collaborating research laboratory. Stored samples may be used later to further evaluate immune responses and to elucidate genetic factors associated with immune response.

4.2.11 Apheresis Eligibility Criteria

Subject must meet all of the following criteria:

1. Afebrile (temperature $\leq 37.5^0$C)
2. Weight ≥110 pounds
3. Adequate bilateral antecubital venous access
4. Hemoglobin ≥ 12.5 g/dL for females; ≥ 13.0 g/dL for men
5. Platelets > 150,000 K/uL
6. No cardiovascular instability as indicated by a) history of medically significant cardiac arrhythmia within the last 12 months, or b) ischemic cardiovascular disease within the last 12 months, or c) heart rate outside of the 50 - 100 beats/minute interval (on 3 successive readings), or d) blood pressure greater than 180 mmHg (systolic) or 100 mmHg (diastolic) on 3 successive readings
7. No current lung or kidney disease
8. No known coagulation disorder
9. No sickle cell disease
10. No active or chronic hepatitis
11. No intravenous injection drug use in the past 5 years
12. Not breast feeding
13. Negative beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) performed by a VRC study clinician within 72 hours prior to the apheresis procedure

4.2.12 Concomitant Medications

Only routine prescription medications will be entered in the database at the time of enrollment. Subsequently, the only concomitant medications that will be recorded or updated in the database are those associated with an AE requiring expedited reporting or the development of a new chronic condition requiring ongoing medical management. Anti-viral medications taken during influenza or influenza-like illnesses will be recorded in the database. Receipt of a FDA-approved vaccine at any time during the study will be recorded in the database (clinicians should work with subjects regarding the timing of licensed vaccines relative to study injection). Otherwise, concomitant medications taken throughout the study will be recorded in the subject's study chart and general medical record for general medical documentation but will not be recorded in the database.

4.3 Criteria for Dose Escalation and Dose Continuation

There will be one dose escalation review and two dose continuation reviews in this study. The Protocol Safety Review Team (PSRT, Section 8.8) will conduct an interim safety data review before dose escalation or repeat dosing may occur. The PSRT must assess the data as showing no

significant safety concerns before enrollment of the next dose level and repeat dosing at the same level may proceed.

Enrollment will begin in Group 1 (20 mcg of VRC-FLUNPF099-00-VP) with no more than one subject enrolled per day. Two weeks after vaccination of the third subject, there will be an interim safety review of available data for three subjects to determine whether to continue enrollment in Group 1 and to proceed to the next dose level. If the 20 mcg dose of VRC-FLUNPF099-00-VP is assessed as safe, enrollment can continue for Group 1 and begin for Group 2A.

In Group 2A (60 mcg of VRC-FLUNPF099-00-VP), enrollment will continue with no more than one subject enrolled per day. Two weeks after vaccination of the third subject, there will be an interim safety review of the available data for three subjects to determine whether to proceed to Groups 2B, 2C, and 2D. If the 60 mcg dose of VRC-FLUNPF099-00-VP is assessed as safe, enrollment can continue for Group 2A and begin for Groups 2B, 2C, and 2D. Additionally, if the 60 mcg dose is assessed as safe, all Group 2 subjects may receive the second vaccination at week 16.

Additional subjects may be enrolled into a group in order to have the requisite data for at least 3 subjects, if first vaccinations are not completed or there are discontinuations from the study before there are sufficient data to conduct the dose escalation and continuation review for that group.

The IRB will be provided with documentation of the safety review process and notification of the dose escalation. Consultation with the IRB and FDA, if needed, as per study pause criteria (Section 4.5) will occur if indicated by the review. One outcome of a dose escalation review may be to recommend evaluation of additional subjects at the current dose level and reassess for safety before proceeding to a higher dose level and repeat dosing at the same dose level.

4.4 Criteria for Discontinuing Study Injections or Protocol Participation

Decisions by the PI or designee to discontinue study injections or protocol participation for a subject will be made with these criteria.

4.4.1 Discontinuation of Study Injections

A subject will be discontinued from receiving study product for the following reasons:

1. Subject voluntarily withdraws
2. Pregnancy
3. Grade 3 unsolicited AE assessed as possibly, probably or definitely related to study product
4. Grade 4 AE assessed as related to study product
5. Immediate hypersensitivity reaction associated with study product
6. Intercurrent illness that is not expected to resolve prior to the next scheduled study injection
7. Treatment with systemic glucocorticoids (e.g., prednisone or other glucocorticoid) or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that study injections may continue per investigator discretion if the next dose occurs at least 2 weeks following completion of glucocorticoid treatment

8. The study PI assesses that it is not in the best interest of the subject to continue the vaccination schedule

Group 2 subjects who do not receive the second injection as scheduled are expected to continue follow-up according to the Schedule of Evaluations for Group 1 subjects, except that research sample collections will be discontinued for pregnant women or others in which it is contraindicated.

4.4.2 Discontinuation from Protocol Participation

A subject will be discontinued from protocol participation for the following reasons:

1. Subject voluntarily withdraws
2. Subject develops a medical condition that is a contraindication to continuing study participation
3. The IND Sponsor or regulatory authority stops the protocol
4. The IND Sponsor or PI assesses that is it not in the best interest of the subject to continue participation in the study or that the subject's compliance with the study is not sufficient

4.5 Criteria for Pausing and Resuming the Study

4.5.1 Plan for Pausing the Study

The PI and Protocol Safety Review Team (PSRT) will closely monitor and analyze study data as they become available and will make determinations regarding the presence and severity of AEs. The administration of study injections and new enrollments will be paused and the IND Sponsor will be promptly notified according to the following criteria:

- **One** (or more) subject experiences a **SAE** or **Grade 4 AE** assessed as related to study product.
- **Two** (or more) subjects experience the same **Grade 3 unsolicited AE** assessed as possibly, probably or definitely related to study product.
- **Three** (or more) subjects experience the same **Grade 2 or higher laboratory abnormality** assessed as possibly, probably or definitely related to study product.
- **One** (or more) subject experiences ulceration, abscess, or necrosis at the injection site.

Self-limited solicited reactogenicity that is not an SAE will not be counted towards pause criteria.

4.5.2 Plan for Review of Pauses and Resuming the Study

The IND Sponsor, with participation by the PI and PSRT, will conduct the review and make the decision to resume, amend or close the study and notify the IRB accordingly. As part of the pause review, the reviewers will also advise on whether the study needs to be paused again for any subsequent AEs of the same type. The pause criterion for the SAE will continue to apply.

The administration of study injections and new enrollments would resume only if review of the events that caused the pause resulted in a recommendation to permit further study injections and enrollments. Safety data reports and changes in study status will be submitted to relevant regulatory authorities in accordance with Section 5 and institutional policy.

5. SAFETY AND ADVERSE EVENT REPORTING

5.1 ADVERSE EVENTS

Adverse Event (AE) - Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research. In the context of FDA-required reporting, an AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Each AE will be graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Food and Drug Administration Guidance – September 2007 (Appendix II). The following guidelines will be used to determine whether or not an AE is recorded in the study database:

- Solicited AEs (i.e., reactogenicity parameters as defined in Section 4.2.6) will be recorded without attribution assessments by the subject on paper or an electronic diary card for 7 days after each injection. If the paper diary card is completed by subject, data will be transcribed by a clinician into the study database. Clinicians will follow and collect resolution information for any reactogenicity symptoms that are not resolved within 7 days.
- All unsolicited AEs will be recorded with attribution assessments in the study database from receipt of the first study injection through completion of the 4 week visit that follows each study injection. At other periods between injections and following the 4 week post-injection visit after the second vaccination through the last study visit, only SAEs (Section 5.2), new chronic medical conditions, and influenza or ILI will be recorded.
- Cases of influenza or influenza-like illness (ILI) will be evaluated as follows:

  ILI is defined as fever (temperature of 100^0F [37.8^0C] or greater) and a cough and/or sore throat in the absence of a known cause other than influenza. Collection of nasopharyngeal swabs will be used for laboratory confirmation of influenza by polymerase chain reaction (PCR) in subjects who meet criteria for ILI. The severity of illness in subjects with laboratory confirmed influenza illness will be recorded on a case report form rather than on an AE form.

5.2 SERIOUS ADVERSE EVENTS

The term "Serious Adverse Event" (SAE) is defined in 21 CFR 312.32 as follows: "An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or the sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency

room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

"Life-threatening" refers to an AE that at occurrence represents an immediate risk of death to the subject. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. Similarly, a hospital admission for an elective procedure is not considered a SAE.

5.3 ADVERSE EVENT REPORTING TO THE IND SPONSOR

AEs that meet SAE criteria must be reported and submitted by the clinical site on an expedited basis to the IND Sponsor, VRC/NIAID/NIH, according to sponsor guidelines as follows:

- Results in death;
- Is life threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect in the offspring of a study subject; OR
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the 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).

In addition, any event, regardless of severity, which in the judgment of an investigator represents a SAE, may be reported on an expedited basis.

An investigator must communicate an initial SAE report within 24 hours of site awareness of occurrence to the IND Sponsor data entry into the database, which triggers an alert to the IND Sponsor MO. Within 3 working days, a written summary by the investigator should be submitted to the IND Sponsor.

In order for the IND Sponsor to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 or 15 calendar days, the investigator must submit additional information as soon as it is available.

5.3.1 IND Sponsor Reporting to the FDA

The IND Sponsor is responsible for making the determination of which SAEs are "serious and unexpected suspected adverse reactions" (SUSARs) as defined in 21 CFR 312.32. The following definitions apply:

- *Suspected adverse reaction* means any AE for which there is a reasonable possibility that the study product caused the AE.
- *Unexpected Adverse Event* means an AE that is not listed in the IB or is not listed at the specificity or severity that has been observed.

All SUSARs (as determined by the IND Sponsor) will be reported to the FDA as IND Safety Reports per 21 CFR 312.32 as soon as possible but not exceeding 7 calendar days for unexpected

fatal or life-threatening events, and not exceeding 15 calendar days for other qualifying events. IND Safety Reports will also be provided to the IRB.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

5.4 REPORTING TO THE INSTITUTIONAL REVIEW BOARD

The following information is consistent with NIH IRB Policy 801: Reporting Research Events, Version 1, effective July 1, 2019.

