RANDOMIZED, DOUBLE BLIND EVALUATION OF LATE BOOST STRATEGIES WITH IHV01 (FLSC IN ALUMINUM PHOSPHATE) AND A244 WITH OR WITHOUT ALFQ FOR HIV-UNINFECTED PARTICIPANTS IN THE HIV VACCINE TRIAL RV306 / WRAIR 1920

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PRINCIPAL INVESTIGATOR'S AGREEMENT

1. I agree to follow this protocol version as approved by the IRBs/ERCs.
2. I will conduct the study in accordance with applicable IRB/ERC requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I will not modify the protocol without first obtaining an IRB/ERC approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants.
5. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the U.S. FDA Modernization Act and NIH policy, I will ensure the registration and reporting results of the trial on the www.clinicaltrials.gov website.
6. In accordance with WRAIR Command Policy 2008-35, I will ensure that the Commanding General receives a pre-brief (or Executive Summary) and approves the study prior to execution.
7. I will ensure that the data (and/or specimens) are maintained in accordance with the data (and/or specimen) disposition outlined in the protocol. Any modifications to this plan should first be reviewed and approved by the applicable IRBs/ERCs.
8. I will promptly report changes to the research or unanticipated problems to the WRAIR IRB immediately via the WRAIR Human Subjects Protection Branch at (301) 319-9940 (during duty hours) or to usarmy.detrick.medcom-wrair.mbx.hspb@health.mil and submit a written report within 10 working days of knowledge of the event.
9. I will prepare continuing review reports at an interval established by the IRB/ERC, and a study closure report when all research activities are completed.
10. I will immediately report to the WRAIR Human Subjects Protection Branch knowledge of any pending compliance inspection by any outside governmental agency.
11. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

_____ _____

Punnee Pitisuttithum, MD, DTM&H Date
Mahidol University Site Principal Investigator

_____ _____

LTG Sorachai Nitayaphan, MD, PhD Date
RTA-AFRIMS Site Principal Investigator

EMERGENCY CONTACT INFORMATION

Table 1: Emergency Contact Information

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Local Drug and Vaccine Authorities	MoPH TSHAVD Thai FDA	See page 8

2. SYNOPSIS

Name of Sponsor:

The Surgeon General, Department of the Army

Name of Investigational Products:

IHV01 (FLSC), A244 (with aluminum hydroxide fluid gel adjuvant), ALFQ Adjuvant, and Placebo

Name of Active Ingredient:

IHV01 consists of the Full-Length Single Chain (FLSC) gp120-CD4 chimera subunit HIV-1 vaccine formulated in aluminum phosphate adjuvant. It is encoded by a synthetic gene, which contains a human codon-optimized HIV (BaL) gp120 sequence followed by human CD4D1D2, with a flexible 20-amino acid linker.

A244 consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01AE A244 derived from the CM244 CRF_01AE. The A244 gp120 envelope has an 11 amino N-terminal deletion, similar to the A244 protein used in AIDSVAX B/E. The aluminum hydroxide fluid gel (AHFG) adjuvant that is mixed with A244 consists of Rehydragel HPA that is diluted with sterile water for injection to a concentration of 5 ± 1mg/mL.

ALFQ (Army Liposomal Formulation) is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (MPLA) with the addition of QS-21.

Normal Saline (0.9% Sodium Chloride for Injection) will be used as a placebo.

Title of Study:

Randomized, Double Blind Evaluation of Late Boost Strategies with IHV01 (FLSC in aluminum phosphate) and A244 with or without ALFQ for HIV-uninfected Participants in the HIV Vaccine Trial RV306 / WRAIR 1920

Study Center(s):

Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University; Royal Thai Army Clinical Research Center, Armed Forces Research Institute of Medical Sciences; Thai Red Cross AIDS Research Centre; Institute of HIV Research and Innovation Foundation; and the Chulalongkorn Memorial Hospital.

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Associate Investigators:

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MAJ Worapong Nasomsong, MD
COL Jessica Cowden, MD, MSPH

Laboratory Investigators:

Siriwat Akapirat, MT, PhD
Alexandra Schuetz, PhD
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Study Period (years):

Estimated date first participant enrolled: June 2021

Estimated date last participant completed: December 2023

Estimated duration of the trial: 5 years (2-3 years screening and clinical activities: 2-3 years laboratory assays and data analysis)

Phase of development: I

Objectives:

Primary:

- To evaluate the safety of 3 novel investigational products in a late vaccine boost setting: the candidate IHV01 and A244/AHFG vaccines and the ALFQ adjuvant

Secondary:

- To evaluate the effect of a full dose of the IHV01 and A244/AHFG vaccines and the ALFQ adjuvant on cellular, humoral, and innate immune responses (peripheral, lymphoid and mucosal).
- To evaluate the effect of fractional dosing of the IHV01 and A244/AHFG vaccines on cellular, humoral, and innate immune responses (peripheral, lymphoid and mucosal)

Methodology:

This study is exploratory in nature.

The purpose of this study is to define the safety and immunogenicity of IHV01 and A244/AHFG with and without ALFQ at a full dose and at a fractional dose (one-fifth of a full dose) in a late boost setting for participants who had previously received a late boost of AIDSVAX®B/E with or without ALVAC in RV306. Safety will be assessed through the frequency of the overall and specific post-vaccination reactions. Blood, lymph nodes, sigmoid tissue, and mucosal specimens/secretions will be collected to assess humoral, cell-mediated, innate, and mucosal immune responses.

Healthy, HIV-uninfected participants, at a low risk for HIV infection, available for 12 months, who were randomized to receive active vaccine in RV306 and completed all vaccinations will be enrolled. A total of 80 participants will be enrolled across four vaccination groups with 20 participants per group. Within each group, participants will be randomized to receive either IHV01 and A244/AHFG at a full or fractional dose with or without ALFQ or placebo at a targeted ratio of 4:1 vaccine to placebo. Participants will receive 2 intramuscular (IM) injections into the quadriceps muscle at Day 0. The same quadriceps muscle will be used for both injections. Participants randomized to receive the vaccines will have one injection of IHV01 and one injection of A244/AHFG at a full or fractional dose with or without ALFQ, whereas participants randomized to receive placebo will get 2 separate injections of Normal Saline. All placebo injection volumes will match the vaccine injection volumes for the group in which a participant has been randomized. Participants will be followed-up for up to 12 months after enrollment. Mucosal secretion collections and endocervical cytobrush/swab

procedures will be performed at Days 0, 14, 168, and 336 on consenting participants. Leukapheresis, sigmoid biopsy, and lymph node biopsy procedures will be performed only at Day 14 on consenting participants.

