**PROTOCOL TITLE:** An Exploratory, Pilot Study of the Safety and Immunogenicity of Reduced Doses of the US Yellow Fever Vaccine (FY19-26)

## SECTION A: RESEARCH TEAM AND LOCATIONS

### A1. RESEARCH TEAM

| Study Role | Institution/Company and Contact Information |
|---|---|
| **Funder** | DHA-Immunization Healthcare Division (DHA-IHD)<br>Office of the Principal Assistant for Acquisition<br>US Army Medical Research and Development Command<br>810 Schreider St., Ste. 204<br>Ft. Detrick, MD 21701<br>Phone: 301-619-4566<br>DSN: 343-4566<br>Fax: 301-619-9793 |
| **Principal Investigator** | Phillip R. Pittman, M.D., MPH<br>Chief, Clinical Research Department<br>Division of Medicine, USAMRIID<br>1425 Porter Street, Fort Detrick, MD 21702-5011<br>Phone: 301-619-2997<br>Email: phillip.r.pittman.civ@health.mil |
| **Associate Investigator(s)** | Fernando B Guerena, M.D., M.P.H., COL<br>Director Core Support, USAMRIID<br>1425 Porter Street, Fort Detrick, MD 21702-5011<br>Telephone: (301) 619-0010<br>Email: fernando.b.guerena.mil@health.mil |
| **Study Coordinator/ Protocol Nurse** | Lawrence Korman, R.N., BSN, CCRP<br>Division of Medicine, USAMRIID<br>1425 Porter Street, Fort Detrick, MD 21702-5011<br>Phone: 301-619-6008<br>Email: lawrence.korman2.civ@health.mil |
| **Recruiter** | Vincent Fulton B.S., CCRP<br>Division of Medicine, USAMRIID<br>1425 Porter Street, Fort Detrick, MD 21702-5011<br>Phone: 301-619-6019<br>Email: vincent.b.fulton2.ctr@health.mil |

**Other Individuals Supporting the Research**

Eric C. Mossel, PhD., MAJ
Microbiologist
Center for Disease Control (CDC)
Ft. Collins Branch
3156 Rampart Rd
Fort Collins, CO 80521
Phone: 970-494-6595
Email: eric.c.mossel.mil@health.mil

Rafi Ahmed, PhD.
Emory University
Emory Vaccine Center
1510 Clifton Road
G211 Rollins Research Building
Atlanta, Georgia 30322

Rafick-Pierre Sekaly, Ph.D.
Emory University
Atlanta, Georgia 30322

## A2. ROLES AND RESPONSIBILITIES

### A2.1 Key Research Personnel

*Name(s):* Phillip R. Pittman, M.D., MPH
*Research Role:* Principal Investigator
*Study Responsibilities:* Supervises research team, trains study staff, obtain informed consent, determine inclusion/exclusion criteria, conducts physical exams, provide medical care for adverse events, collecting data, analyzing data, administration of vaccine, and evaluation of adverse events.

*Name(s):* Fernando B Guerena, M.D., MPH, COL
*Research Role:* Associate Investigator
*Study Responsibilities:* Assists the PI in conduct of study and supervision of study staff, obtain informed consent, determine inclusion/exclusion criteria, conducts physical exams, provide medical care for adverse events, collecting data, administration of vaccine, and evaluation of adverse events.

*Name(s):* Lawrence Korman, R.N., BSN, CCRP
*Research Role:* Protocol Nurse / Study Coordinator
*Study Responsibilities:* obtain informed consent, review inclusion/exclusion criteria, collecting data, administration of vaccine, vaccine accountability, phlebotomy, recruitment, follow-up on adverse events, and trains research team on protocol/protocol changes.

*Name(s):* Vincent Fulton BS, CCRP
*Research Role:* Recruiter
*Study Responsibilities:* Recruitment and collect data.

### A2.2. Others Involved in the Research, as applicable

*Name(s):* Eric C. Mossel, PhD., MAJ, Rafi Ahmed, PhD., Rafick-Pierre Sekaly, Ph.D.
*Research Role:* Microbiologist

*Study Responsibilities:* Performs laboratory testing of de-identified samples.

### A3. RESEARCH LOCATIONS

Department of Clinical Research, Division of Medicine, USAMRIID will serve as the site where subjects will be recruited and enrolled into the study, where study interventions and data analysis will occur.

CDC, Fort Collins Branch will perform PRNT and PCR evaluations.

Emory University will perform peripheral blood mononuclear cell (PBMC) and culture evaluations.

Lab Corp will perform hematology, chemistry, HIV and Hepatitis panel evaluations for all blood samples.

### A4. MULTI-SITE RESEARCH

N/A

## SECTION B: RESEARCH METHODOLOGY

### B1. ABSTRACT

YF-VAX® shortages caused by vaccine manufacturing issues or disease outbreaks are major world-wide safety concern. Outbreaks often occur in areas of the world where the DoD is at risk of deploying it forces and as such the DoD is compelled to examine alternative methods to expand vaccine capacity in order to ensure warfighter immune readiness. The purpose of this open-label, randomized, exploratory, pilot study is to evaluate the human immune response to reduced subcutaneous (SQ) dosing of YF-VAX® compared to the standard FDA approved subcutaneous vaccination dose. The current dose of the US FDA licensed YF-VAX® is approximately 55,000 PFU in 0.5 mL administered SQ. Using the licensed dosage as standard, we propose to evaluate reduced doses of 1/5th and 1/10th or 0.10 mL and 0.05 mL YF-VAX®. There will be a total of 90 subjects divided into 3 Groups of 30 subjects each. As many as 150 healthy volunteers age 18 – 50 years who have not previously received yellow fever vaccine or any flavivirus vaccine nor knowingly has suffered with yellow fever disease or other flavivirus disease may be screened to qualify 90 subjects as meeting inclusion/exclusion criteria. On the day of vaccination, subjects will be randomized to one of 3 groups. Cohorts of approximately 15-20 will be vaccinated until enrollment is complete. Following vaccination, solicited adverse events (AEs) will be recorded on the case report forms, and unsolicited AEs on a diary card for 28 days. SAEs will be recorded through day 365. Blood will be collected at designated time points for antibody titers and vaccine virus viremia. The study will assess the feasibility of launching a large pivotal non-inferiority study of the standard dose vs the optimal reduced dose as determined by this study.

### B2. BACKGROUND AND SIGNIFICANCE

**Background:** The African yellow fever epidemic coupled with vaccine production constraints has led to a worldwide shortage of yellow fever vaccine [1]. On 9 May 2016 DHA-Immunization Healthcare Division (DHA-IHD) issued an information paper describing the yellow fever vaccine (YF-VAX®) shortage and revising US DOD vaccination and ordering procedures [2]. The DHA information paper explained that Sanofi Pasteur, the sole FDA-licensed manufacturer of yellow fever vaccine in the United States was unable to produce sufficient quantity of YF-VAX®. The DHA information paper also outlined the potential impact as “Proof of yellow fever immunization is a requirement for entry into 18 countries within the AFRICOM AOR in addition to, endemic zones located within the SOUTHCOM AOR. Entry into certain countries may be denied to Service members if they do not have a current (within 10 years) yellow fever immunization documented, possibly impacting their mission or resulting in quarantine/detainment [2]. DHA monitors the DOD YF-VAX® supply monthly [3]. To

address the shortage among US travelers, FDA allowed US travelers to receive the Sanofi Pasteur European Yellow Fever Vaccine (Stamaril) under pre-selected travel clinics throughout the country, under the auspices of CDC and Sanofi Pasteur [4].

The current YF vaccine shortage has highlighted the need to pursue alternative solutions to extend existing vaccine supplies, the most rational solution being dose-sparing strategies. The Stamaril vaccine is currently licensed only under an expedited access pathway (EAP) for use at a limited number of sites in the U.S. FDA licensure for Stamaril would need to be initiated by Sanofi and would require funding as well as time to conduct the required clinical trials. Although a new batch of YF-Vax became available in 2019, this does not address the underlying problem of how long it takes to manufacture and qualify new YF-Vax® lots. While licensure of a fractionated dose would take time, data that fractionated dosing induces immunity above the WHO-defined protective threshold could form the basis for an FDA EAP approval in the event of an outbreak. An EAP would also provide a vaccination alternative for U.S. Armed Forces deployed to endemic areas during any future vaccine shortages.