Reportable Event - An event that occurs during the course of human subject research that requires notification to the IRB.

For the purposes of this policy, reportable events include the following:

- Unanticipated Problems (UPs) involving risks to subjects or others
- Non-compliance (including major protocol deviations and noncompliance that is not related to a protocol deviation)
- Deaths related or possibly related to research activities
- New information that might affect the willingness of subjects to enroll or continue participation in the study

5.4.1 Unanticipated Problem

An unanticipated problem (UP) is defined as any incident, experience, or outcome that meets all three of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; and
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others (which may include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.

A UP must be reported within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

5.4.2 Protocol Deviation

A protocol deviation (PD) is defined as any change, divergence, or departure from the IRB-approved study procedures in a research protocol and are further characterized as major and minor as follows:

- Major Deviations – Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor Deviations – Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

For the reporting purposes, failure of subjects to comply with the research protocol does not represent non-compliance unless that failure is due to an action or omission of a member of the research team, for example, the failure to give adequate instruction to the subject.

A major deviation must be reported within 7 calendar days of an investigator becoming aware of an actual or suspected deviation. Although PDs are also non-compliance, these should only be reported once as deviations. Major deviations resulting in death must be reported within 24 hours of the occurrence of the event or of any member of the study team becoming aware of the death.

Researchers are responsible for monitoring their studies throughout the year for adherence to the IRB approved protocol. The purpose of this monitoring is to identify major deviations and to look for trends in minor deviations that may indicate a systemic issue in how the study is being conducted that could potentially negatively impact the rights, safety, or welfare of participants or the study's ability to produce scientifically valid results. A series of minor deviations pointing toward a more global issue that could affect the rights, safety or welfare of the participant or affect the validity of the study should be reported as a major deviation. In all other instances, a summary of minor deviations should be provided to the IRB at the time of continuing review.

5.4.3 Non-Compliance

Non-compliance is the failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

Non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

Non-compliance is further characterized as serious or continuing as follows:

- Serious non-compliance – Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially effects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
- Continuing non-compliance – A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events.

Any actual or suspected non-compliance by any investigator or entity associated with the protocol must be reported to the IRB by the PI/designee within 7 calendar days of any investigator or individual associated with the protocol first becoming aware.

5.4.4 Death

Any death of a research subject that is possibly, probably or definitely related to the research must be reported within 24 hours of an investigator becoming aware of the death.

5.4.5 New Information

New information that might affect the willingness of a subject to enroll or remain in the study should be reported within 7 calendar days of an investigator first becoming aware.

5.4.6 Suspension or Termination of Research Activities

Any suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency must be reported within 7 calendar days of an investigator becoming aware.

5.4.7 Expedited Reporting to the IRB

Death related to research must be reported within 24 hours.

The following will be reported within 7 calendar days of investigator awareness:

- Actual or suspected UPs;
- Actual or suspected non-compliance;
- Actual or suspected Major PDs;
- SAEs that are actual or suspected UPs;
- New information that might affect the willingness of a subject to enroll or remain in the study;
- Suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency.

5.4.8 Annual Reporting to the IRB

The following will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Summary of UPs and non-compliance;
- AEs, including SAEs, that are not UPs, as a narrative summary statement indicating whether these events were within the expected range;
- Minor PDs (aggregate summary);
- Any trends or events which in the opinion of the investigator should be reported.

6. STATISTICAL CONSIDERATIONS

6.1 OVERVIEW

This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of VRC-FLUNPF099-00-VP in two dose regimens. Study objectives can be found in Section 3.

6.2 ENDPOINTS

6.2.1 Primary Endpoints: Safety

Assessment of product safety will include clinical observation and monitoring of hematological and chemical parameters. Reactogenicity will be closely monitored for 7 days after each injection and safety evaluated by clinical visits for 52 weeks following the last vaccine administration and through an influenza season. See Section 4.2 and Appendix I for details and specified time points. The following parameters will be assessed for all study groups:

- Occurrence of solicited local reactogenicity symptoms for 7 days after each injection
- Occurrence of solicited systemic reactogenicity symptoms for 7 days after each injection
- Change from baseline in safety laboratory measures

- Occurrence of AEs of all severities through the 4 week post-injection visit
- Occurrence at any time throughout the study of SAEs or new chronic medical conditions that require ongoing medical management.

6.2.2 Secondary Endpoints: Immunogenicity

The principal immunogenicity endpoints will be assessed by measuring HA-stem specific antibody by pseudo-neutralization assay at:

- Group 1: Baseline (Week 0/Visit 02) and Week 2 (Visit 04) after vaccination, and
- Groups 2A-2D: Baseline (Week 0/Visit 02) and Week 2 (Visit 04) after the first vaccination, and at Week 16 (Visit 07) and Week 18 (Visit 09) after the second vaccination

6.2.3 Exploratory Endpoints: Immunogenicity

Exploratory immunogenicity evaluations may include the detection of antibody by MSD or microneutralization assay, and exploratory B and T cell assays.

6.3 Sample Size and Accrual

Recruitment will target 53 healthy adult participants 18 to 70 years of age, though up to 70 participants may be enrolled if deemed necessary for safety or immunogenicity evaluations.

Enrollment will be spread over multiple groups. Group 1 will target 5 healthy adults 18 to 40 years of age. Groups 2A, 2B, 2C, and 2D will target 12 healthy adult participants each, with each study group corresponding to a different age range.

6.3.1 Power Calculations for Safety

The goal of the safety evaluation for this study is to identify safety concerns associated with injections of the investigational vaccine. Primary sample size calculations for safety are expressed in terms of the ability to detect serious adverse experiences. Other sample size calculations for comparing the two vaccination groups on adverse experiences are similar to the calculations for immunogenicity (Section 6.4.3).

The ability of the study to identify SAEs will be expressed in terms of the probability of observing a certain number of SAEs. Useful values are the minimum true rate such that the probability of observing at least one event is at least 90%, and the maximum true rate such that the probability of not observing any event is at least 90%.

For Group 1 (*dose-escalation*) within each group (n=5), there is a 90% chance to observe at least 1 SAE if the true rate is at least 0.369 and over 90% chance to observe no SAE if the true rate is less than 0.021.

For Groups 2A, 2B, 2C, 2D within each group (n=12), there is greater than a 90% chance to observe at least 1 SAE if the true rate is at least 0.175 and over a 90% chance to observe no SAE if the true rate is no more than 0.009.

If Groups 2A, 2B, 2C, 2D are combined (N=48), there is greater than a 90% chance to observe at least 1 SAE if the true rate is at least 0.047 and greater than a 90% chance to observe no SAE if the true rate is no more than 0.002

Probabilities of observing 0 or more than 1 SAE within each group are presented in **Table 6-1** for a range of possible true event rates and different sample sizes. These calculations provide a

complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-2 gives the upper and lower bounds for 95% exact binomial confidence intervals of the true SAE rate at all possible numbers of events within each group (n=5 and n=12).

For Group 1 (n=5): If none of the 5 participants receiving the vaccines experience SAEs, the 95% exact 2-sided upper confidence bound for the SAE rate is 0.52.

For Groups 2A, 2B, 2C, 2D (n=12): If none of the 12 participants receiving the vaccines experience SAEs, the 95% exact 2-sided upper confidence bound for the SAE rate is 0.265.

Table 6-1: Probability of Events for Different Safety and Immunogenicity Scenarios

	Group 1 (*dose-escalation*): N=5		Groups 2A, 2B, 2C, 2D: N=12		Groups 2A, 2B, 2C, 2D combined: N=48	
True Event Rate	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)
0.01	0.951	0.001	0.886	0.006	0.617	0.083
0.02	0.904	0.004	0.785	0.023	0.379	0.249
0.05	0.774	0.023	0.540	0.118	0.085	0.699
0.1	0.590	0.081	0.282	0.341	0.006	0.960
0.15	0.444	0.165	0.142	0.557	<0.001	0.996
0.2	0.328	0.263	0.069	0.725	<0.001	>0.999
0.3	0.168	0.472	0.014	0.915	<0.001	>0.999
0.4	0.078	0.663	0.002	0.980	<0.001	>0.999
0.5	0.031	0.812	<0.001	0.997	<0.001	>0.999

Table 6-2: 95% Confidence Intervals for the True Rate at All Possible Observed Rates within a Group (n=5 and n=12)

	Group 1: N=5 95% Confidence Interval				Group 2A, 2B, 2C, 2D: N=12 95% Confidence Interval	
Observed Rate	Lower Bound	Upper Bound		Observed Rate	Lower Bound	Upper Bound
0/5	0	0.522		0/12	0	0.265
1/5	0.005	0.716		1/12	0.002	0.385
2/5	0.053	0.853		2/12	0.021	0.484
3/5	0.147	0.947		3/12	0.055	0.572
4/5	0.284	0.995		4/12	0.099	0.651
5/5	0.478	1		5/12	0.152	0.723
				6/12	0.211	0.789
				7/12	0.277	0.848
				8/12	0.349	0.901
				9/12	0.428	0.945
				10/12	0.516	0.979
				11/12	0.615	0.998
				12/12	0.735	1

6.3.2 Power Calculations for Immunogenicity

Table 6-1 gives the probabilities of observing 0 or more than 1 response over a range of underlying response rates and different sample sizes.

Table 6-2 is applicable to the immunogenic response rates and gives the exact 95% confidence interval of the true response rate over possible number of responses out of the 5 and 12 subjects.

6.3.3 Power for Comparison

As exploratory analyses, we will conduct pairwise comparisons between Groups 2A, 2B, 2C, and 2D of the study for possible differences in immunogenicity; a simple comparison on the positive response rate will be used. **Table 6-3** gives the power of Fisher's exact test to compare the schedules over a range of possible response rates. It indicates that a comparison between the groups is not powered by the study design with 12 samples per group.

Table 6-3: Power to Detect Difference in Response Rates between Any Group by Fisher's Exact Test, Where Both Groups are of Size n=12

	Group Y (N=12)						
Group Z (N=12)		0.1	0.2	0.3	0.4	0.5	0.6
	0.1		2	9	23	42	64
	0.2	2		3	9	21	39
	0.3	9	3		4	9	21
	0.4	23	9	4		4	10
	0.5	42	21	9	4		4
	0.6	64	39	21	10	4	

6.4 STATISTICAL ANALYSIS

All statistical analyses will be performed using Statistical Analysis System (SAS), R, or S-Plus statistical software. No formal multiple comparison adjustments will be employed for safety endpoints or secondary endpoints.