Estimated Number of Participants Screened and Enrolled:

Approximately 110 prior RV306 participants will be screened to enroll 80 participants across both sites (anticipated screening to enrollment ratio of 1.375:1)

Note: More than 80 participants may be enrolled to accommodate for participant replacement (see Section 8.6.3).

Main Criteria for Inclusion/Exclusion:

Inclusion Criteria

1. Healthy, HIV-uninfected male and female participants
2. Prior RV306 recipients who were randomized to receive active vaccine with late boosting at month 12, 15, or 18 and who completed all vaccinations
3. Have a Thai identity card
4. Must be at low risk for HIV infection per investigator assessment
5. Must be able to understand and complete the informed consent process
6. Must be capable of reading Thai
7. Must successfully complete a Test of Understanding prior to enrollment
8. Must be in good general health without clinically significant medical history
9. HIV-uninfected per diagnostic algorithm within 45 days of enrollment
10. Laboratory screening analysis:
    a. Hemoglobin: Women ≥11.0 g/dL, Men ≥11.5 g/dL
    b. White cell count: 4,000 to 11,000 cells/mm^3
    c. Platelets: 150,000 to 450,000/mm^3
    d. ALT and AST ≤1.25 institutional upper limit of reference range
    e. Creatinine: ≤1.25 institutional upper limit of reference range
    f. Urinalysis: blood and protein no greater than 1+ and negative glucose

    Note: Each laboratory screening test that is out of acceptable range can be repeated during the screening window to confirm the result. A second screening visit may be conducted outside of the initial screening visit window for individuals who meet certain criteria (see Section 8.3.1) only if study enrollment is ongoing.
11. Female-Specific Criteria:
    a. Not currently pregnant or breastfeeding and not planning to become pregnant during the first 3 months after study vaccine/placebo injections
    b. Negative pregnancy test for women at screening, prior to vaccination (same day), and prior to any of the invasive procedures
    c. Be using an adequate birth control method for 45 days prior to receipt of vaccine/placebo and for at least 3 months after receipt of the vaccine/placebo. Adequate birth control is defined as follows: Contraceptive medications delivered orally, intramuscularly, vaginally, or implanted underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), condoms, diaphragms, intrauterine device, or abstinence
12. Male-Specific Criteria:
    a. Be using an adequate birth control method for at least 3 months after receipt of the vaccine/placebo. For non-vasectomized male participants with female partners of child-bearing potential this includes the use of condoms or abstinence and/or their partner's use of contraceptive medications delivered orally, intramuscularly, vaginally, or implanted underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), diaphragms, or intrauterine device.

Exclusion Criteria

1. Asplenia: any condition resulting in the absence of a functional spleen
2. Bleeding disorder diagnosed by a medical doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions)
3. History of allergic reaction, anaphylaxis, or other serious adverse reaction to vaccines or components of the vaccines
4. Volunteer has received any of the following substances:
    a. Chronic use of therapies that may modify immune response, such as IV immune globulin and systemic corticosteroids (in doses of ≥20 mg/day prednisone equivalent for periods exceeding 10 days)
    Note: The following exceptions are permitted and will not exclude study participation: use of corticosteroid nasal spray for rhinitis, topical corticosteroids for an acute uncomplicated dermatitis; or a short course (duration of 10 days or less, or a single injection) of corticosteroid for a non-chronic condition (based on investigator clinical judgment) at least 14 days prior to enrollment in this study
    b. Blood products within 120 days prior to HIV screening
    c. Immunoglobulins within 30 days prior to HIV screening
    d. Any licensed vaccine within 14 days prior to study vaccine administration in the present study
    e. Receipt of any investigational HIV vaccine other than RV306 products
    f. Investigational research agents or vaccine within 30 days prior to enrollment in the present study
    g. Receipt of a Coronavirus disease 2019 (COVID-19) vaccine that has been given Emergency Use Authorization (or those that become licensed) by the Thai FDA within 14 days prior to study vaccine administration in the present study
    Note: Volunteers receiving a COVID-19 vaccine that requires 2 doses will not be enrolled until 14 days after the second dose has been administered
    h. Anti-tuberculosis prophylaxis or therapy during the past 90 days prior to enrollment
    Note: Participants determined to be ineligible at screening due to receipt of the above listed substances may be re-screened once the applicable window of receipt has expired.
5. Active sexually transmitted infection confirmed by clinical exam and diagnostic test
6. Any medical, psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contradiction to protocol compliance or impairs a volunteer's ability to give informed consent
7. Psychiatric condition that precludes compliance with the protocol; past or present psychoses; past or present bipolar disorder; disorder requiring lithium; or within 5 years prior to enrollment, a history of suicide ideation or attempt
8. Study site employees who are involved in the protocol and/or may have direct access to study related area

Final evaluation of eligibility will be based on the medical judgment of the investigator based on his/her medical and research experience.

Investigational Product Dosage, Schedule, and Mode of Administration:

Participants will be randomized to receive either a full dose of IHV01 (approximately 300µg) and A244 (approximately 300µg) or a fractional dose of IHV01 (approximately 60µg) and A244 (approximately 60µg). Participants in two of the four randomization groups will also receive ALFQ (approximately 0.5ml) as part of the A244 vaccination. Participants will receive 2 IM injections, either vaccine or placebo, into the same quadriceps muscle at Day 0 only. Enrollment into Groups 1 and 2 will begin with no more than two participants per site per week for the first two weeks. Once the first eight participants are enrolled across Groups 1 and 2, the Safety Monitoring Committee

(SMC) will review 7-days of safety data from these participants to determine whether enrollment can continue. Enrollment into Groups 1 and 2 will continue unrestricted after any safety concerns raised by the SMC are addressed and the Sponsor gives their approval for the continuation of enrollment. Enrollment into Groups 3 and 4 will commence after enrollment into Groups 1 and 2 has been completed, the SMC has reviewed 7-days of safety data from all Group 1 and Group 2 participants, and the Sponsor gives their approval. Enrollment into Groups 3 and 4 will follow a similar approach with no more than two participants per site per week for the first two weeks, followed by an SMC review of 7-days of safety data from the first eight participants enrolled across both Groups, and Sponsor approval before enrollment can continue unrestricted in Groups 3 and 4.

For participants randomized to receive full dose IHV01 and A244 in Group 1, the A244 product will be prepared by mixing AHFG with A244 and Normal Saline. Using a sterile syringe, 1.2mL of the mixture will be withdrawn for IM injection. No mixing is necessary to prepare the IHV01 product. Using a sterile syringe, 1.0mL IHV01 will be withdrawn from the vial for IM injection.