The purpose of this study is to evaluate whether SQ dosing at 1/5th (0.1 mL) and 1/10$^{th}$ (0.05 mL) the current dose of YF –VAX® is similar to the FDA approved subcutaneous vaccination dose of 0.5 mL. A number of recently published editorials have suggested that due to the recent epidemic and vaccine shortage, there should be a renewed emphasis on yellow fever vaccine dose reduction [1, 5-8]. Two recent clinical trials have reported favorable immunogenicity with reduced dosing of YF-VAX using alternative routes of administration. Roukens et al. compared YF-VAX SQ at the standard dose to a reduced dose (1/5$^{th}$ of standard dose) administered ID. They observed that both groups met WHO standards of seroprotection, with PRNT80 titers > 1:16 in 77/77 (100%) ID and 78/78 (100%) SQ [9]." While, Slifka et al. [10] used PRNT50 titers and compared YF-VAX transcutaneous (TC) to SQ in subjects with and without atopic dermatitis and found that reduced dose TC yielded a conversion rate of 26/30 (87%) as compared to a conversion rate of 37/38 (97%) with conventional SQ. Similarly, Campi-Azevedo et al. found that with the Brazilian YF vaccine, a dose of 5000 PFU (1/10$^{th}$ routine dose) demonstrated similar immunological correlates and viral kinetics to the standard dose [11]. The latter point is emphasized as it conveys that immune-stimulating antigen exposure is essentially similar. Wu et al. assessed the approach of administering 1/5$^{th}$ dose of yellow fever vaccine with a mathematical model and concluded that it is an effective strategy for a reduction in the infection attack rate of yellow fever [12]. Visser and Roukens support further study of the reduced dose of vaccine with clinical trials [13].

Recently, a study in the NEJM, conducted during a mass vaccination campaign in Kinshasa, examined a dose of 17DD YF vaccine (Bio-Manguinhos) at one fifth of the standard dose (0.1 ml) to 764 participants from one of six lots (14). Of the 716 participants completing the study, 705 (98%; 95% confidence interval [CI], 97 to 99) were seropositive after vaccination and none of the participants who completed follow-up reported symptoms compatible with yellow fever after vaccination. Unfortunately, this study did not collect data from a comparison arm of standard dose YF vaccine. *The authors of this study emphasized that follow-on studies should be done to confirm that other 17D-derived YF vaccines would perform in similar fashion* (14).

In addition, a publication from Bio-Manguinhos in Brazil describes a dose-response study with their 17DD YF vaccine, administering the vaccine in the usual mean dose of 27,476 IU (full dose, reference) and in tapered doses (10,447 IU, 3013 IU, 587 IU, 158 IU, and 31 IU) by the usual subcutaneous route and usual volume (0.5 mL) (15a). Doses down to 587 IU showed similar immunogenicity to the full dose, while the 158 IU and 31 IU doses displayed lower immunogenicity (15a). Seropositivity was maintained at 10 months, except in the group that received the 31 IU dose (15a). Overall, 85.2% of participants (95% CI 80.8; 88.9) remained seropositive to YF eight years after initial vaccination, with no significant differences between the fractional dose and full-dose groups (15b).

The Strategic Advisory Group of Experts (SAGE) on immunization of the World Health Organization (WHO) endorsed the use of reduced dose of yellow fever vaccine administration in order to quell the recent yellow fever epidemic in West Africa [16]. WHO had called for studying the immunogenicity and safety of fractional dosing of all 4 WHO prequalified yellow fever vaccines administered either subcutaneously or intramuscularly, as fractional dosing has been the mainstay in addressing current YF outbreaks.

In their review, Rouken and Visser wrote, “The global yellow fever vaccine supply is insufficient to provide full-dose vaccination to millions threatened by outbreaks. Given the excess of live-attenuated 17D yellow fever virus in the current single dose vials, dose sparing would increase available vaccine doses manifold (17).” At this point, to our knowledge the only vaccine to have not been studied for feasibility of fractional dosing subcutaneously is the only U.S. FDA approved vaccine, YF-VAX®.

*It is critically important that the DOD address this gap in medical knowledge as future vaccine shortages for this disease, which is endemic to many areas of strategic military importance, are a very real possibility. The most efficient and cost-effective path forward is to perform a pilot study to 1. Provide the DOD with an informative knowledge product regarding the performance of fractional YF-VAX and 2. Better position the DOD MRDC for follow-on studies should there be a future prolonged shortage of YF-VAX.*

## B3. MILITARY RELEVANCE

The outcome of this work will provide a basis to support emergency use authorization of the current vaccine YF-VAX® at a reduced dose. Additionally, equivalency in this proof of concept study serves as a framework to conduct a larger non-inferiority study to meet the regulatory requirements for lowering the dose of the FDA approved vaccine, thereby helping to address the US shortage of YF-VAX®.

YF-VAX® is currently in use by the DOD and is the only FDA approved vaccine against yellow fever disease. Thus, YF-VAX® is the only YF vaccine that can be routinely administered to DOD active duty personnel and dependents. YF-VAX® is indicated for active immunization for the prevention of YF in persons 9 months of age and older in the following categories: Persons living in or traveling to endemic areas, persons travelling internationally through countries with YF, and laboratory personnel. YF is endemic in Sub-Saharan Africa, South America, and the Caribbean. YF-VAX® is currently available only in very limited supplies. There is currently no new production of YF-VAX® due to manufacturing issues. Sanofi Pasteur, the vaccine manufacturer, has not determined definitively when YF-VAX® vaccine production will be restarted.

YF disease is a viral hemorrhagic fever that is a re-emerging infectious disease and is likely to continue to be a threat to deploying military forces well into the future. Understanding the safety and biologic/immunologic responses to this vaccine is paramount to the warfighter's immune readiness and the readiness of the Department of Defense to perform its mission. It is essential that the war fighter is provided an effective method of protection from yellow fever virus infection when deployed in an endemic area.

## B4. OBJECTIVES/SPECIFIC AIMS/RESEARCH QUESTIONS

**Hypothesis:** Hypothesis: YF-VAX® administered by the SQ route with a fractional dose will demonstrate similar neutralizing antibody response and adverse events compared to YF-VAX® administered by standard dose SQ route.

**Primary Objective**:

- The primary objective of this study is to explore the safety and immunogenicity of two fractionated doses of YF-VAX (1/5$^{th}$ and 1/10$^{th}$ the standard dose) compared to the standard YF-VAX dose.

**Secondary Objectives:**

- To determine if YF vaccine viremia (as determined by viral plaque assays and/or RT-PCR), for the 14 days following vaccination, is diminished by fractional dose vaccination vs standard dose vaccination.
- To compare the neutralizing antibody response of SQ standard dose YF vaccine to SQ fractional dose YF vaccine up to one year following vaccination.

**Exploratory Objectives:**

- To collect samples to characterize the impact of SQ fractional dose YF vaccine vs SQ standard dose YF vaccine in terms of immune profile.
    - These studies would be funded by follow-on proposals and not the current proposal

**Primary Endpoints**

- **Immunogenicity**
    - Proportion of subjects in each dose-group who develop protective antibody titers at day 28 post-YF vaccination as defined by $PRNT_{50}$ ≥1:10 or higher.
    - Geometric mean $PRNT_{50}$ titers at day 28 post-YF vaccination for each dose group.
    - Proportion of subjects in each dose-group who develop a 4-fold increase in $PRNT_{50}$ titers from day 0 to day 28.
- **Safety**
    - Occurrence of adverse events, in each dose group.
        - Occurrence of solicited adverse events in each dose group for the first 28 days following vaccination.
        - Occurrence of unsolicited adverse events in each dose group for the first 28 days following vaccination.
        - Occurrence of serious adverse events during the study period.

**Secondary Endpoints**

- **Immunogenicity**
    - Proportion of subjects in each dose-group who develop protective antibody titers up to a year (days 0, 3, 7, 10, 14, 90, 180, 365) post-YF vaccination as determined by $PRNT_{50}$ and $PRNT_{80}$ of 1:10 or higher. Days 0, 28 and 365 will be used for analyses addressing the secondary objectives. Days 3, 7, 10, 14, 90 and 180 will be used for exploratory analyses only
    - Geometric mean $PRNT_{50}$ and $PRNT_{80}$ titers up to 1 year (days 0, 3, 7, 10, 14, 90, 180, 365) post-YF vaccination for each dose group. Days 0 and 365 will be used for analyses addressing the secondary objectives. Days 3, 7, 10, 14, 90 and 180 will be used for exploratory analyses only
    - Proportion of subjects in each dose-group who develop a 4-fold increase in titers up to a year (days 3, 7, 10, 14, 90, 180, 365) compared to day 0 as determined by $PRNT_{50}$. Day 365 will be used for analyses addressing the secondary objectives. Days 3, 7, 10, 14, 90, and 180 will be used for exploratory analyses only. All $PRNT_{80}$ analyses, including 4-fold increase from baseline will be considered secondary.
- **Viremia**
    - Mean viral load (copies / mL blood) at day 0, 2, 3, 4, 5 based on day of vaccination (blood draw will occur only once over the weekend—the missed day will not be considered a protocol violation or deviation), 6, 7, 10 and 14 post-vaccination in each dose group, as determined by PCR.