6.4.1 Analysis Variables

The analysis variables consist of baseline variables and safety variables for primary and secondary objective analyses.

6.4.2 Baseline Demographics

Baseline characteristics including demographics and laboratory measurements will be summarized using descriptive statistics.

6.4.3 Safety Analysis

6.5.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all assessments.

6.5.3.2 Adverse Events

AEs are coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentages of participants experiencing each specific AE will be tabulated by severity and relationship to treatment. For the calculations in these tables, each participant's AE will be counted once under the maximum severity or strongest recorded causal relationship to treatment.

A complete listing of AEs for each participant will provide details including severity, relationship to treatment, onset, duration and outcome.

6.5.3.3 Local Laboratory Values

Boxplots, violin plots, or beeswarm plots of local laboratory values will be generated for baseline values and for values measured during the course of the study. Each plot will show the 1st quartile, the median, and the 3rd quartile. Outliers, or values outside the boxplot, will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

6.4.4 Immunogenicity Analysis

The statistical analysis for immunogenicity will employ the intent-to-treat principle whereby all data from enrolled subjects will be analyzed according to the group assignment. However, if during immune assessment on stored samples, a subject is found to have a positive antibody response at baseline, the vaccine immune responses assessment for these subjects will not be included in the final immunogenicity analysis. If needed, a per-protocol analysis will be performed as secondary analysis where subjects will be analyzed according to their actual vaccination scheme if it is different from the assigned or up to the last visit in the study if there are early dropouts. The study is not designed to power the comparison in immune responses between vaccine dosages.

If assay data are qualitative (i.e., positive or negative) then analyses will be performed by tabulating the frequency of positive response for each assay at each time point that an assessment is performed. Binomial response rates will be presented with their corresponding exact 95% confidence interval estimates.

Some immunologic assays have underlying continuous or count-type readout that is often dichotomized into responder/non-responder categories. For these assays, graphical and tabular summaries of the underlying distributions will be made. These summaries may be performed on transformed data (e.g., log transformation) for ease of interpretation.

6.4.5 Missing Data

Missing responses will be assumed to be missing completely at random. Analyses will include all samples available at each study time point. Based on experience from previous trials, we expect missing data to be rare. Regardless, in the event of missing data, we will report the occurrence and extent of missingness. We will also provide plausible explanations for the missingness mechanism, should such information be available.

6.4.6 Interim Analyses

Safety Reviews: The PSRT will review safety data routinely throughout the study. The study will utilize both electronic database features and reviews by designated safety review personnel to identify in a timely manner if any of the safety pause rules of the study are met.

Immunogenicity Review: Analyses of immunogenicity may be performed when pseudo-neutralization assay of samples collected 2 weeks after each vaccination are conducted. This may occur prior to completion of safety follow-up visits or collection of data for secondary and exploratory immunogenicity endpoints. Such an analysis would constitute the final analysis for the primary immunogenicity endpoint, so sample size adjustments are not required. Reports providing results by study group may be provided to VRC solely for the purpose of informing decisions related to future trials in a timely manner. The results should in no way influence the conduct of the VRC 321 trial in terms of early termination or later safety or immunogenicity endpoint assessments. Analyses of secondary and exploratory immunogenicity assays may also be performed as data become available.

7. PHARMACY AND ADMINISTRATION PROCEDURES

The study groups and study agent dosing schedule are shown in **Table 1** in **Section 4**. Refer to the IB for further information about the investigational study products.

7.1 STUDY PRODUCT

The study includes one investigational vaccine described as follows:

- VRC-FLUNPF099-00-VP at 180 mcg/mL. Vials contain 0.7 ± 0.1 mL of a clear, colorless, isotonic buffered (pH=7.2) sterile solution. The formulation buffer consists of 20mM Sodium Phosphate, 100mM Sodium Chloride, 5% Sucrose, and 0.01% PF-68. Vials are intended for single use only and do not contain a preservative. Vials must not be refrozen or reused after thawing.

- VRC-PBSPLA043-00-VP (PBS), diluent, is a clear, colorless, sterile solution at pH 7.2, aseptically filled to a volume of 1.2 mL in a 3 mL glass vial. Vials are intended for single use only and do not contain preservative. Vials must not be refrozen or reused after thawing.

7.2 STUDY PRODUCT PRESENTATION AND STORAGE

7.2.1 Study Product Labels

At the time of study product delivery to the pharmacy, labels on study products VRC-FLUNPF099-00-VP and VRC-PBSPLA043-00-VP will have specific product information (e.g., product description, VRC product number, lot number, fill volume, concentration, fill date, storage condition). Labels will contain an Investigational Use Statement ("Limited by Federal Law to Investigational Use") and manufacturer information.

7.2.2 Study Product Storage

VRC-FLUNPF099-00-VP (H1ssF_3928): Vials will be shipped within the recommended temperature range using appropriate shipping configurations to the study pharmacist or designee. Vials of vaccine are stored until use at -35°C to -15°C in a qualified, continuously monitored, temperature-controlled freezer. As freezer temperatures may fluctuate, a temperature range of -45°C to -10°C is acceptable. Storage below -45ºC is not permitted because of the stopper limitation.

VRC-PBSPLA043-00-VP (PBS): Vials are stored until use at the target temperature of -35°C to -15°C in a qualified, continuously monitored, temperature-controlled freezer. As freezer

temperatures may fluctuate, a temperature range of -45°C to -10°C is acceptable based upon historic stability data from studies of similar products.

7.2.3 Temperature excursions

If deviations in storage temperature occur from the normal allowance for the pharmacy freezer, the site pharmacist or designee must report the storage temperature excursion promptly to the PI and IND Sponsor. The excursion must be evaluated and investigated, and action must be taken to restore and maintain the desired temperature limits. Pending the outcome of the investigation, the IND Sponsor will notify the pharmacist if continued clinical use of the product is acceptable.

7.3 Preparation of Study Products for Injection

This section describes how the site pharmacist will prepare study injections. Clinician instructions on how to select an arm and administer an injection are in Section 4.2.5. Refer to the group assignment for the study subject to prepare the correct dose.

For all study groups, the prepared syringe must be labeled with the subject identifier and the date and time after which the preparation may not be used. Filled syringes should be kept at room temperature and out of direct sunlight until product administration. All injections must be administered within 4 hours after removing the vaccine vial from the freezer.

7.3.1 Preparation of VRC-FLUNPF099-00-VP for Needle and Syringe Administration

Subjects will receive the vaccine via needle and syringe injection. The following instructions apply for VRC-FLUNPF099-00-VP preparation. More information on vaccine preparation is available in the IB.

Group 1 (*20 mcg*):

1. Thaw 1 vial of H1ssF_3928 at room temperature (15 to 30º C) until all ice crystals have melted. Swirl gently to mix.
2. Thaw 1 vial of PBS diluent at room temperature (15 to 30º C) until all ice crystals have melted. Swirl gently to mix.
3. Withdraw 0.11 mL of H1ssF_3928 into the syringe.
4. Withdraw 0.2 mL of PBS into the same syringe.
5. Invert syringe 5x to mix.

Group 2A, Group 2B, Group 2C, and Group 2D (*60 mcg*):

1. Thaw 1 vial of H1ssF_3928 at room temperature (15 to 30º C) until all ice crystals have melted. Swirl gently to mix.
2. Withdraw 0.33 mL of H1ssF_3928 into the syringe.

7.3.2 Administration of Injections

Refer to Section 4.2.5. Product labeling and IM injection procedures will be performed consistent with institutional policies and standard procedures.

7.4 Study Product Accountability

7.4.1 Documentation

The study pharmacist or designee will be responsible for maintaining an accurate record of the codes, inventory, and an accountability record of the investigational study products supplies for this study. Electronic documentation as well as paper copies will be used.

7.4.2 Disposition

The empty vials, the partially-used vials, and any unused, unopened vial that has been removed from freezer will be discarded on the same day as removal from freezer in a biohazard containment bag and incinerated or autoclaved per facility policy. Any unopened vials that remain at the end of the study will be discarded or returned at the discretion of the VRC in accordance with policies that apply to investigational products. Partially used vials or expired prepared doses cannot be administered to other subjects nor used for *in vitro* experimental studies and will be discarded as indicated above.

8. HUMAN SUBJECT PROTECTIONS AND ETHICAL OBLIGATIONS

This research study will be conducted in compliance with the protocol, International Council for Harmonisation Good Clinical Practices (ICH-GCP) guidance, and all applicable regulatory requirements.

8.1 INSTITUTIONAL REVIEW BOARD

A copy of the protocol, ICF, other written subject-facing information, and any advertising material will be submitted to the NIAID IRB for written approval prior to use.

The PI must submit and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the ICF. The PI will notify the IRB of events that occur on study as described in Section 5.4.

The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

8.2 SUBJECT IDENTIFICATION AND ENROLLMENT OF STUDY PARTICIPANTS

Study subjects will be recruited through on-site and off-site advertising done for the screening protocol, VRC 500 (11-I-0164). All study activities will be carried out at the NIH CC. Effort will be made to include women and minorities in proportions similar to that of the community from which they are recruited.

8.2.1 Participation of Children

Children are not eligible to participate in this clinical trial because the investigational vaccine has not been previously evaluated in adults. If the product is assessed as safe and immunogenic, other protocols designed for children may be conducted in the future.

8.2.2 Participation of NIH Employees

NIH employees and members of their immediate families may participate in this protocol. NIH staff may be a vulnerable class of subjects. The VRC will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH Information Sheet on Employee Research Participation" and a copy of the "Leave Policy for NIH Employees Participating in NIH Medical Research studies."

Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation. The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees. The employee subject's privacy and confidentiality will be preserved in accordance

with NIH CC and NIAID policies. For NIH employee subjects, consent will be obtained by an individual who is independent of the employee's team. If the individual obtaining consent is a co-worker to the subject, independent monitoring of the consent process will be included through the Bioethics Consultation Service. Protocol study staff will be trained on obtaining potentially sensitive and private information from co-workers or subordinates.