For participants randomized to receive fractional dose IHV01 and A244 in Group 2, the A244 product will be prepared by mixing Normal Saline, AHFG, and A244. Using a sterile syringe, 1.2mL of the resulting product will be withdrawn for IM injection. The IHV01 product will be prepared by mixing Normal Saline and IHV01. Using a sterile syringe, 1.0mL of the mixture will be withdrawn for IM injection.

For participants randomized to receive full dose IHV01 and A244 + ALFQ in Group 3, the A244 + ALFQ product will be prepared by mixing AHFG and A244 with ALFQ. After mixing, 1.2mL will be withdrawn into a sterile syringe for IM injection. No mixing is necessary to prepare the IHV01 product. Using a sterile syringe, 1.0mL IHV01 will be withdrawn from the vial for IM injection.

For participants randomized to receive fractional dose IHV01 and A244 + ALFQ in Group 4, the A244 + ALFQ product will be prepared by mixing Normal Saline, AHFG, and A244 with ALFQ. After mixing, 1.2mL will be withdrawn into a sterile syringe for IM injection. The IHV01 product will be prepared by mixing Normal Saline and IHV01. Using a sterile syringe, 1.0mL of the mixture will be withdrawn for IM injection.

Normal Saline (0.9% Sodium Chloride for Injection) will serve as a placebo for the trial. All placebo injection volumes will match the vaccine injection volumes for the group in which a participant has been randomized.

Duration of Treatment:

Participants will receive 2 IM injections of either the investigational products or placebo at Day 0, after which they will be followed for 12 months for safety and immunogenicity analyses.

Criteria for Evaluation:
Immunogenicity:

Humoral responses will be assessed by HIV-specific binding antibody assays, HIV-specific neutralizing antibody assays, and non-neutralizing antibody function assays at Days 0, 14, 168, and 336. Humoral mucosal immune responses in the rectal, penile, semen and cervico-vaginal compartments will be assessed using non-invasive sampling methods (sponge, menstrual disc, and masturbation) at the same time points. Genital secretion samples will be assayed for cytokines and inflammatory markers and be used to assess the proteome/microbiome.

HIV-specific cell-mediated responses will be assessed by intracellular cytokine staining, CD4 and CD8 T cell proliferation, and related assays. Innate immune responses and genetic responses will be quantified. In addition, invasive sigmoid and lymph node biopsies performed on a subset of willing participants at Day 14 will be utilized. Cells isolated from sigmoid biopsies and endocervical cytobrush/swab collection will be used to assess vaccine-specific immune responses and the dynamics of key mucosal cell populations and innate responses.

Inguinal lymph node biopsies will be performed on a subset of willing participants at Day 14 to assess the effect of ALFQ on immune activation and HIV-specific immune responses in lymph nodes. Analysis will be done to determine whether ALFQ improves immune responses in the lymphoid follicles and stimulates the generation of T follicular helper cells, which may play a critical role in the generation of broadly neutralizing antibodies through interactions with germinal center B cells in the lymph nodes

Leukapheresis will be performed on a subset of willing participants to allow mapping of innate and HIV-specific functional responses within a given antigen, where in excess of 30 million cells are usually required for each antigen.

Safety:

Safety will be assessed both by direct physical examination and by diary cards, which serve as memory tools for better identification of reactions. Participants will be assessed at pre-vaccination baseline and will remain in clinic under direct observation for at least 30 minutes post vaccination. Participants will return to clinic on Days 1 and 7 post-vaccination to be assessed for symptoms of local and systemic reactogenicity.

Participants will be followed for a total of 12 months post vaccination. Adverse Events (AEs) and Serious Adverse Events (SAEs) will be recorded at all visits along with timing and possible attribution to Investigational Product. Because this clinical trial involves an adjuvant, Adverse Events of Special Interest (AESIs) will also be assessed. Safety laboratory analyses of complete blood count, liver function tests, urinalysis, and pregnancy test in females will also be performed. Safety data for the ALFQ product will be used to supplement safety data produced from other studies in support of its use with experimental COVID-19 vaccine candidates.

Statistical Methods:

This study intends to descriptively characterize safety and immune responses following delayed boosting of individuals previously receiving a late boost of AIDSVAX®B/E with or without ALVAC in the RV306 protocol. Comparisons of interest include full dose vs. fractional dose (Groups 1+3 vs. Groups 2+4) and effect of ALFQ (Groups 1+2 vs. Groups 3+4). For safety analyses, the occurrence of local and systemic reactogenicity symptoms, AEs and SAEs will be assessed as the proportions of participants experiencing such safety events. The primary immunogenicity endpoint is the magnitude of immune response to the endpoint titer of plasma IgG, the same variable assessed in RV306. Immune response over time (durability) will be assessed by the positive incremental area under the curve (AUC) based on a graph with log endpoint titer on the y-axis and visit day on the x-axis. Safety and immunogenicity analyses will also be sub-stratified by gender to explore gender-specific differences in immune responses.

3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations are used in this study protocol.

Table 2: Abbreviations

Abbreviation	Explanation
ADCC	Antibody-dependent Cellular Cytotoxicity
ADCP	Antibody-dependent Cellular Phagocytosis
AE	Adverse Event
AESI	Adverse Event of Special Interest
AFRIMS	Armed Forces Research Institute of Medical Sciences
AHFG	Aluminum Hydroxide Fluid Gel
ALF	Army Liposomal Formulation
AR	Army Regulation
AUC	Area Under the Curve
C	Celsius
CFR	Code of Federal Regulation
cm	Centimeter
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRR	Continuing Review Report
DA	Department of the Army
DCAC	Data Coordinating and Analysis Center
DoD	Department of Defense
EC	Ethical Committee
F	Fahrenheit
FDA	Food and Drug Administration
FLSC	Full Length Single Chain
FGT	Female Genital Tract
FTM	Faculty of Tropical Medicine
GCP	Good Clinical Practice
GP	Glycoprotein
Hg	Mercury
HIPAA	Health Insurance Portability Accountability Act
HIV	Human Immunodeficiency Virus
HJF	Henry M. Jackson Foundation
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICS	Intra-cellular Cytokine Staining
ID	Infectious Disease
IEC	Independent Ethics Committee
IHV	Institute of Human Virology, University of Maryland School of Medicine
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
ISCOM	Immune Stimulating Complex
mg	Milligram
MHRP	US Military HIV Research Program