**Exploratory Endpoints**

- **Immunogenicity**
    - Frequency and phenotype of YF-specific T and B cells for each dose group up to 1 year post-vaccination (days 0, 7, 14, 28, 90, 365) as determined via ELISPOT and / or ICS/flow cytometry (up to 40 members from the last 3 cohorts only - the exact number will be determined by the number of subjects in these cohorts who volunteer to participate in this part of the study).

- Cytokine profiling for YF-specific peripheral blood mononuclear cells via Mesoscale multiplex assay or comparable (up to 40 members from the last 3 cohorts only).
- Microbiome—blood samples will be collected (days 0 (baseline), 2, 7, 14, 28, 3 months, 1 year) for determination of how vaccination with the live, attenuated YF-VAX effects the microbiome (up to 40 members from the last 3 cohorts only).

# B5. RESEARCH PLAN

## B5.1 Research Design

This is an open label, randomized, exploratory pilot study to assess proof of concept for reduced subcutaneous doses of the FDA licensed YF vaccine, YF-VAX®.

## B5.2 Research Subjects/Population(s)

### B5.2.1 Subject Population(s)

The study population will consist of males and non-pregnant or non-breastfeeding females 18 to 50 years of age who reside in the Washington-Baltimore-Frederick area to include active duty military personnel, government and non-government (including contractors) civilians.

### B5.2.2 Number of Subjects, Records, and/or Specimens

Up to 150 individuals will be screened in order to randomize 90 eligible individuals to one of three groups: Group 1— YF-VAX® standard dose 0.5 mL SQ = 30 subjects; Group 2—0.10 mL (1/5$^{th}$) SQ = 30 subjects; Group 3—0.05 mL (1/10$^{th}$) SQ = 30 subjects.

| Group | Number of Subjects | YF-VAX Dose | Volume (mL) |
|---|---|---|---|
| Group 1 | 30 | Standard dose | 0.5 mL |
| Group 2 | 30 | 1/5$^{th}$ | 0.1 mL |
| Group 3 | 30 | 1/10$^{th}$ | 0.05 mL |

### B5.2.3 Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

1. Males and Females 18 to 50 years of age.
2. In good health, as determined by pertinent medical history, physical examination, vital signs, and clinical safety laboratory evaluations.
3. Female of child bearing potential: Has a negative pregnancy test and is willing to use a reliable form of contraception for the duration of the study after vaccination.
4. Negative human immunodeficiency virus (HIV) antibody screen, seronegative for hepatitis B surface antigen (HBsAg) and hepatitis C antibody (following HIV and hepatitis testing, subjects will be provided with counseling and referral for health care if any test is positive).
5. Ability to comprehend and a willingness to sign an informed consent, which includes the Health Insurance Portability and Accountability Act (HIPAA) Authorization, and a separate consent form for HIV testing.

6. Be willing to comply with all follow-up visits, testing, AE reporting, and completion of diary card.

### B5.2.4 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. Receipt of any other investigational vaccine or investigational drug within 28 days prior to or after vaccination with YF-VAX® vaccine.
2. Have had any known flavivirus disease or receipt of any flavivirus vaccine, licensed or investigational at any time; in addition to any yellow fever vaccine, these include; Japanese Encephalitis (JE), St. Louis Encephalitis, Tick Borne Encephalitis (TBE), West Nile, Dengue, Zika virus
3. Anticipates receipt of any other vaccine within 28 days of YF-VAX®. COVID-19 vaccination and Influenza vaccination will be permitted but not within 14 days of YF-VAX®.
4. Acute or chronic medical conditions, or medications that, in the Principal Investigator's (PI) opinion, would impair the subject's ability to respond to vaccination.
5. Hypersensitivity to any vaccine, eggs or egg products, or allergy to any component of the YF-VAX® (sorbitol, gelatin) or latex.
6. Corticosteroids even ≥20 mg/day of prednisone for ≥ 2 weeks suppresses the immune system. Low-dose corticosteroid topical products and nasal sprays used sporadically (i.e. prn--according to circumstances) are permissible.
7. History of immunosuppression, by any cause--primary or acquired immunodeficiencies, transplantation, malignant neoplasm, lymphoma, leukemia, thymoma, mysthenia gravis, radiation, immunosuppressive drugs, including antimetabolites, TNF-alpha inhibitors (etanercept), IL-1 blocking agents and other monoclonal antibodies targeting immune cells (e.g., rituximab, alemtuzumab, etc), etc.
8. Receipt of or anticipates receipt of/or donation of blood or blood products for 2 months after receipt of YF-VAX®. **(Note: Blood banks require a minimum 2 week interval between the receipt of this FDA licensed vaccine and blood donations; however because of the blood collections in this study, an interval of 2 months is requested).**
9. Female: Pregnant (or planning to become pregnant) or breastfeeding for the duration of the study after receipt of YF-VAX®.
10. Clinically significant abnormal laboratory tests (generally ≥2 times the upper limit of normal or as determined by the PI).

## B5.3 Research Procedures

After informed consent is obtained, prospective participants will be screened for study eligibility criteria to include medical history, physical examination including vital signs, review of concomitant medications including vaccination history, lab tests including complete blood count (CBC) with differential (4 mL), comprehensive metabolic panel (CMP) (8.5 mL), urinalysis, hepatitis panel (Hepatitis B surface antigen [HBsAg] and antibodies to Hepatitis C [8.5 mL]), human immunodeficiency virus (HIV) antibody screen (8.5 mL), pregnancy test (for all females to confirm non-pregnant status), and Yellow Fever PRNT 50/80 (8.5 mL). Maximum amount of blood drawn for screening tests is 38 mL. The results of all screening labs, vaccine titers and safety labs (CBC, CMP) will be reviewed with each subject and a copy of the results will be provided if requested by the subject.

In the event of a screening failure secondary to mild or limited acute illness or abnormal laboratory values, the subject may be re-screened after resolution of the event. Re-screening may require only an additional blood draw or may require a full re-screening evaluation, depending on the circumstances of and the time interval from the initial screening failure.
If a subject is screened and cannot be vaccinated because of a certain transient condition, (e.g. abnormal lab value due to an acute condition or a missing lab evaluation) then the subject can be re-screened on one further occasion only and the respective test(s) should be repeated as a "partial" re-screening rather than a "full" re-

screening. The partial re-screening visit must be within the 60-day window started by the first Screening Visit and the window -60 to -1 day before the vaccination must not be exceeded.

If a subject could not be vaccinated and the 60-day period is over, the subject is withdrawn from the study, a "full" re-screening assessment including re-consenting, physical examination, blood tests must be performed and the subject will be assigned a new study ID number. The clock then re-starts at the re-screening visit with Day -60 before 1st vaccination.

The PI or Associate Investigator will review the eligibility criteria and the screening laboratory test results to determine a candidate's eligibility to participate in this study. Blood tests that show a subject is HIV, hepatitis B or C positive are required by law to be reported to the Maryland Health Department. Subjects will be notified of this reporting requirement. The test results reported to Maryland Health Department will contain the participant's name, contact information, including address and telephone numbers, and the type of testing that was done. If the participant is in the military, study staff are required to report the same kind of information to military preventive medicine service. As a result, this information may end up in the subject's military medical record and may be reported to their chain of command. The study doctors will discuss abnormal testing results face-to-face with the study participant (and notify their primary doctor at their request). Participants will be advised of this in the informed consent form. Civilian subjects who are positive for HIV, Hepatitis B surface antigen, or Hepatitis C will be referred to their private physician if they wish. Military personnel who test positive for HIV, Hepatitis B surface antigen, or Hepatitis C will be referred to an Army Health Clinic. Study personnel will also inform subjects if they do not qualify to participate in this study and why.

On vaccination day prior to administration of the vaccine, review of current concomitant medications, pregnancy test (for all females to confirm non-pregnant status), eligibility criteria review and research lab tests to include, Yellow Fever PRNT 50/80, PBMC, and Yellow Fever Vaccine viremia.

Cohorts of participants should be randomized for vaccination to one of three (3) groups with individuals assigned to each group so that a proportional of each sex is roughly equivalent between groups. For each cohort of subjects, a Randomization Worksheet with subject ID and sex for each subject will either be transmitted electronically or by hand to statistician for randomization once all subjects have been approved for study participation. YF-VAX® will be administered in the clinic area according to the assigned dosing regimen of one (1) subcutaneous (SQ) injection documented on the Randomization Worksheet supplied by the statistician on the day of vaccination.

After the vaccination, subjects will remain in the clinic area for at least 30 minutes for vital signs, review of any post vaccination adverse events and will receive a diary card to document all adverse events that occur over the next 28 days.

Post vaccination visit procedures to include vital signs, review of current concomitant medications, lab tests, focused physical exam directed at vaccination site and review of adverse events as documented in the Study Event Schedule. Follow-up visits will take approximately 15 to 30 minutes.