8.3 INFORMED CONSENT

The study ICF describes the investigational product to be used and all aspects involved in protocol participation.

Before a subject's participation in the study, the investigators must obtain written informed consent from the subject, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or study products are administered.

An IRB-approved AoU, intended to assist in the evaluation of the subject's understanding of this study, is administered as part of the consent process.

The acquisition of informed consent will be documented in the subject's medical records, as required by 21 CFR 312.62, and the ICF will be signed and personally dated by the subject and by the person who conducted the informed consent discussion. The original, signed ICF will be placed in the medical record and a copy of the signed ICF will be provided to the subject.

8.4 SUBJECT CONFIDENTIALITY

The investigator must ensure that the subject's anonymity is maintained and will ensure that no information identifying the subject will be released to any unauthorized party. Subjects will not be identified in any reports on this study. All records will be kept confidential to the extent provided by federal, state and local law. Medical records are made available for review when required by the FDA or other authorized users, such as the vaccine manufacturer, only under the guidelines set by the Federal Privacy Act. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects. Stored study research samples are labeled by a code (such as a number) that only the study team can link to the subject. The requirement to maintain subject confidentiality is discussed in the study ICF.

8.5 RISKS AND BENEFITS

8.5.1 Risks of the VRC-FLUNPF099-00-VP vaccine

This is the first study in humans of the investigational vaccine, VRC-FLUNPF099-00-VP. This product is derived from *H. pylori* ferritin and not human ferritin, for this reason it is not expected to interfere with iron storage, transport or iron serum levels [18].

Potential side effects resulting from IM injection include stinging, arm discomfort, redness of the skin or mild bruising at vaccine injection sites.

Subjects may exhibit general signs and symptoms associated with administration of a vaccine, including fever, chills, rash, aches and pains, nausea, headache, dizziness and fatigue. These side effects will be monitored, but are generally short term, mild to moderate severity and

usually do not require treatment.

In another VRC study of a similar ferritin vaccine (VRC 316), only 23.4% of participants were noted to have occasional asymptomatic and self-limited changes in laboratory tests such as mild leukocytosis, mild leukopenia, and mild increase in ALP as of March 24, 2019.

There may be other unknown side effects.

8.5.2 Risks of Specimen Collections

- *Blood drawing*: The risks of blood sample collection are minimal and consist of mild discomfort at the sample collection site. The procedure may cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken.
- *Apheresis*: The procedure may cause pain, bruising, and discomfort in the arms where the needles are placed. It may also cause chills, nausea, heartburn, mild muscle cramps and tingling sensation around the mouth or in the fingers, however this can usually be relieved by slowing or temporarily interrupting the apheresis procedure or taking a calcium containing antacid, such as Tums®. Other possible side effects are anxiety, vomiting and lightheadedness. Temporary lowering of the blood pressure may develop. There is the rare possibility of infection, fainting or seizure. Very rarely a nerve problem at the needle placement site may occur. Also, very rarely, a machine malfunction may occur, resulting in the loss of about one unit of blood. There may be additional risks of apheresis that are unknown at this time.
- *Mucosa samples*: Collection of samples by nasopharyngeal swabs rubbed over the mucosal surfaces may cause momentary discomfort and, in some cases, minor bleeding.

8.5.3 Risks of study vaccines on the fetus or nursing infant

We do not know the possible effects of the study vaccine on the fetus or nursing infant. Women of reproductive potential will be required to agree to use an effective method of birth control beginning 21 days prior to enrollment and continuing through end of study.

Because this is a research study, women of reproductive potential will be tested for pregnancy prior to administration of each study injection and asked to notify the site immediately upon learning of a pregnancy during this study. In the case of pregnancy, subjects will no longer receive additional vaccine but will continue to be followed for safety. Research sample collections will be discontinued for pregnant women. The subject will be contacted to ask about the outcome of a pregnancy that begins during the study.

8.5.4 Risks of New Diagnoses

It is possible that the standard medical tests performed as part of this research protocol will result in new diagnoses. Depending upon the medical findings and consequences of being provided with the new medical information about health status, the study subject may view this aspect of study participation as either a risk or a benefit. Any such information will be shared and discussed with the subject and, if requested by the subject, will be forwarded to the subject's primary health care provider for further workup and management.

8.5.5 Study Benefits

Study subjects will not receive direct health benefit from study participation. This protocol is not designed to provide treatment for any condition. Others may benefit from knowledge gained in

this study that may aid in the development of an H1 (or universal) influenza virus vaccine. The investigational vaccine is not expected to provide protection from influenza.

8.6 PLAN FOR USE AND STORAGE OF BIOLOGICAL SAMPLES

The plan for use and storage of biological samples from this protocol is as outlined in the following sections.

8.6.1 Use of Samples, Specimens and Data

Samples, specimens and data collected under this protocol may be used to conduct protocol related safety and immune response evaluations, exploratory laboratory evaluations related to the type of infection the study product was designed to prevent, exploratory laboratory evaluations related to vaccine or infectious disease research in general and for research assay validation.

Genetic testing may be performed in accordance with the genetic testing information that is included in the study ICF. HLA testing may be done in association with identifying factors linked with the immune response development or progression of infections.

Additional optional genetic testing, including transcriptome sequencing may be done on collected specimens in an effort to assess the expression of genes involved in the immune response to vaccination.

Results of genetic testing may have psychological implications for patients such as revelations regarding future health risks, incurable conditions, and/or information contradictory to stated biological relationships. Genetics counseling and advice will be available from the NIH to help subjects at all study sites with the implications of findings, where appropriate.

Following genetic testing, the data will be shared in a controlled-access public database for other investigators to benefit from it (e.g. the Database of Genotypes and Phenotypes dbGAP). However, no personal identifiable information will be shared in this process as the results will only be shared with a code. Other optional analysis, including proteome, lipidome, metabolome, and exosome may be done on collected specimens to evaluate some proteins, lipids, metabolites, and low molecular weight molecules involved in the immune response to vaccination.

8.6.2 Storage and Tracking of Blood Samples and Other Specimens

All of the stored study research samples are labeled by a code that only the site can link to the subject. Samples are stored at the VIP, Gaithersburg, MD or VRC laboratories in Building 40, Bethesda, MD, which are both secure facilities with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data. Samples will be tracked in the Laboratory Information Management System (LIMS) database or using another software designed for this purpose (e.g., Freezerworks).

8.6.3 Disposition of Samples, Specimens and Data at Completion of the Protocol

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. IRB approval must be sought prior to any sharing of samples. Any clinical information shared about those samples would similarly require prior IRB approval. The research use of stored, unlinked or unidentified samples may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the NIH Office of Human Subjects Research, which is authorized to determine whether a research activity is exempt.

At the time of protocol termination, samples will remain in the VIP facility or VRC laboratories or, after IRB approval, transferred to another repository. Regulatory oversight of the stored samples and data may be transferred to a stored samples protocol as part of the IRB-approved termination plan. Data will be archived by the VRC in compliance with requirements for retention of research records, or after IRB and study sponsor approval, it may be either destroyed or transferred to another repository.

8.6.4 Loss or Destruction of Samples, Specimens or Data

The NIH Intramural Protocol Deviation definition related to loss of or destruction of samples or data will be followed. Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB in accordance with institutional policies. The PI will also notify the IRB if the decision is made to destroy the remaining samples.

8.7 COMPENSATION

Subjects will be compensated for time and inconvenience in accordance with the standards for compensation of the NIH Clinical Research Volunteer Program. The compensation per visit will be $275 for visits that include injections and $175 for clinic visits that include a blood draw. Any visit that includes mucosal sample collection will result in an additional $50 in compensation. The compensation for any clinic visit that does not include a blood draw or mucosal sample collection will be $75. The compensation for timely completion of the electronic diary card will be $25. Compensation will be $250 for apheresis, if performed. The total compensation for the subject is based on the number of study clinic visits, injections completed and if optional research blood collections are performed.

Subjects will receive compensation by direct deposit within approximately 1 or 2 weeks after each completed visit. Compensation may need to be reported to the Internal Revenue Service (IRS) as taxable income.

The approximate total compensation is as follows:

Group	Without Apheresis	With Apheresis
Group 1	$1,875	NA
Group 2A Group 2B Group 2C Group 2D	$2,525	$2,600

8.8 SAFETY MONITORING

8.8.1 Protocol Safety Review Team

Close cooperation between the designated members of the Protocol Team will occur to evaluate and respond to individual AEs in a timely manner. The VRC designated Safety Officer for the day conducts a daily safety review of clinical data per VRC Standard Operating Procedures. The

PSRT, comprised of the PI, Associate Investigators, Study Coordinator, Protocol Specialists, and other Study Clinicians, will review the summary study safety data reports on a weekly basis through 4 weeks after the last subject receives the final study injection. After this time, the PSRT will monitor the safety data reports on a monthly basis through completion of the last study visit.

9. ADMINISTRATIVE AND LEGAL OBLIGATIONS

9.1 PROTOCOL AMENDMENTS AND STUDY TERMINATION

Agreement from the PI must be obtained for all amendments to the protocol and the informed consent document. All study amendments will be submitted to the IRB for approval.

The VRC, NIAID IRB, Office of Human Research Protections, study PI, and FDA reserves the right to terminate the study. The PI will notify the IRB in writing of the study's completion or early termination.

9.2 STUDY DOCUMENTATION AND STORAGE

The PI will maintain a list of appropriately qualified persons to whom trial duties have been delegated.

Source documents are original documents, data, and records from which the subject's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, and correspondence.

The PI and staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the VRC, IRB, FDA, and/or applicable regulatory authorities. Elements include but not limited to:

- Subject files containing completed informed consent forms, and supporting copies of source documentation (if kept)
- Study files containing the protocol with all amendments, Investigator Brochures, copies of all correspondence with the IRB and VRC

In addition, all original source documentation must be maintained and be readily available.

All essential documentation will be retained by the institution for the same period of time required for medical records retention. The FDA requires study records to be retained for up to two years after marketing approval or refusal (21 CFR 312.62). No study document will be destroyed without prior written agreement between the VRC and the PI.