Abbreviation	Explanation
mL	Milliliter
mm	Millimeter
MOP	Manual of Procedures
MoPH	Ministry of Public Health
MPLA	Monophosphoryl Lipid A
NAAT	Nucleic Acid Testing
OHRP	Office for Human Research Protections, Department of Health and Human Services
ORA	Office of Regulated Activities, USAMRDC
OHARO OHRO	Office of Human and Animal Research Oversight, Office of Human Research Oversight
PI	Principal investigator The investigator who leads the study conduct at an individual study center. Every study center has a principal investigator.
PSRT	Protocol Safety Review Team
PSSO	Product Safety Surveillance Office, USAMRDC ORA
PVG	Pharmacovigilance
RTA	Royal Thai Army
SAE	Serious adverse event
SAP	Statistical analysis plan
SMC	Safety Monitoring Committee
SOE	Schedule of Evaluations
SOP	Standard operating procedure
STI	Sexually Transmitted Infection
TOU	Test of Understanding
TSG-DA	The Surgeon General, Department of the Army
TSHAVD	Technical Subcommittee on HIV/AIDS Vaccine Development, MoPH
UPIRTSO	Unanticipated Problem Involving Risk to Subjects or Others
USAMC	US Army Medical Component
USAMMDA	United States Army Medical Materiel Development Activity
USAMRDC	United States Army Medical Research and Development Command
VTC	Vaccine Trials Center
WRAIR	Walter Reed Army Institute of Research

5. INTRODUCTION

In 2019, an estimated 38 million people [31.6–44.5] were living with HIV worldwide. This reflects the continued large number of new HIV infections and a significant expansion of access to antiretroviral therapy, which has helped reduce AIDS-related deaths, especially in more recent years (UNAIDS, 2020). Despite promising but still fragile successes in prevention and care and treatment, the development of a safe and efficacious preventive HIV vaccine as part of a comprehensive prevention program remains among the highest global health priorities and the best long-term tool for the control of the HIV-1 (Barouch, 2008; Johnston, 2008; Koff, 2010).

In response to the epidemic, the Royal Thai Government developed and implemented a comprehensive plan for prevention and control of HIV; preventive HIV vaccines are an integral component of this plan (National AIDS Committee, 1997). Consequently, the National AIDS Commission of Thailand established a Subcommittee for HIV Vaccine Development and Trials with responsibility for coordinating and overseeing efforts in this area. In 1991, the World Health Organization (WHO) selected Thailand as a site for evaluation of candidate HIV vaccines (Esparza, 1993). Since then, WHO/UNAIDS has provided consultation to the Subcommittee for HIV Vaccine Development and Trials of the National Commission for the Prevention and Control of AIDS. Through multiple partnerships and collaborations, Thailand has actively carried out its National Plan and implemented several vaccine clinical trials including the first Phase III trial in the developing world (Pitisuttithum, 2010). The combination of planning, collaboration and commitment to HIV vaccine development has put Thailand in a position of international leadership concerning HIV vaccine development.

To date, 5 efficacy trials of candidate HIV vaccines have been completed: VAX003 (Pitisuttithum, 2006), VAX004 (Flynn, 2005), RV144 (Rerks-Ngarm, 2009), STEP (Buchbinder, 2008), and HVTN505 (Hammer, 2013). Of these, only the RV144 phase III trial demonstrated any efficacy. Healthy participants who received the 6-month vaccination regimen containing ALVAC-HIV (vCP1521) (ALVAC) and AIDSVAX® B/E, formulated in aluminum hydroxide, were 31% less likely to become HIV infected over 3.5 years of follow-up than participants who received placebo. In a post-hoc analysis, efficacy was 60% at 12 months, indicating that protective immunity may have waned rapidly (Robb, 2012). A study of immunologic correlates found that the magnitude of plasma IgG antibodies to the scaffolded variable regions 1 and 2 (V1V2) of HIV-1 envelope was inversely correlated with risk, while plasma IgA antibody titers to HIV-1 gp120 envelope were directly correlated with risk (Haynes, 2012a).

To build upon the demonstrated efficacy in RV144, the RV305 clinical trial enrolled 162 participants who received the active vaccine regimen in the RV144 trial 6 to 8 years earlier and boosted these individuals twice at months 0 and 6 with ALVAC and AIDSVAX® B/E, either alone or in combination, in a randomized, double-blinded placebo-controlled manner. Long-term memory responses to HIV-1 antigens persisted, allowing boosting of HIV-specific plasma antibody responses to levels higher than observed in the RV144 trial (Rerks-Ngarm, 2017). Furthermore, late boosting expanded a subdominant pool of envelope CD4 binding site-reactive memory B cells with increased somatic hypermutation, long third heavy chain complementarity determining regions (HCDR3), and Tier 2 neutralization capacity (Easterhoff, 2017), all features of broadly neutralizing antibodies (Bonsignori, 2011; Walker, 2009; Walker, 2011; Zhou, 2015). Mucosal sampling demonstrated that HIV-specific antibody responses were also present in cervicovaginal secretions, rectal secretions and seminal plasma (Akapirat, 2018),

the initial point of defense from sexually transmitted infections. Interestingly, immune responses to AIDSVAX® B/E were higher after the initial boost than after the second boost 6 months later, suggesting that a delayed interval between priming and boosting may generate stronger immunity to booster vaccinations. Thus, boosting with a protein-based candidate HIV vaccine after a delay of several years can boost memory and elicit high magnitude immune responses in multiple compartments.

To further examine the impact of the interval between priming and boosting, the RV306 Phase II clinical trial was conducted in 360 healthy, unvaccinated participants to determine whether similar improvements in quality, magnitude or duration of humoral, cellular and mucosal responses could be afforded by boosting the RV144 regimen at either 12, 15, or 18 months post initial vaccination series, and to determine the optimal boosting interval for further clinical development. As with the primary RV144 vaccination series, boosting the RV144 vaccine regimen was safe overall, as there were no vaccine-related serious adverse events. Vaccination at month 12 with AIDSVAX® B/E with or without ALVAC, or at Month 15 or 18 with both vaccines, significantly improved humoral and cellular immunogenicity relative to participants who did not receive a late boost to the RV144 vaccine regimen. Overall, boosting at Month 15 or 18 resulted in stronger humoral and cellular responses than boosting at Month 12. Specifically, peak plasma IgG responses were higher in participants boosted at Month 15 or 18 than at Month 12 for all antigens. In addition, late boosting increased the durability of plasma IgG responses, as the median decrease of plasma IgG was 32-fold over 6 months without late boosting, but only 8-fold over 6 months in participants receiving late boost vaccinations. Longer boosting intervals also preferentially improved neutralization breadth. While vaccine boosts at any time point improved neutralization of the Tier 1 pseudoviruses MW965.26 (Subtype C); SF162.LS (Subtype B) and TH023.6 (Subtype AE), only boosting at Month 15 or Month 18 improved neutralization of subtype B MN.3.