If a female subject becomes pregnant during the study, she will be advised to contact the principal investigator. The Investigators will follow the subject until the baby's birth regarding the outcome of her pregnancy. The following information will be requested from the subject concerning the pregnancy outcome: term or early delivery; natural birth or C-section; child's birth weight and length; child's physical examination (normal or any birth defects).

Subjects who receive less than the full dose during study participation should receive, when appropriate, a full dose of YF-VAX® before either deploying or travelling as a tourist to an endemic area as documented in the YF-VAX® package insert. Data suggest that vaccination in the presence of neutralizing YFV antibodies does not increase the risk for adverse events—SAEs occur primarily after primary YF immunization (18). The level

of YFV-specific antibodies following booster vaccinations appear to be correlated inversely to the amount of pre-existing antibodies (19), however, no data suggest that there is a negative impact on sero-protection.

### Study Events Schedule

| Study Event | Screening | Day of Vaccination | Post-Vaccination Day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days -60 to -1 | 0 | 2 | 3 | 5 | 6 | 7 (+ 1) | 10 (± 1) | 14 (± 2) | 28 (± 3) | 90 (± 7) | 180 (± 14) | 365 (± 28) |
| Informed Consent | X | | | | | | | | | | | | |
| Inclusion/Exclusion Criteria Review | X | X | | | | | | | | | | | |
| Medical History | X | | | | | | | | | | | | X [c] |
| Concomitant Medication Review | X | X | | | | | X | | X | X | | X | X |
| Physical Examination | X | | | | | | | | | | | | X [c] |
| Vital Signs [d] | X | X[e] | X | | | | X | | | X | | | X |
| **CLIN LABS** | | | | | | | | | | | | | |
| Complete Blood Count (CBC) with Differential | X | | | | | | X | | X | | | | |
| Comprehensive Metabolic Panel (CMP) | X | | | | | | X | | X | | | | |
| Urinalysis | X | | | | | | | | | | | | |
| Hepatitis Panel | X | | | | | | | | | | | | |
| HIV Antibody Screen | X | | | | | | | | | | | | |
| Serum/Urine Pregnancy Test for Females of Childbearing Potential | X | X | | | | | | | | | | | |
| **Research LABS** | | | | | | | | | | | | | |
| PRNT 50/80 | X | X | | X | | | X | X | X | X | X | X | X |
| YFV viremia | | X | X | X | X | X | X | X | X | | | | |
| Amount of Blood (mL) | 38 | 17 | 9 | 17 | 9 | 9 | 30 | 17 | 30 | 9 | 9 | 9 | 9 |
| Additional Tests on Last 40 Subjects | | | | | | | | | | | | | |
| PBMC's | | X | | | | | X | | X | X | X | | X |
| Microbiome | | X | X | | | | X | | X | X | X | | X |
| Amount of Blood (mL) | | 42 | | | | | 42 | | 42 | 42 | 42 | | 76 |
| | | | | | | | | | | | | | |
| Approx. Total Amt. of Blood (mL) | 38 | 59 | 9 | 17 | 9 | 9 | 72 | 17 | 72 | 51 | 51 | 9 | 85 |
| | | | | | | | | | | | | | |
| YF-VAX® Administration | | X | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| Injection Site Observation | | X | X | | | | X | | | X | | | |
| AE Review | | X[f,g] | X | | | | X[g] | | | X[g] | | | |
| SAE Review | | X | X | X | X | | X | | | X | X | X | X |

[c] Targeted examination at principal investigator's discretion.
[d] Blood pressure, pulse, respiration rate, and temperature; not to be done on weekends.
[e] Pre vaccination and ≥ 30 minutes post-vaccination.
[f] Subjects will be observed in the clinic for AEs for at least 30 minutes following vaccination
[g] Diary Card Review

## B5.4 Data Collection

Demographic information to include gender, race and date of birth along with the information/labs listed in the Schedule of Events table.

### B5.5 Managing Data and/or Human Biological Specimens for this Research

For this study, an EDC database system will be used for the collection of study data in an electronic format. The EDC database system will be designed based on protocol requirements, approved eCRF layouts and specifications, and IAW 21 CFR Part 11. The eCRF layouts, generated by the Data Manager, and source documents and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDC database system. The investigator is ultimately responsible for the accuracy of data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the clinical monitor and the designated Data Management Group. The data management plan will describe data storage, security, database user access management.

Subjects will be identified on eCRFs by a unique subject identification number. No personal identifier will be used in any publication or communication used to support this research study. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

The electronic case report form (eCRF) data will be transcribed from source documentation. No source data will be recorded directly in the eCRF (ie, without prior written or electronic record of data). The transcribed data will be consistent with the source documents or the discrepancies will be explained

Data generated by this study may be available for inspection upon request by representatives of the USAMRIID Office of Human Research Oversight (OHRO), HQ USAMRMC IRB, and other DoD offices charged with oversight of human subjects research.

Blood specimens not designated for future use in the subject's informed consent document (ICD) will be destroyed upon the completion of research in accordance with USAMRIID SOP MD-09-11.

Clinical members of the team will be able to identify subjects because of the written consent process. HIPAA requires that researchers obtain the subject's permission (authorization) to use health information about the subject that is either created by or used in connection with this research. For this study, PHI will include a subject's research record, immunization record and, clinical/laboratory data obtained during the course of this study. This authorization has no expiration date.

Data that are considered PHI under HIPAA legislation as described earlier are coded, linked to subjects only via a SIN, and acceptable under HIPAA as a limited data set. Identified data (signed consent documents and enrollment logs linking subject names and SINs) collected during the course of the study will be given to the PI (or designee) and maintained in a secure fashion in accordance with standard procedures at USAMRIID.

The research team (Investigators, Study Coordinator) will have access to the data/specimens and to the link between the subjects and their data/specimens. The enrollment/withdrawal log linking the data/specimens to the subjects will be destroyed after the closure of the research study by the IRB.

### B5.6 Managing Data and/or Human Biological Specimens for <u>Future</u> Research

With written consent from the subject, unused serum specimens will be stored for future study use to further our understanding of the immune response to YF-VAX®. These samples will be stored indefinitely at USAMRIID.

### B5.7 Devices, Drugs, Dietary Supplements, Nutritional Supplements, And Biologics

### B5.7.1 Devices

#### 5.7.1.1 FDA-approved device being used in this research according to the approved labeling

N/A

#### 5.7.1.2 FDA-approved device being used in this research in a manner other than its approved labeling

N/A

### B5.7.2 Drugs

#### B5.7.2.1 FDA-approved and used in accordance with the approved labeling

N/A

#### B5.7.2.2 FDA-approved and used in a manner not in accordance with its approved labeling

Drug name: **YF-VAX.** Source: Sanofi Pasteur, Inc. Per Package Insert, "YF-VAX®, Yellow Fever Vaccine, for subcutaneous use, is prepared by culturing the 17D-204 strain of yellow fever virus in living avian leucosis virus-free (ALV-free) chicken embryos. The vaccine contains sorbitol and gelatin as a stabilizer, is lyophilized, and is hermetically sealed under nitrogen. No preservative is added. Each vial of vaccine is supplied with a separate vial of sterile diluent, which contains Sodium Chloride Injection USP—without a preservative. YF-Vax is formulated to contain not less than 4.74 log 10 plaque forming units (PFU) per 0.5 mL dose throughout the life of the product. Before reconstitution, YF-VAX is a pinkish color. After reconstitution, YF-VAX is a slight pink-brown suspension. The vial stoppers for YF-VAX and diluent are not made with natural rubber latex."

Store lyophilized vaccine at 2° to 8° C (35° to 46° F). **DO NOT FREEZE!**
**Cold Chain Precaution:** Half-life is reduced from approximately 14 days at 35° to 37° C to 3-4 days at 45° to 47° C.

**Vaccine Preparation**

Reconstitute the vaccine using only the diluent supplied (0.6 mL vial of Sodium Chloride injection USP for single dose vial of vaccine. After removing the "flip-off" caps, cleanse the vaccine and diluent vial stoppers with a suitable germicide. **Do not remove the vial stoppers or metal seals holding them in place.** Using aseptic technique, use a suitable sterile needle and syringe to withdraw the volume of supplied diluent shown on the diluent label and slowly inject the diluent into the vial containing the vaccine. Allow the reconstituted vaccine to sit for one to two minutes and then carefully swirl mixture until a uniform suspension is achieved. Avoid vigorous shaking as this tends to cause foaming of the suspension. Do not dilute reconstituted vaccine. Use aseptic technique and a separate sterile needle and syringe to withdraw each vaccine dose from the single dose vial of reconstituted vaccine. The proper dose of reconstituted vaccine will be withdrawn directly from each "single dose vial" and used to vaccinate a single subject, the remaining vaccine, if any will be disposed of as Medical Waste.