9.3 DATA COLLECTION, DATA SHARING AND PROTOCOL MONITORING

9.3.1 Data Collection

Clinical research data will be collected in a secure electronic web-based clinical data management system (CDMS) through a contract research organization, The Emmes Company, LLC (Rockville, MD). Extracted data without patient identifiers will be sent to the Protocol Statistician for statistical analysis.

9.3.2 Data Sharing Plan

Data generated in this study will be shared as de-identified data in the government-funded public repository, www.ClinicalTrials.gov. Data may be shared prior to publication at approved public presentations or for collaborative development and will be shared at the time of publication or within 1 year of the primary completion date.

9.3.3 Source Documents

The site will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP, regulatory and institutional requirements for the protection of confidentiality of subjects.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include but are not limited to medical records, laboratory reports, pharmacy records and other research records maintained for the clinical trial.

9.3.4 Protocol Monitoring Plan

Site investigators will allow the study monitors, the NIAID IRB, and the FDA to inspect study documents (e.g., consent forms, drug distribution forms, and case report forms) and pertinent hospital or clinic records for confirmation of the study data.

Site visits by study monitors will be made in accordance with the study monitoring plan to monitor the following: study operations, the quality of data collected in the research records, the accuracy and timeliness of data entered in the database, and to determine that all process and regulatory requirements are met. Study monitoring visits will occur as defined by the IND Sponsor approved monitoring plan.

9.4 LANGUAGE

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood by the subject.

9.5 POLICY REGARDING RESEARCH-RELATED INJURIES

The NIH CC will provide short-term medical care for any injury resulting from participation in this research. In general, the NIH, the NIH CC, or the U.S. Federal Government will provide no long-term medical care or financial compensation for research-related injuries.

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APPENDIX I: SCHEDULE OF EVALUATIONS

		VRC 500	VRC 321 Schedule of Evaluations: Group 1										
Visit Number		*01	02	02A	03	04	05	06	07	09	11	12	13
Week of Study		-4 to 0	W0	W1	W1	W2	W4	W12	W16	W18	W28	W40	W52
Day of Study		-28 to 0	[1]D0	D1	D6	D14	D28	D84	D112	D126	D196	D280	D364
Clinical	Tube												
*VRC 500 Screening Consent		X											
VRC 321 AoU; Consent			X										
[2]Physical exam for eligibility, height /weight/ vitals at screening; vital signs and targeted exam (as needed) other visits.		X	X		X	X	X	X	X	X	X	X	X
Medical history targeted to eligibility at screening; then interim medical history		X	X		X	X	X	X	X	X	X	X	X
[3]Study Product Administration: **Group 1**			**X**										
Phone evaluation (clinic visit as needed)				X									
Begin diary card			X										
[4]Pregnancy test: urine or serum		X	X				X					X	X
[4]Pregnancy prevention counseling/ Reproductive Information Form		X	X				X					X	X
CBC with differential	EDTA	3	3			3	3					3	3
Iron and serum ferritin		X					X						
Total bilirubin, AST, ALT, and ALP	GLT	4	4			4	4					4	4
Creatinine		X	X			X							
[5]HLA type	EDTA										20		
HIV Ab/Ag Combo (other tests, if needed)	EDTA	3											
Research Samples													
H. pylori ferritin antibody and human ferritin antibody	SST	X					X						
Serum	SST	32	16		16	16	16	16	16	16	16	16	16
PBMC and plasma	EDTA	80	80		[6]80	80	80	80	80	40	80	80	80
Daily Volume (mL)		122	103	0	96	103	103	96	96	56	116	103	103
Max. Cumulative Volume (mL)		122	225	225	321	424	527	623	719	775	891	994	1,097

* VRC 500: Screening evaluations must be no more than 28 days prior to Day 0 to be used for eligibility (negative pregnancy test from Day 0 must be used for eligibility). If clinical assessment on Day 0 suggests significant changes may have occurred since screening, then physical examination & laboratory studies done on Day 0 are used for eligibility.

[1] Day 0=day of enrollment and first vaccine injection. Day 0 evaluations prior to first injection are the baseline for assessing adverse events subsequently.

[2] Screening visit includes physical exam with vital signs. At other visits, physical exam is done if indicated. Otherwise only blood pressure (BP), pulse, temperature, and respiration are required.

[3] Study Product Administration: **Group 1** will receive H1ssF_3928 20 mcg IM at Day 0. Complete post vaccination evaluations (BP, pulse, temperature, respiration and injection site assessment) at **1 hour** or longer after injection.

[4] Negative pregnancy test results must be confirmed for women of reproductive potential prior to each study injection.

[5] HLA type blood sample is collected once at any time-point in the study and is shown as a Visit 05 evaluation for convenience; however, if HLA type is already available in the medical record, it does not need to be repeated. HLA type may also be obtained from a frozen sample.

[6] Visit 03: Two tubes of PBMC collected in EDTA tubes will be sent to Building 40, while the remainder of the blood collected will be sent to VIPVITL.

Visit windows: Schedule Visits 02A - 13 with respect to Day 0 per the following visit windows: Visit 02A (+1 day). Visit 03 (±1 day). Visits 04, 05, 09 (±2 days). Visits 07 (± 7 days). Visit 06 (±14 days). Visits 11, 12 and 13 (± 14 days).

		VRC 500	VRC 321 Schedule of Evaluations: Groups 2A, 2B, 2C, 2D														
Visit Number		*01	02	02A	03	04	05	06	07	07A	08	09	10	11	12	13	14
Week of Study		-4 to 0	W0	W1	W1	W2	W4	W12	W16	W17	W17	W18	W20	W28	W40	W52	W68
Day of Study		-28 to 0	[1]D0	D1	D6	D14	D28	D84	D112	D113	D118	D126	D140	D196	D280	D364	D476
Clinical	Tube																
*VRC 500 Screening Consent		X															
VRC 321 AoU; Consent			X														
[2]Physical exam for eligibility, height /weight/ vitals at screening; vital signs and targeted exam (as needed) other visits.		X	X		X	X	X	X	X		X	X	X	X	X	X	X
Medical history targeted to eligibility at screening; then interim medical history		X	X		X	X	X	X	X		X	X	X	X	X	X	X
[3]Study Product Administration: **Group 2A-2D**			**X**						**X**								
Phone evaluation (clinic visit as needed)				X						X							
Begin diary card			X														
[4]Pregnancy test: urine or serum		X	X				X		X			[8][X]	X		X	X	X
[4]Pregnancy prevention counseling/ Reproductive Information Form		X	X				X		X			[8][X]	X		X	X	X
CBC with differential	EDTA	3	3			3	3		3				3		3	3	3
Iron and serum ferritin		X							X				X				
Total bilirubin, AST, ALT, and ALP	GLT	4	4			4	4		4				4		4	4	4
Creatinine		X	X			X							X				
[5]HLA type	EDTA													20			
HIV Ab/Ag Combo (other tests, if needed)	EDTA	3															
Research Samples																	
H. pylori ferritin antibody and human ferritin antibody	SST	X					X						X				
Serum	SST	32	16		16	16	16	16	16		16	16	16	16	16	16	16
PBMC and plasma	EDTA	80	80		[6]80	80	80	80	80		80	[7]120 or Apheresis (6)	80	80	80	80	80
Daily Volume (mL)		122	103	0	96	103	103	96	103	0	96	136	103	116	103	103	103
Max. Cumulative Volume (mL)		122	225	225	321	424	527	623	726	727	822	958	1,061	1,177	1,280	1,383	1,486

* VRC 500: Screening evaluations must be no more than 28 days prior to Day 0 to be used for eligibility (pregnancy test from Day 0 must be used for eligibility). If clinical assessment on Day 0 suggests significant changes may have occurred since screening, then physical examination & laboratory studies done on Day 0 are used for eligibility.

[1] Day 0=day of enrollment and first vaccine injection. Day 0 evaluations prior to first injection are the baseline for assessing adverse events subsequently.

[2] Screening visit includes physical exam with vital signs. At other visits, physical examination is done if indicated. Otherwise only blood pressure (BP), pulse, temperature, and respiration are required.

[3] Study Product Administration: **Groups 2A-2D** will receive H1ssF_3928 60 mcg IM at Day 0 and Week 16. Complete post vaccination evaluations (BP, pulse, temperature, respiration and injection site assessment) at **30 minutes** or longer after each injection for **Group 2A-2D**.

[4] Negative pregnancy test results must be confirmed for women of reproductive potential prior to each study injection and prior to apheresis.

[5] HLA type blood sample is collected once at any time-point in the study and is shown as a Visit 05 evaluation for convenience; however, if HLA type is already available in the medical record, it does not need to be repeated. HLA type may also be obtained from a frozen sample.

[6] Visit 03: Two EDTA tubes for PBMC collected will be sent to Building 40, while the remainder of the blood collected will be sent to VIP.

[7] If optional Apheresis occurs, ONLY draw 16 mL in SST (DO NOT draw 120 ml in EDTA tubes).

[8] For women of reproductive potential, pregnancy test must be negative within 72 hours prior to apheresis procedure.

Visit windows: Schedule Visits 02A - 07 with respect to Day 0; Schedule Visits 07A - 13 with respect to Visit 07. The following visit windows apply: Visits 02A and 07A (+1 day). Visits 03 and 08 (±1 day). Visits 04 and 09 (±2 days). Visits 05 and 10 (±2 days). Visits 06 and 11 (±14 days). Visit 07 (±14 days). Visits 12, 13 and 14 (±14 days).

APPENDIX II: ASSESSMENT OF RELATIONSHIP TO VACCINE AND GRADING SEVERITY OF ADVERSE EVENTS

Assessment of Relationship of an Adverse Event to Study Vaccine:

The relationship between an AE and the vaccine will be assessed by the investigator on the basis of his or her clinical judgment and the definitions below.

- **Definitely Related**. The AE and administration of study agent are related in time, and a direct association can be demonstrated.
- **Probably Related**. The AE and administration of study agent are reasonably related in time, and the AE is more likely explained by study agent than other causes.
- **Possibly Related**. The AE and administration of study agent are reasonably related in time, but the AE can be explained equally well by causes other than study agent.
- **Not Related**. The AE is clearly explained by another cause not related to the study product.