The A244 protein, a candidate vaccine that consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01AE A244 with an 11 amino N-terminal deletion, has demonstrated its ability to improve the antigenicity and immunogenicity in humans as a component of AIDSVAX® B/E and alone in non-human primate (NHP) studies (Alam, 2013). It has been re-manufactured for use as an additional boost to RV306 late boost volunteers in this clinical trial. An increasing body of literature now supports the concept of serial boosting with heterologous proteins to guide the evolution of broadly neutralizing antibodies from germline to mature B cells towards epitopes that confer protection. Dr. Haynes and others at Duke University have shown that evolution of broadly neutralizing antibodies occurs in a staged manner, and can be induced by serial protein immunogens (Bonsignori, 2017; Tian, 2016).

The FLSC candidate vaccine, which consists of the FLSC gp120-CD4 chimera subunit HIV-1 vaccine formulated in Aluminum phosphate adjuvant (Alum), has been manufactured for human use as IHV01 and has been safely administered to healthy participants in a Phase I trial. IHV01 would provide a heterologous boost to prior RV306 participants, as it provides exposure to HIV envelope epitopes at the CD4 binding site (DeVico, 2007), distinct from AIDSVAX® B/E.

Manufacture of protein-based vaccine antigens can be technically complex and expensive, both of which can limit vaccine supply, particularly in times of high demand during an epidemic outbreak. Optimization to reduce vaccine dosing can help to alleviate these issues. In a controlled human malaria parasite infection model, reducing the dose of the third boost of the candidate

malaria vaccine RTS/S, AS-01 improved vaccine efficacy, accompanied by increased antibody somatic hypermutation and avidity, both characteristics of broadly neutralizing HIV antibodies (Regules, 2016). A separate study evaluating fractional dosing of inactivated polio administered intradermally demonstrated similar immunogenicity to full dose intramuscular vaccination (Resik, 2010). In a recent outbreak of yellow fever in Angola and the Democratic Republic of the Congo, vaccine shortage necessitated widespread deployment of fractional dosing of yellow fever vaccine at one fifth of the full dose to children and adults. Fractional dosing led to seroconversion in 98% of seronegative vaccine recipients, and immune responses in 66% of previously seroreactive vaccine recipients (Ahuka-Mundeke, 2018). Even a similar rather than superior immunogenicity of fractional dosing would result in significant cost savings, thereby facilitating scale-up and delivery to populations at greatest need for a preventive HIV vaccine.

Relative to vaccine antigens from other pathogens, there is a particularly rapid decline of immune responses to HIV gp120 envelope proteins. Modulation of the response to vaccines with immunostimulatory adjuvants is one very promising approach to improve durability. The Army Liposome Formulation (ALF) family of adjuvants are ALFA (ALF adsorbed to aluminum salt), ALFQ (ALF mixed with the saponin QS21; US patent pending) (Beck, 2015), and ALFQA (ALFQ adsorbed to aluminum salt). Both ALFA and ALFQ are potent adjuvants, non-pyrogenic, and nontoxic in rabbit pyrogenicity and toxicology studies. ALFQ is an alternative to the successful but proprietary adjuvants, AS01B (liposomes containing both MPLA and the saponin QS21). ALFQ is potent in preclinical studies and contains 4 times the amount of MPLA and twice the amount of QS21 when compared to AS01B. An ongoing NHP study comparing HIV gp120 A244 formulated with either ALFQ or with aluminum hydroxide fluid gel (AHFG) has shown that animals that received ALFQ as the adjuvant compared to animals that received AHFG mounted significantly higher binding antibody responses to A244, gp70V1V2 A244, and V2 peptide (unpublished data). The responses were significantly higher after each immunization, as well as in ADCC responses, quantified using gp120 coated target cells. Given that antibody functions such as ADCC may have contributed to protection by non-neutralizing antibodies in RV144 vaccine recipients, ALFQ may contribute to increasing protective vaccine efficacy. In addition, data generated regarding the ALFQ adjuvant in this trial will help contribute to the safety database supporting its use with experimental antigens targeted towards other pathogens, including SARS-CoV-2.

5.1. Military Relevance

Historically, infectious diseases have had a major impact on US Armed Forces. With an estimated 34 million infected individuals worldwide at the end of 2011, HIV poses a significant and persistent threat in terms of force readiness and protection and may act as a war-starter by affecting the stability and security of nation-states.

HIV's relevance to the US Military has been recognized from the very beginning of the pandemic. In 1985, the US Military recognized the emerging HIV-1 epidemic as a new threat to US and allied forces worldwide. The United States Congress mandated the establishment of the US Military HIV Research Program to develop effective preventive measures to include prevention education, vaccine development and implementation of novel anti-viral therapies and clinical management tools for the US DoD and Allied Forces. In 2001, the Armed Forces Epidemiology Board identified HIV as a disease of military importance, and Army FOC 09-07,

Global Casualty Prevention, requires detection, identification, and vaccination in order to protect US personnel against potential infectious disease (ID) threats. The 2001 DoD Report on Biological Warfare Defense Vaccine Research and Development identified HIV as the 4th greatest infectious disease threat to DoD forces. Department of Army Headquarters designated HIV vaccine development as an Army Technology Objective (ATO), a status reserves for the highest priority science and technology efforts. Furthermore, The National Security Strategy of the United States (2002, 2006, and 2010) clearly identifies the threat of HIV/AIDS as a destabilizing force that threatens US National Security. Finally, in January 2011, The Department of the Army approved the HIV Vaccines Capability Development Document for HIV Vaccine, which addresses the need to provide service members with vaccine protection against HIV and AIDS.

Medical care for HIV infection is extremely costly to the US Defense Health Program. The estimated lifetime cost of HIV infection is at least USD $400,000 and the estimated average yearly cost varies from $20,000 to $25,000, bringing the estimated lifetime healthcare cost of the 5,000 HIV infected servicemen/women to $8B to $10B dollars. Total annual cost of treatment for HIV-infected service members ranges from $21 to $54 million per year.

Infection rates could rise precipitously if forces are deployed to areas of high HIV prevalence. We developed a projection based on the US Army Surgeon General's estimate of gonorrhea cases/year during the Vietnam War and determined the estimated number of visits to gonorrhea infected commercial sex workers necessary to generate that number. This equates to roughly one visit per service member per year during the height of the Vietnam War (this is widely viewed as an underestimate, since most cases of Sexually Transmitted Infections (STIs) were treated without being reported). Given the known infection rate of 1/300 and estimating commercial sex worker HIV prevalence at 30% (lower than currently estimated in Thailand, Vietnam or many African nations) this equates to 1,200 to 1,800 potential new HIV infections per year for a deployed force of 400,000. Developing a highly efficacious vaccine to protect the Warfighter thus remains of significant military relevance.