Parental drug products should be inspected visually for particulate matter and discoloration prior to administration. If either of these conditions exists, do not administer the vaccine.

**Use YF-VAX within 60 minutes of reconstituting the single dose or multi-dose vial.**

Properly dispose of all reconstituted vaccine and containers that remain unused after one hour according to locally approved guidelines (e, sterilized or disposed in red hazardous waste containers) (18).

**Dosing information:**

**Syringes will be used with 25 gauge, 5/8 inch needle for fractional dosing.**

Group 1: A single subcutaneous injection of 0.5 mL of reconstituted vaccine (standard dose)
Group 2: A single subcutaneous injection of 0.1 mL of reconstituted vaccine (1/5$^{th}$ dose)
Group 3: A single subcutaneous injection of 0.05 mL of reconstituted vaccine (1/10$^{th}$ dose)

The vaccine preparation process is described in detail in the Package Insert (Appendix C). The handling of reconstituted vaccine and its administration is described in a Study Specific Procedure (SSP).

#### B5.7.2.3 Any drug not approved by the FDA

N/A

## B5.8 Statistical Analysis

### B5.8.1 Sample Size Estimation

The primary objective of this study is to explore the safety and immunogenicity of two fractionated doses of YF-VAX (1/5$^{th}$ and 1/10$^{th}$ the standard dose) compared to the standard YF-VAX dose. As this is a pilot study, no formal statistical hypothesis testing is planned. A target enrollment of 30 participants per treatment group is planned and assuming a drop-out rate of 30%, the final sample size for analysis will be approximately 21 participants per treatment group. The primary objective will be evaluating proportions of subjects that seroconverted and the proportion of subjects experiencing each type of adverse event . Proportions will be presented with the 95% confidence interval using an exact binomial proportion. The following table displays the width of the 95% confidence intervals under different sample sizes and expected proportions.

| Expected Proportion | 95% CI width (lower bound – higher bound) | 95% CI width (lower bound – higher bound) |
|---|---|---|
| | N=20/group | N=30/group |
| 0.05 | 0.247 (0.001 – 0.249) | 0.193 (0.004 – 0.197) |
| 0.10 | 0.305 (0.012 – 0.317) | 0.244 (0.021 – 0.265) |
| 0.15 | 0.347 (0.032 – 0.379) | 0.281 (0.047 – 0.327) |
| 0.25 | 0.404 (0.087 – 0.491) | 0.330 (0.111 – 0.441) |
| 0.50 | 0.456 (0.272 – 0.728) | 0.374 (0.313 – 0.687) |
| 0.95 | 0.247 (0.751 – 0.999) | 0.193 (0.803 – 0.996) |
| 1.00 | 0.168 (0.832 – 1.000) | 0.116 (0.884 – 1.000) |

With the sample sizes of between 20 and 30 per treatment group, if the expected seroconversion of the fractionated doses is 100% as seen in previous studies, there will be 95% confidence that the actual seroconversion rate will be above 83% and 88% respectively. This is above the WHO recommendation of 80% efficacy in yellow-fever vaccination sero-protection and would help justify a larger non-inferiority study of the fractionated doses to the standard dose.

### B5.8.2 Data analysis

Detailed statistical procedures and proposed tables, listings, and figures will be provided in a separate statistical analysis plan (SAP) written shortly after protocol approval. The SAP will be finalized before study closeout and database lock. The following key statistical components will be considered, and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured,
- Statistical methods and tests that will be used to analyze the endpoints,
- Strategy that will be used if the statistical test assumptions are not satisfied,
- Indication of whether the comparisons will be one-tailed or two-tailed (with justification of the choice) and the level of significance to be used,
- Identification of whether any adjustments to the significance level or the overall *P* value will be made to account for any planned or unplanned subgroup analyses or multiple testing,
- Specification of potential adjusted analyses and a statement of which covariates or factors will be included,
- Planned exploratory analyses and justification of their importance, and
- Any subgroup effects with biological justification and support from within and outside the study.

An electronic data capture (EDC) database system will be used for the collection of clinical data in an electronic format. After the clinical database has been locked at the conclusion of the study, database management personnel will extract a data file in SAS® data format using data transfer programs specific to the EDC database and will provide the study data from the database to the statistician at USAMRIID. A statistician at USAMRIID will analyze these data. Data in this study will be assessed IAW USAMRIID SOPs relating to statistical analyses of data in a GCP environment.

All analyses, including any hypotheses suggested by these data, will be tested using the statistical methods as specified in the SAP at the two-tailed 95% confidence level. Adjustments for multiple comparisons will be considered only in the context of sets of like variables (such as reactions) using either a bootstrap approach or other methods deemed appropriate. All computations will be completed using the SAS package of standard biostatistical tools or other validated statistical software (SAS Institute Inc., SAS System Version 9.4 [or later version], Cary, NC 27513, 2013).

Safety endpoint measurements in this vaccine study will be evaluated for all vaccinated subjects regardless of adherence to the protocol. Immunogenicity endpoints will be evaluated for all per-protocol subjects.

This study is designed to be an exploratory analysis comparing the standard of care SQ route at full dose to SQ administration that utilizes significantly decreased doses (1/5th or 1/10$^{th}$). The primary immunogenicity assessment will be based on the exact (Clopper-Pearson) 95% confidence interval of the observed sample rates of immune response (PRNT50/PRNT80 ≥ 1:10). The emphasis of the study is on descriptive rather than analytical statistics in order to inform future non-inferiority studies. A final sample size of no less than 20 per group produces a two-sided 95% confidence interval with a width equal to 0.168 when the observed immunogenicity response rate 1.00.

The number of subjects enrolled, completed, or withdrawn will be summarized for each group and overall. Reasons for withdrawal, when known, will be provided. Subject demographics including gender, age, and race will be summarized by group and overall. Summary statistics will include mean, standard deviation, median,

and range for continuous variables and number and percent for categorical variables. Descriptive tables of demographics, subject disposition, vaccinations administered, compliance with protocol, study deviations, any local injection site measurements or vitals taken will be compiled.

Only subjects vaccinated in compliance with the protocol and having blood for titers drawn in compliance with the protocol will be included in the immunogenicity analyses. The primary analysis variable for assessing immunogenicity will be the proportion of subjects who develop PRNT50 and PRNT80 titers ≥ 1:10 at each scheduled time point for which blood samples are drawn and over the entire study period to study completion. Binomial proportions and 95% confidence intervals will be calculated for subjects who develop PRNT50 and PRNT80 titers ≥ 1:10 at each scheduled time point for which blood samples are drawn and over the entire study period to study completion and exact 95% confidence intervals of these rates will be calculated. The effect of demographic variables on immunogenicity rates may be tested by Fisher exact tests when permitted by data. Geometric means of PRNT80 titers and standard deviations of the geometric means will be calculated using log-transformed titers, replacing any titers below the limit of detection with a value equal to the lower limit of detection divided by the square root of 2 (LLOD/√2).

Rates of adverse events (AEs) will be tabulated overall, by type (local or systemic), by system organ class, by severity, by relationship, and by sex. Binomial proportions of these rates and exact 95% confidence intervals will be calculated. If the same reaction occurs on multiple occasions after a given vaccination for a given subject, the highest severity will be assumed and will be counted only once. Thus, individuals receiving a vaccination and reporting a specific reaction, not reactions themselves, will be counted in the calculation of reaction rates for a specific reaction type. Duration of local and systemic reactions will be calculated. Mean, median, and range of duration of local and systemic reactions will be tabulated. Serious and/or unexpected AEs reported during the study period will be listed. All vaccinated subjects will be included in the safety analyses regardless of compliance with this protocol. The effect of demographic variables on AE rates may be tested by Fisher exact tests when permitted by data.

## SECTION C: HUMAN RESEARCH PROTECTIONS

### C1. RECRUITMENT AND CONSENT

#### C1.1 Identification and Selection of Subjects

Potentially eligible individuals will be recruited using the institutional review board (IRB)–approved recruitment material/advertisement to include flyers and e-mail documenting subject eligibility criteria, number of study/blood draw visits and participant's study duration time frame.

#### C1.2 Recruitment Process

Potentially eligible individuals will be recruited using the institutional review board (IRB)–approved recruitment material/advertisement.

Interested individuals will initiate contact involving either the study recruiter, investigators or study coordinator to discuss study participation requirements. All prospective study subjects will be briefed individually by study personnel on the nature of the study and will be provided the currently approved informed consent document describing all aspects of the research study and procedures (including, but not exclusive to, the timing of blood draws and the amount of blood to be drawn). Prospective subjects will be provided the opportunity to discuss the study with the study investigator(s), research nurse, or study coordinator and will have any/all questions answered.