For purposes of preparing summary data reports in which AE attributions are simplified to "Related" or "Not Related", in this protocol, the "Definitely, Probably and Possibly" attributions above will be mapped to the "Related" category, while the "Unlikely/Probably Not Related" and "Not Related" attributions above will be mapped to the "Not Related" category. The definitions that apply when these two attribution categories alone are used are as follows:

- **Related** – There is a reasonable possibility that the AE may be related to the study product(s).
- **Not Related** – There is not a reasonable possibility that the AE is related to the study product(s).

Grading the Severity of Adverse Events:

The FDA Guidance for Industry (September 2007): "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" is the basis for the severity grading of AEs in this protocol. Several modifications were made to the table as follows:

- "Emergency room visit" is not automatically considered a life-threatening event; these words have been removed from any "Grade 4" definition where they appear in the table copied from the guidance document.
- Laboratory value shown as a "graded" value in the table that is within the institutional normal range will not be severity graded or recorded as an AE.
- Severity grading for hemoglobin decrease on the basis of the magnitude of decrease from baseline is not applicable at the Grade 1 level; only absolute hemoglobin will be used to define Grade 1.
- Severity grading for Grade 4 local reaction to injectable product (Erythema/Redness and Induration/Swelling) refer to necrosis or exfoliative dermatitis "requiring medical attention."
- Bruising or skin lesion associated with study injection will be assessed using the same severity grading as for erythema/redness.

When not otherwise specified, the following guidance will be used to assign a severity grade:

- **Grade 1 (Mild)**: No effect on activities of daily living
- **Grade 2 (Moderate)**: Some interference with activity not requiring medical intervention
- **Grade 3 (Severe)**: Prevents daily activity and requires medical intervention
- **Grade 4 (Potentially Life-threatening)**: Hospitalization; immediate medical intervention or therapy required to prevent death.
- **Grade 5 (Death)**: Death is assigned a Grade 5 severity. Only the single AE that is assessed as the primary cause of death should be assigned "Grade 5" severity.

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials
Modified from FDA Guidance - September 2007

A. Tables for Clinical Abnormalities

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Hospitalization
[1,2]Erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis requiring medical attention
[3] Induration/Swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis requiring medical attention
[4] Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
[5]Fever (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104

[4] **Vital Signs**	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia
[6]Bradycardia - beats per Minute	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	Hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

1. In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
2. Bruising or skin lesion associated with study injection will be assessed using the same severity grading as for erythema/redness.
3. Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.
4. Subject should be at rest for all vital sign measurements.
5. Oral temperature; no recent hot or cold beverages or smoking.
6. When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing
   Bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	Hospitalization for hypotensive shock
Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**

Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	Hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	Hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	Hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	Hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Hospitalization

B. Tables for Laboratory Abnormalities

The laboratory values provided in the tables below serve as guidelines and are dependent upon the institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4) ****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	>1.5 – 3.0 x ULN	>3.0 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 – 10 x ULN	> 10 x ULN

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4) ****
Liver Function Tests – ALT, AST increase by factor	1.1 – 2.5 x ULN	> 2.6 – 5.0 x ULN	> 5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	> 1.26 – 1.5 x ULN	> 1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***ULN" is the upper limit of the normal range.

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) decrease from baseline value - gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) decrease from baseline value – gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.10 x ULN**	> 1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.10 – 1.20 x ULN	1.21 – 1.4 x ULN	1.4 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

**ULN" is the upper limit of the normal range.

PRINCIPAL INVESTIGATOR: Alicia Widge, MD, MS

STUDY TITLE: VRC 321: A Phase I Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability, and Immunogenicity of an Influenza H1 Stabilized Stem Ferritin Vaccine, VRCFLUNPF099-00-VP, in Healthy Adults

STUDY SITE: NIAID/VRC

Cohort: *Healthy volunteer*

Consent Version: *3.0, April 20, 2020*

WHO DO YOU CONTACT ABOUT THIS STUDY?

Principal Investigator: Alicia Widge, MD, MS; 301-761-7968; alicia.widge@nih.gov

Study Coordinator: Floreliz Mendoza, RN; 301-451-8715; mendozaf@mail.nih.gov

This consent form describes a research study and is designed to help you decide if you would like to be a part of the research study.

You are being asked to take part in a research study at the National Institutes of Health (NIH). Members of the study team will talk with you about the information described in this document. Some people have personal, religious, or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). Take the time needed to ask any questions and discuss this study with NIH staff, and with your family, friends, and personal health care providers. Taking part in research at the NIH is your choice.

IT IS YOUR CHOICE TO TAKE PART IN THE STUDY

You may choose not to take part in this study for any reason. If you join this study, you may change your mind and stop participating in the study at any time and for any reason. In either case, you will not lose any benefits to which you are otherwise entitled. However, to be seen at the NIH, you must be taking part in a study or are being considered for a study. If you do choose to leave the study, please inform your study team to ensure a safe withdrawal from the research.

WHY IS THIS STUDY BEING DONE?

This is the first study in people of this experimental vaccine for prevention of H1 influenza (flu). "Experimental" means that the study vaccine has not been approved by the Food and Drug

IRB NUMBER: 19I0032
IRB APPROVAL DATE: 05/18/2020

Administration (FDA). The FDA allows this vaccine to be used for research purposes only. We do not know if this vaccine works. The main purpose of this study is to see if the experimental vaccine is safe and if there are any side effects. We also want to study immune responses to this vaccine.

STUDY VACCINE

Vaccines are given to teach the body to prevent or fight an infection. In this study, we are testing one experimental vaccine that was developed by the Vaccine Research Center (VRC) at the NIH: H1 Stabilized Stem Ferritin vaccine, VRC-FLUNPF099-00-VP. In this consent form, the study vaccine will be called the "H1 Ferritin vaccine."

This vaccine is intended to help the body to make an immune response to H1 flu, which is a strain of flu that infects humans.

Most vaccines are made of proteins that are injected into a muscle. Proteins are natural substances that the body uses as building blocks. This vaccine is made in the laboratory with two proteins: one protein from the H1 flu virus and one protein from a type of bacteria called *Helicobacter pylori* (*H. pylori*). These two proteins have been modified in the laboratory. When combined, they make a particle that looks like the outside of the H1 flu virus. The body's immune system may respond to this particle. There is no virus or bacteria in the vaccine, so you cannot get an H1 flu infection or *H. pylori* infection from this vaccine.

We do not know if the H1 Ferritin vaccine will protect you from flu.

ELIGIBILITY

You are eligible to take part in this study because you have completed the screening process and are:

- 18-70 years old
- In general good health without significant medical problems as determined at screening
- Willing to get the experimental vaccine
- Willing to donate blood samples for future research
- Willing to use birth control from at least 21 days prior to enrollment through the end of the study, if female and able to become pregnant
- Must have received a licensed influenza (flu) vaccine at least once since January 1, 2014.

STUDY PLAN

About 53 people will take part in this study at the NIH Clinical Center in Bethesda, MD.

If you are enrolled in Group 1, you will get one vaccination and remain in the study for about one year.

If you are enrolled in Groups 2A, Group 2B, Group 2C, or Group 2D you will get two vaccinations and remain in the study for about one year and three months.

You will get the experimental vaccine by injections (shots) in the upper arm muscle. This is called an intramuscular "IM" injection. We will use a needle and syringe to give you the H1 Ferritin vaccine. We will check you for any side effects from the vaccine.

We will tell you if we learn anything new during this study that might cause you to change your mind about staying in the study. At the end of the study, we will tell you when study results may be available and how to learn about them.

There will be two different doses of vaccine given in this study, a lower dose (20 mcg) and a higher dose (60 mcg).

Group 1 will get the lower dose of the H1 Ferritin vaccine one time on the day of enrollment. If there are no safety concerns at this dose after 2 weeks of follow-up, we will start enrolling people to get the higher dose.

Groups 2A, 2B, 2C, 2D will get the higher dose of the H1 Ferritin vaccine on the day of the enrollment and about 4 months later (week 16). Groups are based on age. This is done so that researchers may compare the immune response of people of different ages.

The table below shows the study plan:

Group	Age	Subjects	Day 0	Week 16
1	18-40	**5**	20 mcg	
2A	18-40	**12**	60 mcg	60 mcg
2B	41-49	**12**	60 mcg	60 mcg
2C	50-59	**12**	60 mcg	60 mcg
2D	60-70	**12**	60 mcg	60 mcg
Total		**53**		

STUDY PROCEDURES

If you agree to take part in the study and are enrolled in:

Group 1: you will have 1 vaccination visit, 1 phone follow-up, and 9 follow-up clinic visits.

Groups 2A, 2B, 2C, 2D: you will have 2 vaccination visits, a phone follow-up after each vaccination, and 11 follow-up clinic visits.

If you are a woman who can get pregnant, we will do a pregnancy test before each injection. The test must show that you are not pregnant before you can get the injection.

Each vaccination visit will take about 4 to 6 hours. Most follow-up clinic visits will take about 1 to 2 hours; the optional apheresis visit will take 2 to 4 hours.

If you are enrolled in Group 1 you will need to stay in the clinic for at least 1 hour after your vaccination. If you are enrolled in Group 2A, 2B, 2C, or 2D, you will need to stay in the clinic for at least 30 minutes after each vaccination.

After each vaccination, the clinic staff will call to check on you. Also, after each vaccination, you will need to complete a diary card for 7 days. The purpose of the diary card is to record any symptoms that you may have for data analysis and not because of any risk of getting the flu from this vaccine. We will give you a thermometer to record your temperature even if you feel well. We will also give you a ruler to measure any skin changes at the injection site. You will get a password to a secure website where you can enter this data online. If you do not have access to the internet, you can use a paper diary card instead.

If you have any symptoms or feel unwell, you should tell a clinic nurse or doctor as soon as possible. You can reach the staff by phone 24 hours a day. If you have symptoms, you may be asked to come to the clinic for a checkup. It is very important that you follow the instructions from the clinic staff.

At each visit, we will check you for any health changes or problems. We will ask you how you are feeling and if you have taken any new medications. We will draw your blood at each visit, taking about 6 to 14 tubes of blood per visit. We will tell you right away if any of your test results show a health problem. Some blood samples will be used for research only so that we can study your immune response to the vaccine. Results of these research tests are not used to check your health and will not be given to you.