5.2. Thailand Relevance

Thailand has one of the highest rates of HIV in Asia and the Pacific, accounting for 9% of the region's total population of people living with HIV. Although the epidemic is in decline, the prevalence remains high among key affected groups, with young people from key populations particularly at risk of acquiring HIV. In 2018, around half of new HIV infections in Thailand occurred among people aged 15-24. In 2019, 5,400 people in Thailand became HIV positive. Unprotected sex is estimated to account for 90% of all new HIV infections. The government has made important progress against HIV, but this epidemic will not end without a vaccine. The experimental vaccines that are in development present the greatest opportunity to save lives in human history as the work done in previous vaccine studies have helped to develop improvements in vaccine/adjuvant breadth and duration in order to protect more people for longer periods of time. Until the goal of a safe and effective vaccine is achieved, we must continue to use all available tools to help curb the HIV epidemic and accelerate progress towards that goal. Vaccines save lives today, and a new generation of vaccines can save even more lives tomorrow. There is still a lot of work to be done to achieve the goal of a safe and effective vaccine given the various HIV subtypes that are present across the world, however, there has

been a great deal of attention given to the HIV subtypes circulating in Thailand. In this study, A244 is subtype AE and is designed specifically to match circulating HIV subtypes in Thailand. If the vaccine is shown to improve immune responses in Thai people, the development of this vaccine will be monumentally beneficial to the Thai people.

5.3. Rationale for Study

A vaccine to prevent HIV infection remains of paramount importance that both to diminish the global epidemic (Medlock, 2017) and to reduce the burden to the DoD, given the cost of treatment for its HIV-infected service members ranges from $21 to $54 million per year. Similarly, improving the rapidity of our response to new emerging infectious diseases will be facilitated by development of a potent adjuvant that can be broadly coupled to various candidate vaccines, unencumbered by external intellectual property. RV546 addresses both of these goals by administering the IHV01 and A244/AHFG candidate vaccines with or without ALFQ in participants who have a "head start" on their vaccination series through participation in the prior RV306 trial, allowing for evaluation of safety and immunogenicity after a single vaccination to quickly inform product development efforts.

5.4. Name and Description of the Investigational Product

IHV01, an Institute of Human Virology (IHV) product, consists of the FLSC gp120-CD4 chimera subunit HIV-1 vaccine formulated in Aluminum phosphate adjuvant (Alum). The formulation consists of 0.3 mg/mL of FLSC, 2.4 mg/mL of Alum, 5 mM NaOAc, 40 mg/mL mannitol, pH 6.2.

A244, a Duke University product, consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01AE A244 derived from the CM244 CRF_01AE. The A244 gp120 envelop has an 11-amino N-terminal deletion, similar to the bivalent AIDVAX® B/E protein used in RV144 (Alam, 2013), which has been safely administered to over 9,000 participants. The AHFG adjuvant that is mixed with A244 consists of Rehydragel HPA that has been diluted with sterile water and has an aluminum concentration of 5 ± 1 mg/mL.

ALFQ, a US Army product, is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (3D-PHAD®) with the addition of QS-21.

Normal Saline (0.9% Sodium Chloride for Injection) will serve as a placebo for the trial.

Refer to Section 7.4 for additional information.

5.5. Summary of Nonclinical and Clinical Trials

5.5.1. IHV01 (FLSC) Vaccine

Prior to clinical testing in humans, a rhesus macaque analogue, rhFLSC, was created containing rhesus macaque CD4D1D2 sequences matched to the immunized host to avoid xenogeneic responses against human CD4 domains. In separate experiments, rhesus macaques were immunized repeatedly with rhFLSC formulated with various adjuvants: ISCOMATRIX, a saponin-containing lipid immune stimulating complex (ISCOM), or RC529-SE, a stable emulsion of squalene, glycerol, phosphatidyl choline, and synthetic monophosphoryl lipid A (a TLR-4 agonist), or tat toxoid. Animals were assessed for protection from acquisition after

repeated low dose mucosal challenges with either SIVmac251 or SHIV162P3 to more accurately mimic exposure during sexual transmission in humans. Immunization with rhFLSC in various formulations afforded partial protection to challenges with either virus, allowing investigation of correlates of protection. Protection from acquisition was preferentially highest in those animals that developed antibody-dependent cellular cytotoxicity activity from antibodies directed to CD4i epitopes preferentially presented by the FLSC (Fouts, 2015). FLSC is vialed for human use with aluminum phosphate as IHV01.

NCT02756208 is a recently completed Phase I dose escalation clinical trial to determine the optimal safe dose of IHV01 in healthy participants. This Phase I clinical trial was a randomized, placebo-controlled trial which enrolled 65 healthy HIV-1 negative adults who were at low risk of infection with HIV-1 in one of 3 different dose groups (75µg, 150µg, and 300µg). Of the 65 enrolled participants, 49 received IHV01 and 16 received a Normal Saline placebo. Both groups received injections at four different time points. The primary endpoint of the trial was the safety and tolerability of the IHV01 vaccine product as measured by local and systemic vaccine reactogenicity, laboratory safety evaluation and medical history and physical examination. No vaccine-related SAEs had occurred and CD4+ T-cell counts remained stable throughout the trial (Chua, 2021). Of the 241 injections given, participants reported 26 instances of mild local reactogenicity (7 placebo, 19 vaccine), 3 instances of moderate local reactogenicity (0 placebo, 3 vaccine), and 1 instance of severe local reactogenicity (0 placebo, 1 vaccine). The severe local reactogenicity was reported by an individual in the 300µg vaccine group. Despite this one instance of severe local reactogenicity, a majority of the injections were associated with no reactogenicity (196 of the 241 injections). The most common AEs related to the injections included injection site pain, pruritis, and headache. These AEs were not significantly different between the vaccinated and placebo groups. Additional safety information can be found in Section 5.3 of the IHV01 IB.

In this study, IHV01 will be administered as a late boost to prior RV306 vaccine recipients. The unique conformational structure of FLSC provides exposure to HIV-1 envelope epitopes at the CD4 binding site that are not exposed in the monomeric gp120 expressed in the AIDSVAX® B/E vaccine used in RV144, RV305, and RV306. Exposure to these epitopes may likely improve the breadth of humoral responses, particularly to CD4i epitopes.

5.5.2. A244 Vaccine

Clinical trials involving Thai adults have demonstrated that gp120 vaccines, both monovalent and bivalent, are safe and immunogenic (Migasena, 2000; Pitisuttithum, 2003). This demonstrated proof of the safety and immunogenicity led to the VAX003 phase III randomized, double-blind, placebo-controlled vaccine efficacy trial of the AIDSVAX® B/E vaccine, which is a bivalent HIV gp120 envelope glycoprotein vaccine containing a subtype B envelope from the HIV-1 strain MN and a subtype E envelope from the HIV-1 strain A244. Although the trial was completed successfully, the vaccine did not show its ability to prevent HIV-1 infection or delay HIV-1 disease progression (Pitisuttithum, 2006). A subsequent RV144 efficacy study of AIDSVAX® B/E primed with ALVAC-HIV (vCP1521) demonstrated a 31% vaccine efficacy of this combination.