For active military, Unit officers and senior NCOs in the chain of command will not be present at the time of research subject solicitation and consent during any research recruitment sessions in which members of units under their command are afforded the opportunity to participate as research subjects (DODI 3216.02). However, it is not the intent to recruit from assembled units.

### C1.3 Eligibility

Participation in this study will be determined by either the Principal Investigator, Associate Investigator or designee upon review of the eligibility criteria requirements, history and physical, and all screening lab tests.

### C1.4 Consent Process

A combined written informed consent and HIPAA Authorization document will be signed by each subject before any study-related procedures are initiated. This informed consent document will be retained by the investigator as part of the study records. The investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI. No subject should grant consent until questions have been answered to his or her satisfaction.

Should the protocol be modified, the ICD must be revised to reflect the changes to the protocol. If the subject is directly affected by the change, the subject will receive a copy of the revised ICD for review. If the subject decides to continue participation, then the subject must sign and date the revised ICD. Each subject will receive a copy of the signed and dated ICD.

#### C1.4.1 Research involving subjects with cognitive impairment or who lack capacity to provide informed consent

N/A

#### C1.4.2 Research involving non-English speaking subjects

N/A

#### C1.4.3 Research involving a waiver of the requirement to obtain informed consent OR alteration of the elements of informed consent

N/A

#### C1.4.4 Research involving a waiver of the requirement for investigator to obtain a signed consent form

N/A

#### C1.4.5 Waivers of assent or parental permission when the research involves children

N/A

#### C1.4.6 Research involving data collection for the USAMRDC Volunteer Registry Database

N/A

## C2. COMPENSATION FOR PARTICIPATION

Subjects will be compensated for participation in this study.

Utilizing a preloaded gift card, subjects will be compensated on the same day the blood sample is obtained with $50.00 for each blood draw. Study subjects who complete vaccination day activities will be compensated an additional $200.00 for a total of $850.00. (This is in addition to the $50 study participant receives for the blood draw on the vaccination day). On vaccination days subjects may be required to be present at the facility for up to 1-2 hours. Post vaccination vital signs and clinical checks must be obtained no less than 30 minutes after receipt of the vaccination. These additional funds are to compensate for the additional time necessary on vaccination days. All military and federal/civilian employees will be off duty or on leave on the day of vaccination in order to receive the additional $200, day of vaccination compensation.

## C3. WITHDRAWAL FROM RESEARCH PARTICIPATION

Subjects may withdraw from this protocol at any time without penalty or loss of benefits to which they are otherwise entitled. Reasons for premature withdrawal from the study will be recorded on the appropriate source documents.

Subjects may be withdrawn from the study by an investigator for any of the following reasons:

- Failure to comply with study procedures, as outlined in the ICD, at the discretion of the principal investigator (PI).
- Any problem wherein the safety or health of the subject may be compromised by further participation, as determined by the PI, or Headquarters US Army Medical Research and Development Command (HQ USMRDC) IRB.
- Any problem that may preclude drawing blood.
- Pregnancy.

## C4. PRIVACY FOR SUBJECTS

The PI, investigator or designee will present the protocol to the subject singly. Questions regarding the nature of the protocol, the means by which the study is to be conducted, and the risks to the participants will be solicited on an individual basis during the consent process and at each follow-up study visit.

## C5. CONFIDENTIALITY PROCEDURES FOR RESEARCH RECORDS, DATA, HUMAN BIOLOGICAL SPECIMENS

Each individual enrolled in the study will be assigned a subject identification number (SIN). Upon receipt of an individual's signed and dated consent, the subject will be considered officially enrolled as a study subject. Subject information will be entered onto the current version of the study enrollment/withdrawal log. In addition, SINs will be used in the event that it becomes necessary to identify data specific to a single subject. All data and medical information obtained about subjects during the course of this research will be considered privileged and confidential. Subjects will not be identified by name in any published report, presentation of results, or provision of PHI to collaborating institutions (Privacy Act of 1974 [5 USC 552a] and AR 25-22). Completed study records will be stored in a secure fashion by the PI (or a designee) at USAMRIID.

Representatives of the USAMRIID OHRO, the HQ USAMRMC IRB and other DoD offices may review research records as a part of their responsibility to protect human subjects in research.

Subjects will be identified in study documentation by a study identification number assigned according to USAMRIID SOPs.

The enrollment/withdrawal log will be securely stored separately from the research data in the study specific regulatory file and will be destroyed once the IRB approves the final report. This will completely sever any links between the coded information in the subject study files and render the data anonymized.

No personal identifier will be used in any publication or communication used to support this research study. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

## C6. RISKS OF HARM, MEASURES TO REDUCE THE RISKS OF HARM, AND BENEFITS OF PARTICIPATION

### C6.1 Risks of Harm

The risks directly related to this study are those associated with the following:

**Vaccine Administration**

Common side effects after vaccination include: headache, tiredness or weakness, injection site reactions (pain, tenderness, redness, swelling), and muscle pains, fever, stomach problems, and joint pain.

Other reported reactions include an abnormal sensation, typically tingling or pricking ("pins and needles") and flu-like illness, injection site blisters and syncope.

The most serious adverse reactions that may happen after vaccination with a yellow fever vaccine are the occurrence of Yellow Fever Vaccine-Associated Acute Viscerotropic Disease (YEL-AVD) affecting vital organs similarly to yellow fever infection, and Yellow Fever Vaccine-Associated Acute Neurotropic Disease (YEL-AND) affecting the brain and nerves. These complications have occurred in individuals over the age of 60 years. This study limits the age range to 18-50 years of age.

As with any vaccination, there is a possibility of an allergic reaction, such as rash, itching or hives, swelling of the lips or face, swelling of the throat, fast pulse, sweating, feeling of dread, difficulty breathing, sudden drop in blood pressure causing dizziness or lightheadedness, and inability to breathe without assistance. YF-VAX® will not be administered to anyone with a history of acute hypersensitivity to eggs or egg products due to a risk of anaphylaxis (a severe potentially life-threatening allergic reaction).

Vaccine will be administered by trained professionals; emergency kits are located in vaccination area.

**Pregnancy and Breastfeeding Risks**

The vaccine may be dangerous to an embryo or fetus or to a breastfed infant. Vaccination with YF-VAX® is also contraindicated in lactating women who are providing breastmilk to infants less than 9 months of age due to the potential for transmission of vaccine virus in breastmilk. Pregnant and/or breastfeeding women are not eligible to participate in the study because of the potential risks.

**Phlebotomy Risk**

Subjects may experience local discomfort or bleeding under the skin from needle sticks, resulting in a localized hematoma that generally resolves within 1 to 2 weeks. A rare complication may be localized infection at the

phlebotomy site or thrombophlebitis. This would be treated as medically indicated. Some subjects may feel lightheaded, queasy, or nauseated; have chills; develop a fast heartbeat; and/or faint during blood collection. These symptoms can be resolved by having the subject lie down and/or by stopping the procedure. To minimize risks, only qualified personnel will draw blood.

**Confidentiality**

Each individual enrolled in the study will be assigned a SIN as noted in section C5. Subjects will not be identified by name in any published report, presentation of results, or provision of PHI. Completed study records will be stored in a secure fashion by the PI (or a designee) at USAMRIID. Representatives of the USAMRIID OHRO, the HQ USAMRDC IRB, and other DoD offices may review research records as a part of their responsibility to protect human subjects in research.

Samples sent to CDC laboratory and Emory University will be stored in a secure manner. When sample testing is completed, all samples will be either destroyed at testing site or shipped back to USAMRIID for either destruction or for possible testing in future studies to further our understanding of the immune response to YF-VAX®.

**Risks to Study Personnel and the Environment**

The principal risks to study personnel in the clinical setting are associated with needle sticks. Standard operating procedures (SOPs) and universal precautions will reduce the risk of exposure for these individuals. This study will have no known risks to the environment: all biohazardous wastes will be disposed of as stipulated by local, state, and federal regulations.

### C6.2 Incidental or Unexpected Findings

Unexpected or incidental findings will be duly evaluated for severity and relatedness and reported to the IRB and VAERS if greater qualified.

### C6.3 Potential Benefits

Subjects may benefit from participating in the study by learning about their health from the screening tests. Subjects who receive the full dose of YF-VAX® are considered protected from Yellow Fever disease per FDA.

## C7. DATA AND SAFETY MONITORING

### C7.1 Monitoring

Safety monitoring will occur throughout the study; therefore safety concerns will be identified by continuous review of the data as represented by the Study Event Schedule table by the PI, research staff, and research monitor.

A CRO will be contracted to perform data monitoring services. Monitoring will be conducted according to a monitoring plan approved by the PI. The monitoring plan will specify in detail the items for source data verification and other tasks to be performed during the clinical trial site visits.
All documents in the context with this clinical trial will be handled confidentially at all times.
The monitor will be given access to relevant clinical records to confirm their consistency with the CRF entries and to obtain an adequate overview of the course of the trial. The monitor will verify that the entries in the CRF are complete, accurate, correct and supported by source documents. In addition the monitor will verify that all required data documented in the source were transferred accurately in the CRF. This will be done under preservation of data protection.