Mucosal Swab: It is very important to report symptoms including fever of 100^0 F or higher, runny nose, sore throat, headache, feeling more tired than usual or muscle aches. This group of symptoms may be the flu or a flu-like illness. You will need to come to the clinic so that we can do a nose and throat swab. We will use a thin disposable swab to collect this sample. Each swab is new and sterile and is safe for use in sensitive parts of the body.

Apheresis: For subjects enrolled in Group 2A, Group 2B, Group 2C, and Group 2D, after your last study injection, we would like to collect your blood by a method called "apheresis." This procedure is optional and choosing not to take part in it will not affect your study participation.

To be eligible for apheresis:

- You must not have an unstable heart as indicated by your medical history and test results
- You must not have blood pressure greater than 180/100
- You must not have a known blood clotting disorder
- You must not be pregnant or breast feeding
- You must not have a condition that the attending physician or the apheresis clinic staff considers a reason to not do an apheresis procedure.

Before apheresis, we will check your weight, pulse and blood pressure. We will ask questions about your general health and medical history. If you are a woman who can get pregnant, we will do a pregnancy test before the apheresis procedure. The test must show that you are not pregnant.

During the procedure, you will lie on a recliner, couch, or hospital bed. The procedure requires that a needle be placed into a vein in both arms using a sterile method. The kits used to collect apheresis samples are sterile, single-use, disposable sets that are not in contact with any person's bodily fluids other than yours. No blood products are given to you during these procedures. The apheresis is done at the NIH Clinical Center, and a physician from the NIH Department of Transfusion Medicine will be available in or near the apheresis area at all times.

In the apheresis procedure, blood is removed through a needle in the vein of one arm, spun in a machine that separates the desired blood components (white blood cells) and then the rest of your blood is returned to you through a needle in the other arm. Citrate, a medication to prevent blood from clotting, is added to the blood while in the machine to prevent it from clotting.

The purpose of this procedure is to allow us to get and study a larger number of white blood cells that cannot be collected by simple blood drawing. The number of white blood cells collected is a small fraction of the total amount in your body. The body quickly replaces cells that have been removed. The Blood Bank of the NIH Clinical Center and other blood banks use similar procedures every day to collect blood samples from donors and as a form of therapy for some diseases. However, we will not use your samples for transfusion or therapy. The procedure will take approximately 1-3 hours.

MONITORING OF THE STUDY

This study will be monitored by a group of physicians and scientists at NIH. This group will review the study information and will pay close attention to any reactions. If there are serious side effects, study injections may be delayed or canceled.

GENETIC TESTING

Some of the blood drawn from you during this study will be used for genetic tests. Some genetic tests are done in research studies to see if genetic differences in people cause different types of immune responses. Your blood sample used in these genetic tests will not have your name on it and the results will not be in your medical record except for Human Leukocyte Antigen (HLA) typing results. These tests are not used to check your health and we will not tell you the results.

A special genetic test, called HLA typing, is done by the NIH Clinical Center medical laboratory. These results will be in your medical record, but they will not be used to check your health. Any genetic testing, including HLA testing, is for research purposes only. Any genetic information collected or learned about you will be kept confidential. Medical records, including HLA test results are kept securely. We will not give any genetic information that is in your medical record to anyone without your permission.

STORED SAMPLES

We will collect blood samples from you during the study. We will keep these samples for future research indefinitely to learn more about flu virus, vaccines, the immune system, and other research questions. Results from research with your samples will not be in your medical record or reported to you.

Labeling of Stored Samples: Your stored samples will be labeled by a special code or number and not your personal information. Only the study team can link this code to you. Any identifying information about you (like name or date of birth) will be kept as confidential as allowable by law.

Risks of Stored Samples: There is a risk of unplanned release of information from your medical record. The chance that this information will be given to an unauthorized person without your permission is very small. Possible problems with the unplanned release of information include discrimination when applying for insurance or employment. Similar problems may occur if you give information about yourself or agree to have your medical record released.

Future studies: In the future, other investigators at NIH or outside of NIH may wish to study your stored samples. When your stored samples are shared, they will be marked with a code. Your samples will not have any identifying information on them. Some information about you, like your gender, age, health history, or ethnicity may be shared with other researchers. Any future research

studies using your samples will be conducted in a way that protects the rights and privacy of study participants.

Your stored samples will only be used for research and will not be sold. The research done with your materials may be used to develop new products in the future, but you will not receive payment for such products.

Making your Choice: You cannot take part in this study if you do not want us to collect or store your blood samples. If you agree to take part in this study, you must also agree to let us keep any of your samples and data for future research.

If you decide not to take part in this study, you may still take part in other studies at NIH.

POSSIBLE STUDY RISKS

***Possible* risks of injections:** Temporary stinging, pain, redness, soreness, itchiness, swelling, or bruising.

***Possible* risks of any vaccine:** Fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired and/or unwell. These types of reactions are usually greatest within the first 24 hours after vaccination and typically last 1 to 3 days. Over-the-counter medicine, like acetaminophen (Tylenol) or ibuprofen, may be used to help these symptoms.

Very rarely, a serious allergic reaction with symptoms like hives, trouble breathing, or sudden weakness may occur shortly after any vaccination. This is called "anaphylaxis" and may be life-threatening. While you are waiting in the clinic after the vaccination, we will monitor you for anaphylaxis. Treatment for anaphylaxis will be given right away if it occurs.

***Possible* risks of the H1 Ferritin vaccine**: The H1 Ferritin vaccine has not been given to people before. It may have unknown risks. It has been tested in mice and rabbits. The vaccine did not cause any unusual side effects and met the safety criteria to be tested in humans.

***Possible* risks of blood drawing:** Pain, bleeding, bruising, feeling lightheaded, and fainting. Rarely, infection may occur at the site where the blood is taken.

***Possible* risks of mucosal sample collection:** Samples collected by rubbing swabs over mucosal surfaces in the mouth or in the nose can cause brief discomfort, or a little bleeding.

***Possible* risks of apheresis**: Apheresis is generally safe and side effects are rare. Pain, bruising or discomfort at the needle placement site may occur. Sometimes apheresis causes a tingling sensation around the lips, nose and mouth, coolness all over and slight nausea. This can usually be relieved by slowing or temporarily stopping the apheresis or taking an antacid with calcium pill, like Tums®. Other possible side effects are anxiety, vomiting and lightheadedness. Temporary lowering of the blood pressure may develop. There is the rare possibility of infection, fainting or

seizure. Very rarely a nerve problem at the needle placement site may occur. Also, very rarely, a machine malfunction may occur and result in the loss of about one unit (one pint) of blood.

There are theoretical risks from re-infusion of the blood after processing by the machine such as infection or an adverse reaction to the blood components. However, this has not been seen in many thousands of volunteers who have undergone this or similar procedures to date. There may be other risks of apheresis that are unknown at this time.

***Unknown* safety risks**: There may be side effects from the study vaccines - even serious or life-threatening ones- that we do not yet know about. Please tell the study staff about any side effect you think you are having. This is important for your safety.

***Possible* risks of genetic testing**: Unplanned release of information that could be used by insurers or employers to discriminate against you or your family; discovering a gene that suggests risk of disease for you or your family; discovering unknown family relationships.

***Possible* risks of data sharing:** Information in the shared databases could be linked back to you and used to discriminate against you or your family. State and federal laws provide some protections against genetic and pre-existing conditions discrimination.

***Possible* risks during pregnancy**: We do not know how the experimental vaccines may affect a fetus or nursing infant. Therefore, women who can become pregnant must have a negative pregnancy test before each injection and agree to use effective birth control beginning at least 21 days before the first injection until the end of the study. We will discuss effective methods of birth control with you.

You must tell the clinic staff right away if you become pregnant or think that you might be pregnant during the study. If you are pregnant, you will not get any more vaccinations. You will be asked to continue with follow-up visits so that we can check your health. We will ask you the outcome of the pregnancy.

***Possible* other risks**: We do not know if the study vaccine will change how your body responds to flu virus infections in the future.

You may not donate blood at a blood bank while taking part in this study. You may not donate blood for one year after the last experimental vaccine injection.

POSSIBLE BENEFITS

This study is not designed to benefit you. We do not know if the vaccines will work. The study is not designed to protect you from flu. You and others may benefit in the future from the information that will be learned from the study. The study visits are used to check your health for research purposes, not to provide health care. However, we will tell you right away if any of your test results show a possible health problem.

REASONS FOR STOPPING STUDY INJECTIONS

You may not get all of your planned study vaccinations. Reasons for this may include:

- You don't keep appointments or follow procedures
- You get a serious illness that needs ongoing medical care.
- You have a serious side effect, thought to be due to the study vaccine.
- You become pregnant.
- You need to get treatment with a medication that affects your immune system (such as a steroid like prednisone).
- The study is stopped by regulatory agencies, the study sponsor or study investigators. If this happens, we will tell you why.

If you agree to take part in this study, it is important for you to keep all of your appointments. Your participation in this study is completely voluntary. You can choose to stop taking part in the study at any time. There is no penalty or loss of benefits if you choose to leave the study.

If you are not able to get the second injection (for Groups 2A, Group 2B, Group 2C, and Group 2D), we will adjust your schedule but will ask that you still have study visits. It is important that we continue to check your health even if you do not get a second injection.

ALTERNATIVES

This study is not designed to treat or prevent any disease. You may choose to not take part in this study. You may be eligible for other studies.

CONFLICT OF INTEREST

The NIH research staff is checked yearly for conflicts of interest. You may ask the research team for more information. This study may have investigators who are not NIH employees. Non-NIH investigators are expected to follow the principles of the Protocol Review Guide but are not required to report their personal financial holdings to the NIH.

The NIH, including some members of the VRC scientific staff, developed the investigational vaccines being used in this research study. The results of this study could play a role in whether the FDA will approve the vaccine for sale at some time in the future. If approved, the future sale of the vaccine could lead to payments to NIH and some NIH scientists. By U.S. law, government scientists are required to receive such payments for their inventions. You will not get any money from the development or sale of the product.

COMPENSATION, REIMBURSEMENT, AND PAYMENT

Will you receive compensation for participation in the study?

Some NIH Clinical Center studies offer compensation for participation in research. The amount of compensation, if any, is guided by NIH policies and guidelines.

You will be compensated for your time and inconvenience by the NIH Clinical Research Volunteer Program. It is possible that you may have some expenses that are not covered by the compensation provided.