The A244 construct to be used in RV546 has been remanufactured by the Duke Human Vaccine Institute (Durham, NC) and will utilize the A244 gp120 protein as a monovalent vaccine. This

protein retains the same 11 amino acid deletion (A244 Δ11 gp120) as in AIDSVAX® B/E, which was shown in nonhuman primates to improve both envelope antigenicity and immunogenicity (Alam, 2013). Though the A244 construct has been extensively tested in humans, this is the first time that the remanufactured version will be administered to humans. Given the extensive experience and positive safety profile of the A244 protein in prior studies, the U.S. FDA has agreed that a toxicology study is not necessary.

The A244 construct will be mixed with an aluminum hydroxide gel fluid adjuvant. Aluminum hydroxide and aluminum phosphate have been extensively used as adjuvants for HIV-1 vaccines since the 1990s (Gamble, 2010; Ling Munier, 2011; Moody, 2014; Walker, 1995). A review of clinicaltrials.gov has 38 trials listed in which aluminum is or was being used as an adjuvant for HIV-1 Env antigens. The most well-known trials were the phase 3 trials with AIDSVAX®: VAX003 (Pitisuttithum, 2006), VAX004 (Flynn, 2005) and RV144 (Rerks-Ngarm, 2009). Both VAX003 and VAX004 were conducted in high-risk populations, IV injection drug users and men having sex with men, respectively. VAX003 assessed the efficacy of AIDSVAX® B/E and VAX004 assessed the efficacy of AIDSVAX® B/B immunization alone and failed to demonstrate vaccine efficacy against the acquisition of HIV-1. Both had high titer antibody responses. In contrast, the RV144 trial was conducted in a community-risk cohort that received both ALVAC and AIDSVAX® B/E. A modest 31.2% efficacy was observed in RV144. Antibodies to gp70V12 were identified as the inverse correlate for the acquisition of HIV-1 (Haynes, 2012b). These antibodies also waned rapidly.

5.5.3. ALFQ Adjuvant

The WRAIR Laboratory of Antigen and Adjuvant Research (LAAR) developed the original multilamellar Walter Reed Liposomes formulation containing monophosphoryl lipid A (MPLA), saturated dimyristoyl fatty acids, and cholesterol as carriers of vaccines, adjuvants, and drugs have been used with several different antigens in 14 phase I and 2 phase II clinical vaccine trials with an excellent safety profile (Alving, 2012).

Of these, the trial with the most direct relevance to this study was AVEG 015 (NCT00001042), a phase I randomized, double blind placebo controlled, clinical trial conducted in the US in healthy HIV-1 uninfected individuals. The purpose of this trial was to evaluate immune responses to SF-2 gp120 protein formulated with 7 different adjuvants including AH (gp120 + AH) and AH-adsorbed-L(MPLA) [L(gp120 + MPLA) + AH; Walter Reed Liposomes], wherein the antigen was encapsulated in multilamellar liposomes rather than mixing with unilamellar liposomes (McElrath, 1995). In a strikingly consistent pattern, 3 to 12-fold higher titers of IgG Abs were induced not only against the homologous clade B gp120 protein, but also against cross-clade A/E and C, gp120 proteins (Rao, 2018). The liposomal arm also induced 2.5 to 13-fold higher titers of V1V2-specific IgG Abs compared to the AH arm. Even 10 months after the last boost, the titers remained high with only a 5-fold or a 3-fold drop in the Ab titers. In addition, significantly higher levels of neutralizing antibodies and T-cell lymphoproliferation were observed in the liposomal arm compared to gp120 formulated with AH, the MF59 adjuvant, or monophosphoryl lipid A (MPLA) alone (Rao, 2018).

The next generation formulations of liposomes, known as The Army Liposome Formulation (ALF) contain the same components as the Walter Reed Liposomes but are unilamellar and contain synthetic phospholipids, including MPLA (3D-PHAD®). The members of the ALF

family of adjuvants are ALFA (ALF adsorbed to aluminum salt; US patent pending), ALFQ (ALF mixed with the saponin QS21; US patent pending) (Beck, 2015), and ALFQA (ALFQ adsorbed to aluminum salt). Both ALFA and ALFQ are potent adjuvants, non-pyrogenic, and nontoxic in rabbit pyrogenicity and toxicology studies. ALFA and ALFQ are alternatives to the successful but proprietary adjuvants, AS04 (MPLA adsorbed to aluminum salt) and AS01B (liposomes containing both MPLA and the saponin QS21). ALFQ is potent in preclinical studies and contains 4 times the amount of MPLA and twice the amount of QS21 when compared to AS01B. This improved formulation has been demonstrated to increase the immunogenicity and protective efficacy of the candidate malaria vaccine P. falciparum circumsporozite protein (CSP) (FMP013) relative to the adjuvants Montanide, ALF, and ALFA (Genito, 2017). Similarly, ALFQ increased the immunogenicity and protective efficacy of a conjugate *Campylobacter jejuni* capsular polysaccharide antigen conjugated to CRM197 (CPS) in separate challenge experiments in mice and *Aotus nancymaae* monkeys relative to AH and ALF adjuvants (unpublished).

The most direct evidence to support use of ALFQ with HIV envelope antigens arises from an ongoing study in rhesus macaques led by Dr. Bart Haynes at Duke University, which compares HIV gp120 A244 formulated with either ALFQ or with AHFG. Animals that received ALFQ as the adjuvant compared to animals that received AHFG mounted significantly higher binding antibody responses to A244, gp70V1V2 A244, and V2 peptide. The responses were significantly higher after each immunization, as well as in ADCC responses, quantified using gp120 coated target cells. Given that antibody functions such as ADCC may have contributed to protection by non-neutralizing antibodies in RV144 vaccine recipients, ALFQ may contribute to increasing protective vaccine efficacy. Challenge results in this experiment are pending.