The source data verification must be performed by direct inspection of the subject's source documents. If a subject refuses to consent to this procedure, he/she may not be enrolled in the trial. The clinical trial site will provide direct access to all trial related data for the purpose of monitoring and auditing by regulatory authorities. The PI (or a representative) has further agreed to support the monitor in solving any problems he/she discovers during his/her visits.

## C8. REPORTABLE EVENTS

### C8.1 Expected adverse events

The most frequently reported reactions with YF-VAX® include: headache, tiredness or weakness, injection site reactions (pain, tenderness, redness, swelling), and muscle pains.

Other common symptoms include: fever, stomach problems, and joint pain. These reactions usually occur within the first 3 days following vaccination (except fever, which is likely to occur between the 4th and the 14th day after vaccination), and usually last for not more than 3 days.

Other reported reactions include an abnormal sensation, typically tingling or pricking (“pins and needles”) and flu-like illness, injection site blisters and syncope.

The most serious adverse reactions that may happen after vaccination with a yellow fever vaccine are the occurrence of Yellow Fever Vaccine-Associated Acute Viscerotropic Disease (YEL-AVD) affecting vital organs similarly to yellow fever infection, and Yellow Fever Vaccine-Associated Acute Neurotropic Disease (YEL-AND) affecting the brain and nerves.

As with any vaccination, there is a possibility of an allergic reaction, such as rash, itching or hives on the skin, swelling of the lips or face, swelling of the throat, fast pulse, sweating, feeling of dread, difficulty breathing, a sudden drop in blood pressure causing dizziness or lightheadedness, and inability to breathe without assistance. YF-VAX® will not be administered to anyone with a history of acute hypersensitivity to eggs or egg products due to a risk of anaphylaxis.

Following vaccination, solicited adverse events (AEs) will be recorded on the case report forms, and unsolicited AEs on a diary card for 28 days.

### C8.2 Unexpected adverse events and unanticipated problems

All unanticipated problems involving risk to subjects or others and serious adverse events related to study participation will be promptly reported to the HQ USAMRDC IRB by phone (301-619-6240), by e-mail (usarmy.detrick.medcom-usamrmc.other.irb-office@health.mil), by facsimile (301-619-4165), or sent to the U.S. Army Medical Research and Development Command, ATTN: MCMR-RPI, 810 Schreider Street, Fort Detrick, Maryland 21702-5012. A complete written report will follow the initial notification.

### C8.3 Adverse device effects

N/A

### C8.4 FDA-regulated research under IND and IDE

N/A

## D. Assays

**PRNTs:** The WHO sanctioned PRNT will be used to determine YF neutralizing antibodies in sera collected from subjects participating on this protocol. This assay will be performed by the CDC's Fort Collins' laboratory, which is one of a few laboratories recognized by the WHO to perform this assay. CDC-Ft. Collins is in the GYFLN at the level of Global Specialized Lab, along with Erasmus MC, Robert Koch Institute, and Institut Pasteur de Dakar. The results from the CDC-Fort Collins' lab will serve as the gold standard and will be the assay for which group responses will be compared. Other laboratories, including USAMRIID labs have expressed interest in testing samples for comparison with their assays. Such assays will be considered experimental.

**RT-PCR:** The CDC-Fort Collins diagnostic lab does YF RT-PCRs using the Domingo primer set and protocol.

**YFV Viral Plaque Assay:** CDC-Fort Collins lab

Assay procedure/protocol/SOPs will be obtained from CDC-Fort Collins as allowable.

**PBMCs and Microbiome:** Emory University

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## SECTION E: REFERENCES

[1] Vasconcelos PFC and Monath TP. Yellow Fever Remains a Potential Threat to Public Health *Vector-Borne and Zoonotic Diseases*. 2016; 16 (8): 566- 567.
[2] https://health.mil/Military-Health-Topics/Health-Readiness/Immunization-Healthcare/Vaccine-Preventable-Diseases/Yellow-Fever?type=All#RefFeed, accessed July 10, 2017
[3] Medical Material Quality Control Message MMQC-17-1727, http://www.usamma.amedd.army.mil/assets/apps/nala_qaweb/message.cfm?MSG=MMQC-17-1727
[4] Gershman MD, Angelo KM, Ritchey J, et al. Addressing a Yellow Fever Vaccine Shortage — United States, 2016–2017. MMWR Morb Mortal Wkly Rep 2017;66:457–459. DOI: http://dx.doi.org/10.15585/mmwr.mm6617e2.
[5] Monath TP, Woodall JP, Gubler DJ. Yellow fever vaccine supply: a possible solution. *Lancet* 2016; 387: 1600.
[6] WHO Strategic Advisory Group of Experts on Immunization (SAGE), Fractional dose yellow fever vaccine as a dose-sparing option for outbreak response http://www.who.int/immunization/sage/meetings/2016/october/3_Fractional_dose_secretariat_report_full_version.pdf?ua=1, accessed July 14, 2017.
[7] CH Calisher and Woodall JP. Yellow Fever—More a Policy and Planning Problem than a Biological One. *Emerging Infectious Diseases.* 2016; 22 (10): 1859 – 1860. DOI: http://dx.doi.org/10.3201/eid2210.160875
[8] Elachola H, Ditekemena J, Zhuo J et al., Yellow fever outbreaks, vaccine shortages and the Hajj and Olympics: call for global vigilance *Lancet* 2016; 388: 1155. http://dx.doi.org/10.1016/ S0140-6736(16)31546-X.
[9] Roukens AH, Vossen AC, Bredenbeek PJ, van Dissel JT, Visser LG. Intradermally administered yellow fever vaccine at reduced dose induces a protective immune response: a randomized controlled non-inferiority trial. *PLoS ONE.* 2008; 3:e1993. [PubMed: 18431480]
[10] Slifka MK, Leung DYM, Hammarlund E, et al. Transcutaneous Yellow Fever Vaccination of Subjects with or without Atopic Dermatitis. *J Allergy Clin Immunol*. 2014 February ; 133(2): 439–447. doi:10.1016/j.jaci.2013.10.037.
[11] Campi-Azevedo AC, de Almeida Estevam P, Coelho-dos-Reis JG, et al. Subdoses of 17DD yellow fever vaccine elicit similar virological/immunological kinetics timeline. *BMC Infectious Diseases*. 2014;14:391. doi:10.1186/1471-2334-14-391.
[12] Wu JT, Peak CM, Leung GM, Lipsitch M, Fractional dosing of yellow fever vaccine to extend supply: a modeling study, *The Lancet*, Volume 388, Issue 10062, 10–16 December 2016, Pages 2904-2911, http://dx.doi.org/10.1016/S0140-6736(16)31838-4.
[13] Visser LG, Roukens AHE, Modeling a way out of yellow fever, *The Lancet,* Volume 388, Issue 10062, 10–16 December 2016, Pages 2847-2848, http://dx.doi.org/10.1016/S0140-6736(16)31330-7.

[14] Casey RM, Harris JB, Ahuka-Mundeke S, Dixon MG, Nsele PM, Kizito GM, Umutesi G, Laven J, Paluku G, Gueye AS, Hyde TB, Sheria GKM, Muyembe-Tanfum JJ, Staples JE. Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak -Final Report. NEJM 2019; 381:444-54.
[15a] Martins RM, Maia MLS, Farias RHG, et al. 17DD Yellow Fever vaccine: A double blind, randomized clinical trial of immunogenicity and safety on a dose-response study. Hu Vac & Immunoth 9: 4, 879-888, 2013.
[15b] Martins R, Maia MLS, de Lima SMB, de Noronha TG, Xavier JR, Camacho LAB, de Albuquerque EM, Farias RHG, da Matta de Castro T, Homma A; Collaborative Group for Studies on Duration of Immunity from Yellow Fever Vaccine. Duration of post-vaccination immunity to yellow fever in volunteers eight years after a dose-response study. Vaccine. 2018 Jun 27; 36(28):4112-4117.
[16] Weekly Epidemiological Record, 23 June 2017, vol. 92, 25 (pp. 345–356) Yellow fever vaccine: WHO position on the use of fractional doses – June 2017 Global polio eradication: progress towards containment of poliovirus type 2, worldwide 2017.
[17] Roukens AHE, LG Visser. Fractional-dose Yellow Fever vaccination: an expert review. J Trav Med, 2019: 26(6): 1-5.
[18] Recommendations of the Advisory Committee on Immunization Practices (ACIP). Yellow Fever Vaccine. MMWR 2010; 59(RR-7): 1-32.
[19] Hepburn MJ, Kortepeter MG, Pittman PR, et al. Neutralizing antibody response to booster vaccination with the 17D yellow fever vaccine. Vaccine 2006; 24:2843-9.