The compensation is $275 for each vaccination visit. You will get $25 total for the timely completion of all 7 days of an electronic diary. You will get $175 for each scheduled follow-up visit that includes a research blood draw. You will get $75 for all other clinic visits that do not include research blood draws or mucosal sample collection. You will get $250 for apheresis procedure. You will get an additional $50 for any visit that also includes a nose and throat swab.

If you are enrolled in Group 1, the total compensation for completion of all study visits is about $1,875.

If you are enrolled in Groups 2A, 2B, 2C, or 2D, the total compensation for completion of all study visits is about $2,525 without apheresis and about $2,600 with apheresis.

If you are unable to finish the study, you will receive compensation for the visits and for the parts you completed.

With few exceptions, study compensation is considered taxable income that is reportable to the Internal Revenue Service (IRS). A "Form 1099-Other Income" will be sent to you if your total payments for research participation are $600 or more in a calendar year. If you have unpaid debt to the federal government, please be aware that some or all of your compensation may be automatically reduced to repay that debt on your behalf.

Will you receive reimbursement or direct payment by NIH as part of your participation?

Some NIH Clinical Center studies offer reimbursement or payment for travel, lodging or meals while participating in the research. The amount, if any, is guided by NIH policies and guidelines.

This study does not offer reimbursement for, or payment of, travel, lodging or meals.

Will taking part in this research study cost you anything?

NIH does not bill health insurance companies or participants for any research or related clinical care that you receive at the NIH Clinical Center.

There are no costs to you for taking part in this study. We will not charge you or your insurance carrier for any health evaluations or services. You or your health insurance will have to pay for all costs for medical care that you get outside this study. It is possible that you may have some costs that are not covered by the study compensation we give you.

CLINICAL TRIAL REGISTRATION AND RESULTS REPORTING

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

HUMAN DATA SHARING

To advance science, it is helpful for researchers to share information they get from studying humans by putting it into shared scientific databases. Researchers can then study the information combined from many studies to learn even more about health and diseases.

If you agree to take part in this study, some of your data will be put into one or more scientific databases. We will remove identifying information like your name, address, and birth date. The data may then be used for future research and shared broadly for research purposes.

Only researchers who are approved to access the database may be able to see and use your information. You will not get any direct benefits from future research that uses your data and information.

You may stop participating in this study at any time and withdraw permission for your individual data, specimens, and health information to be used for additional or future research. You may ask to have your research data destroyed. However, it may not be possible to withdraw or delete data once they have been shared with other researchers.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The NIH and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Institutes of Health Intramural Institutional Review Board
- The study Sponsor VRC or their agent(s)

When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

If we share your specimens or data with other researchers, in most circumstances we will remove your identifiers before sharing your specimens or data. You should be aware that there is a slight possibility that someone could figure out the information is about you.

Further, the information collected for this study is protected by NIH under a Certificate of Confidentiality and the Privacy Act.

Certificate of Confidentiality

To help us protect your privacy, the NIH Intramural Program has received a Certificate of Confidentiality (Certificate). With this certificate, researchers may not release or use data or information about you except in certain circumstances.

NIH researchers must not share information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if requested by a court.

The Certificate does not protect your information when it:

1. is disclosed to people connected with the research, for example, information may be used for auditing or program evaluation internally by the NIH; or
2. is required to be disclosed by Federal, State, or local laws, for example, when information must be disclosed to meet the legal requirements of the federal Food and Drug Administration (FDA);
3. is for other research;
4. is disclosed with your consent.

The Certificate does not prevent you from voluntarily releasing information about yourself or your involvement in this research.

The Certificate will not be used to prevent disclosure to state or local authorities of harm to self or others including, for example, child abuse and neglect, and by signing below you consent to those disclosures. Other permissions for release may be made by signing NIH forms, such as the Notice and Acknowledgement of Information Practices consent.

Privacy Act

The Federal Privacy Act generally protects the confidentiality of your NIH medical records we collect under the authority of the Public Health Service Act. In some cases, the Privacy Act protections differ from the Certificate of Confidentiality. For example, sometimes the Privacy Act allows release of information from your medical record without your permission, for example, if it is requested by Congress. Information may also be released for certain research purposes with due consideration and protection, to those engaged by the agency for research purposes, to certain federal and state agencies, for HIV partner notification, for infectious disease or abuse or neglect reporting, to tumor registries, for quality assessment and medical audits, or when the NIH is

involved in a lawsuit. However, NIH will only release information from your medical record if it is permitted by both the Certificate of Confidentiality and the Privacy Act.

POLICY REGARDING RESEARCH-RELATED INJURIES

The NIH Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the NIH, the NIH Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

PROBLEMS OR QUESTIONS

If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Alicia Widge, M.D., MS alicia.widge@nih.gov 301-761-7968. Other researchers you may call are: [Floreliz Mendoza, RN, at 301-451-8715. You may also call the NIH Clinical Center Patient Representative at 301-496-2626, or the NIH Office of IRB Operations at 301-402-3713, if you have a research-related complaint or concern.

CONSENT DOCUMENT

Please keep a copy of this document in case you want to read it again.

IRB NUMBER: 19I0032
IRB APPROVAL DATE: 05/18/2020

Adult Research Participant: I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I consent to participate in this study.

Signature of Research Participant	Print Name of Research Participant	Date

Witness to the oral short-form consent process only: This section is only required if you are doing the oral short-consent process and this English consent form has been approved by the IRB for use as the basis of translation.

Witness:

Signature of Witness*	Print Name of Witness	Date

***NIH ADMINISTRATIVE SECTION TO BE COMPLETED REGARDING THE USE OF AN INTERPRETER:**

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent and served as a witness. The investigator obtaining consent may not also serve as the witness.

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent but did not serve as a witness. The name or ID code of the person providing interpretive support is: _____.

PRINCIPAL INVESTIGATOR: Alicia Widge, MD, MS

STUDY TITLE: VRC 321: A Phase I Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability, and Immunogenicity of an Influenza H1 Stabilized Stem Ferritin Vaccine, VRCFLUNPF099-00-VP, in Healthy Adults

STUDY SITE: NIAID/VRC

Cohort: *Healthy volunteer*

Supplementary Consent Version: *3.0, April 20, 2020*

WHO DO YOU CONTACT ABOUT THIS STUDY?

Principal Investigator: Alicia Widge, MD, MS: [redacted]

Study Coordinator: Floreliz Mendoza, RN; [redacted]

Supplementary consent for extended genetic testing (optional)

You agreed to participate in VRC 321 study for testing of an investigational vaccine to prevent H1 influenza (flu) virus infection. This additional consent covers optional genetic testing that you may agree to. If you do not agree to this additional testing, you still can participate in the VRC 321 study.

There is a new type of genetic test that lets us look at the expressions of genes, called transcriptome sequencing. This test lets us look at the genes that are actively expressed at any given moment. However, it does not measure the amount of protein produced. Also, this new genetic test is still in development and researchers are working on understanding the data and how that data can be utilized in various clinical applications, such as in medicine to help prevent disease.

This test may take a long time to understand, and we may not have any news to give you about it. Since we are looking for genes that control infection or immune response, we will not report to you or your doctors anything that we find that is not related to infection or immunity. However, if we find something in your DNA that we think is urgent to deal with because of your health, we will confirm the result and then tell you about it. We think this sort of problem will be very rare.

Genetic Data Sharing

Following genetic testing for transcriptome sequencing, your sequence data may be shared in a controlled access public database, for other investigators to benefit from it. However, no personal, identifiable information will be shared in this process, as the shared results will be coded with no link back to you.

Certificate of Confidentiality

To help us protect your privacy, the NIH Intramural Program has received a Certificate of Confidentiality (Certificate). With this certificate, researchers may not release or use data or information about you except in certain circumstances.

NIH researchers must not share information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if requested by a court.

The Certificate does not protect your information when it:

1. is disclosed to people connected with the research, for example, information may be used for auditing or program evaluation internally by the NIH; or
2. is required to be disclosed by Federal, State, or local laws, for example, when information must be disclosed to meet the legal requirements of the federal Food and Drug Administration (FDA);
3. is for other research;
4. is disclosed with your consent.

The Certificate does not prevent you from voluntarily releasing information about yourself or your involvement in this research.

The Certificate will not be used to prevent disclosure to state or local authorities of harm to self or others including, for example, child abuse and neglect, and by signing below you consent to those disclosures. Other permissions for release may be made by signing NIH forms, such as the Notice and Acknowledgement of Information Practices consent.

Privacy Act

The Federal Privacy Act generally protects the confidentiality of your NIH medical information that we collect under the authority of the Public Health Service Act. In some cases, the Privacy Act protections differ from the Certificate of Confidentiality. For example, sometimes the Privacy Act allows release of information from your record without your permission, for example, if it is requested by Congress. Information may also be released for certain research purposes with due consideration and protection, to those engaged by the agency for research purposes, to certain federal and state agencies, for HIV partner notification, for infectious disease or abuse or neglect reporting, to tumor registries, for quality assessment and medical audits, or when the NIH is involved in a lawsuit. However, NIH will only release information from your medical record if it is permitted by both the Certificate of Confidentiality and the Privacy Act.

POLICY REGARDING RESEARCH-RELATED INJURIES

The NIH Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the NIH, the NIH Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

PROBLEMS OR QUESTIONS

If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Alicia Widge, M.D., MS alicia.widge@nih.gov [redacted] Another researcher you may call is: Floreliz Mendoza, RN, at [redacted] You may also call the NIH Clinical Center Patient Representative at 301-496-2626, or the NIH Office of IRB Operations at 301-402-3713, if you have a research-related complaint or concern.

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Signature of Research Participant	Print Name of Research Participant	Date

Witness to the oral short-form consent process only: This section is only required if you are doing the oral short-consent process with a non-English speaking subject and this English consent form has been approved by the IRB for use as the basis of translation.

Witness:

Signature of Witness*	Print Name of Witness	Date

***NIH ADMINISTRATIVE SECTION TO BE COMPLETED REGARDING THE USE OF AN INTERPRETER:**

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent and served as a witness. The investigator obtaining consent may not also serve as the witness.

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent but did not serve as a witness. The name or ID code of the person providing interpretive support is: _____.