## SECTION E: ABBREVIATIONS AND ACRONY

| Abbreviation | Explanation |
|---|---|
| AE | adverse event, adverse experience |
| AFRICOM AOR | US Africa Command Area of Responsibility |
| C | Celsius |
| CDC | US Centers for Disease Control and Prevention |
| CFR | Code of Federal Regulations |
| Cm | Centimeter(s) |
| DHA-IHB | Defense Health Agency Defense Health Agency-Immunization Healthcare Branch |
| DoD | Department of Defense |
| eCRF | electronic case report form |
| EDC | electronic data capture |
| F | Fahrenheit |
| FDA | US Food and Drug Administration |
| FWA | Federal Wide Assurance |
| GCP | Good Clinical Practice |
| HbsAg | Hepatitis B Surface Antigen |
| Hg | mercury |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | human immunodeficiency virus |
| HQ | Headquarters |
| IAW | in accordance with |
| IB | investigator's brochure |
| ICD | informed consent document |
| ICH | International Conference on Harmonisation |
| ID | Intradermal |
| IRB | Institutional Review Board |
| mg | milligram(s) |
| mL | milliliter(s) |
| mm | millimeter(s) |
| OHRO | Office of Human Research Oversight (USAMRIID) |

| Abbreviation | Explanation |
|---|---|
| PBMC | peripheral blood mononuclear cell |
| pfu | plaque-forming unit(s) |
| PI | principal investigator |
| PHI | protected health information |
| PRNT50 | plaque reduction neutralization 50% titer |
| PRNT80 | plaque reduction neutralization 80% titer |
| SAE | serious adverse event |
| SAGE | Strategic Advisory Group of Experts |
| SAP | statistical analysis plan |
| SIN | subject identification number |
| SOP | standard operating procedure |
| SOUTHCOM AOR | US Southern Command Area of Responsibility |
| SQ | Subcutaneous |
| TC | Transcutaneous |
| USAMRIID | US Army Medical Research Institute of Infectious Diseases |
| USAMRDC/ MRDC | US Army Medical Research and Development Command |
| WHO | World Health Organization |
| YF | Yellow Fever |
| YFV/ YF-VAX | Yellow Fever vaccine |

## SECTION F: DoD PRIVACY RULE AND PROTECTED HEALTH INFORMATION (HIPAA)

☐ NA – institution is not a covered entity

☐ NA – will not use or disclose protected health information

☒ HIPAA authorization will be obtained

☐ An application for waiver/alteration of HIPAA authorization will be submitted

## APPENDIX A: STUDY RELATED ROLES AND RESPONSIBILITIES

### Vaccination Room Team

- May prepare vaccine for administration.
- May administer YF-VAX® to study participants.

### Data Manager

- Provides study database end-user training to users requiring access to the study database.
- Oversees the data entry and data discrepancy management activities.
- Ensures data consistency, quality, and integrity.
- Performs Medical Dictionary for Regulatory Affairs (MedDRA) and World Health Organization (WHO) coding.
- Manages and maintains the study database.

# APPENDIX B: TOXICITY GRADING SCALE

## Criteria for Rating Local Adverse Events

| Local Reaction to Injectable Product | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) | Fatal (Grade 5) |
|---|---|---|---|---|---|
| Pain | Does not interfere with activity | Interferes with activity or repeated use of non-narcotic pain reliever | Prevents daily activity or repeated use of narcotic pain reliever | Emergency room (ER) visit or hospitalization | Death |
| Tenderness | Mild pain to touch | Pain with movement | Significant pain at rest | ER visit or hospitalization | Death |
| Erythema/ Redness[a] | 2.5-5 cm | 5.1-10 cm | > 10 cm | Necrosis or exfoliative dermatitis | Death |
| Swelling[b] | 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 | Death |

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[b] Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

## Criteria for Rating Systemic Adverse Events (Vital Signs)

| Vital Signs[a] | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) | Fatal (Grade 5) |
|---|---|---|---|---|---|
| Fever (°C)[b]<br>(°F) | 38.0-38.4<br>100.4-101.1 | 38.5-38.9<br>101.2-102.0 | 39.0-40<br>102.1-104 | > 40<br>> 104 | Death |
| Tachycardia - beats per minute | 101-115 | 116-130 | > 130 | ER visit or hospitalization for arrhythmia | Death |
| Bradycardia - beats per minute | 50-54 | 45-49 | < 45 | | Death |
| Hypertension (systolic) - mm Hg | 141-150 | 151-155 | > 155 | ER visit or hospitalization for malignant hypertension | Death |
| Hypertension (diastolic) - mm Hg | 91-95 | 96-100 | > 100 | | Death |
| Hypotension (systolic) - mm Hg | 85-89 | 80-84 | < 80 | ER visit or hospitalization for hypotensive shock | Death |
| Respiratory Rate - breaths per minute | 17-20 | 21-25 | > 25 | Intubation | Death |

[a] Subject should be at rest for all vital sign measurements.

[b] Oral temperature; no recent hot or cold beverages or smoking.

## Criteria for Rating Systemic Adverse Events (Symptoms)

| Systemic (General) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) | Fatal (Grade 5) |
|---|---|---|---|---|---|
| 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 | ER visit or hospitalization for hypotensive shock | Death |
| 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 > 800 gms/ 24 hours or requires outpatient IV hydration | ER visit or hospitalization for hypotensive shock | Death |
| Headache | No interference with activity | Some interference with activity or repeated use of non-narcotic pain reliever | Significant, prevents daily activity or repeated use of narcotic pain reliever | ER visit or hospitalization | Death |
| Fatigue | No interference with activity | Some interference with activity | Significant, prevents daily activity | ER visit or hospitalization | Death |
| Myalgia | No interference with activity | Some interference with activity | Significant, prevents daily activity | ER visit or hospitalization | Death |
| Illness or clinical adverse event (as defined according to applicable regulation) | No interference with activity | Some interference with activity not requiring medical intervention | Prevents daily activity and requires medical intervention | ER visit or hospitalization | Death |

# Criteria for Rating Systemic Adverse Events (Chemistry)

| Lab Value | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Sodium (mmol/L) Hyponatremia | <LLN to 132 | 130-131 | 125-129 | <125 |
| Sodium (mmol/L) Hypernatremia | >ULN to 149 | 150-154 | 155-159 | ≥160 |
| Potassium (mmol/L) Hypokalemia | <LLN to 3.1 | 2.5-3.0 | 2.0-2.4 | <2.0 |
| Potassium (mmol/L) Hyperkalemia | >ULN to 5.9 | 6.0-6.5 | 6.6-7.0 | >7.0 |
| Calcium (mmol/L) Hypocalcaemia | <LLN to 2.00 | 1.76-2.00 | 1.50-1.75 | <1.50 |
| Calcium (mmol/L) Hypercalcaemia | >ULN to 2.89 | 2.90-3.09 | 3.10-3.30 | >3.30 |
| Serum creatinine (mg/dL) | >ULN to <1.5x ULN | ≥1.5 to <3x ULN | ≥3 to 6x ULN | >6x ULN |
| Alkaline phosphatase (increase by factor) | >1.25x to <2x ULN | ≥2x to <3x ULN | ≥3x ULN | >10x ULN |
| Liver function tests (increase by factor) | >1x to <2.5x ULN | ≥2.5x to <4x ULN | ≥4x ULN | >10x ULN |
| Total bilirubin (increase by factor) | >ULN to 1.5x ULN | >1.5x to 3x ULN | >3x to 10x ULN | >10x ULN |
| (increase by factor) | >ULN to <2xULN | ≥2x to <5x ULN | ≥5x ULN | |
| Total cholesterol (mg/dL) | > ULN to 300 | >300 to 400 | >400 | |

# Criteria for Rating Systemic Adverse Events (Hematology)

| Lab Value | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Hemoglobin (g/dL) Female | < LLN to ≥10.5 | <10.5 to ≥10.0 | < 10.0 | |
| Hemoglobin (g/dL) Male | <LLN to ≥12.5 | <12.5 to ≥11.0 | < 11.0 | |
| WBC (cells/$mm^3$) Decrease | <LLN to ≥2,500 | <2,500 to ≥1,500 | <1,500 | |
| WBC (cells/$mm^3$) Increase | >ULN to < 15,000 | ≥15,000 to <20,000 | ≥ 20,000 | |
| Lymphocytes (cells/$mm^3$) Decrease | <LLN to ≥750 | <750 to ≥500 | <500 | |
| Neutrophils (cells/$mm^3$) Decrease | <LLN to ≥1,500 | <1,500 to ≥1,000 | <1,000 | |
| Platelets (cells/$mm^3$) Decrease | <LLN to ≥75,000 | <75,000 to ≥ 50,000 | <50,000 | |