In light of the highly favorable data from non-clinical studies, ALFQ moved into human clinical trials in separate studies involving candidate malaria and COVID-19 vaccines. In the malaria vaccine trials, the ALFQ adjuvant was administered with the FMP013 (NCT04268420) and FMP014 (NCT04296279) formulations in low (20 µg protein antigen, 100 µg 3D-PHAD®, 50 µg QS-21) and high doses (40 µg protein antigen, 200 µg 3D-PHAD®, 100 µg QS-21). In the COVID-19 vaccine trial (NCT04784767), the ALFQ adjuvant was administered with a spike-ferritin nanoparticle (SpFN) antigen at consistent dosages in both the low SpFN dose (25 µg SpFN antigen, 200 µg 3D-PHAD®, 100 µg QS-21) and high SpFN dose (50 µg SpFN antigen, 200 µg 3D-PHAD®, 100 µg QS-21) groups. Except for the vaccinations in Part B of FMP014, vaccinations in these studies have been completed and post-vaccination safety data is available. In summary, ALFQ local reactogenicity in these studies appears to be comprised primarily of local pain and tenderness that is omnipresent, mild to moderate, and short-lived across both products and doses. Systemic reactogenicity was mostly mild to moderate with the most commonly reported symptoms consisting of headache, fever, chills, fatigue, and myalgia. In addition, there was one case of severe fatigue in FMP013 that occurred 24 hours post-injection and lasted less than 6 hours and one case of severe flu-like symptoms (severe fever chills, myalgias, and moderate temperature) in a FMP014 high dose participant that lasted less than 24 hours. No SAEs were reported in the FMP014 and EID030 trials; however, there were two SAEs reported in FMP013, Grade 3 right ankle hemarthrosis and Grade 4 acute appendicitis, both of which were assessed by the investigator as not related to the investigational products. No deaths or related SAEs were reported in these studies.

Safety data from this study, the malaria vaccine studies, and the SpFN + ALFQ vaccine (Joyce, 2021) study will contribute to the ALFQ safety profile, which will support its use with future experimental vaccine candidates.

5.6. Known and Potential Risks and Benefits to Human Participants

5.6.1. Risks/Discomfort to Participants and Precautions to Minimize Risk

Outlined below are anticipated and unexpected adverse reactions, and a brief description of procedures to ameliorate risks and symptoms. All known risks and precautions described here are explained in detail in the main study informed consent (Appendix C) and optional procedures informed consent (Appendix D), as applicable. All possible safety precautions will be employed, however if a participant is injured or become ill as a direct result of their participation in the study procedures listed below, they will receive care and treatment for the injury with treatment costs covered by research-related injury insurance (see Section 15.2.3).

5.6.1.1. Local Reactions

Participants may exhibit post-vaccination reactions including local reactions at the injection sites such as erythema, induration, pain/tenderness, swelling and limitation of leg movement. These reactions are generally of short duration and rarely require treatment. Should such reaction persist and require treatment, the participant will be referred to appropriate medical care services.

5.6.1.2. Systemic Reactions

Participants may exhibit general signs and symptoms associated with administration of a vaccine injection, including fever, tiredness, chills, rash, myalgia, arthralgia, nausea, and headache. These side effects will be monitored but are generally of short duration and rarely require treatment. Should such reaction persist and require treatment, the participant will be referred to appropriate medical care services. As with all vaccines, an allergic reaction is possible and will be managed as per site SOP for anaphylaxis. To mitigate this risk, participants will be observed in the clinic for at least 30 minutes post-injections.

5.6.1.3. Pregnancy

The effect of the candidate HIV vaccines on pregnancy and fetus are unknown as they have not been specifically studied in pregnant women. Female participants who are pregnant or plan to become pregnant during the first 3 months post-vaccination will be excluded from enrollment. Female participants must use an adequate form of birth control from 45 days prior to the study injections until at least 3 months after receipt of the study injections. Adequate birth control is defined as follows: Contraceptive medications delivered orally, intramuscularly, vaginally, or implanted underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), condoms, diaphragms, intrauterine device, or abstinence. A pregnancy test will be performed on all female participants at screening; prior to the study injections (same day); at Visits 4, 5, and 6; and prior to any of the invasive procedures as indicated in the SOE. Should a female participant become pregnant within the 3 months post-study injections, she will be followed for safety for the remainder of the study period per the SOE. Every effort will be made to remain in contact with the pregnant participant until birth or termination of the pregnancy in order to record the outcome.

The possible effects of these vaccines on the offspring of a male participant is unknown. Male participants who have not had a vasectomy must use an adequate form of birth control for at least 3 months after receipt of the study injections. Adequate forms of birth control will depend on the child-bearing potential of the participant's female partner but will generally be the same as for female participants. For male participants who have undergone a vasectomy, no additional contraception will be required.

Male and female participants with reproductive potential will be advised to use multiple methods of contraception to ensure their effectiveness, including but not limited to using barrier methods of contraception in combination (e.g., condom with diaphragm) or a male's use of condoms and his female partner's use of contraceptive medication or an intrauterine device.

5.6.1.4. Lactation

These candidate HIV vaccines have not been specifically studied in pregnant and lactating women. No data on lactating women are available from previous vaccine trials. There is no information about harm to an unborn child or a child who is breastfeeding. Breastfeeding women will not be enrolled.

5.6.1.5. Allergic Reaction

As with any Investigational New Drug (IND) product administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. Medical emergency equipment is located at each study clinic. This is available to handle emergencies, such as anaphylaxis and cardiac arrest.

5.6.1.6. Vaccine Induced Seroreactivity

As with every candidate HIV vaccine trial, there is a risk that participants will mount an antibody response to the vaccine, which may cause them to test positive on a standard clinical diagnostic assay for HIV. In order to protect participants from any issues that may arise due to a false-positive HIV tests that can be attributed to receiving the vaccinations, the PI will provide an explanatory letter (Appendix N) and the study will provide re-testing. In order to maintain blinding, the letter will be given to all participants at their vaccination visit regardless of whether they received the vaccines or placebo. The clinical study team will support and counsel any participant who remains seroreactive after study closure and will perform repeat diagnostic testing every 6 months until antibodies are negative.

5.6.1.7. Venipuncture

Blood sampling carries the risk of minor discomfort, bruising, fainting and, rarely, infection at the needle puncture site.

5.6.1.8. Mucosal Secretion Collections

Mucosal secretion samples will be collected in the clinic (semen, penile swab, rectal sponges, rectal swab, cervico-vaginal menstrual discs, vaginal swab, endocervical cytobrush/swab) by trained study personnel. Alternately, for the collection of cervical-vaginal secretions, women may elect to insert and remove cervical menstrual discs themselves. For the collection of penile swabs, men may elect to collect the swabs themselves during the study visit. Inserting an

instrument (speculum, anoscope, sponge, swab, menstrual disc, cytobrush) into the anus or the vagina may cause discomfort and slight irritation. There is no evidence of rectal sponge/swab, penile swab, cervical menstrual disc, or vaginal swab sampling contributing to risk of HIV or other sexually transmitted infection. Semen will be collected by masturbation.

Cytobrush/swab collection: A cytobrush/swab is a small flexible plastic instrument with bristles at the tip used in gynecologic procedures to collect cervical cells for subsequent laboratory analysis.

For these mucosal collections, men and women will be asked to refrain from receptive vaginal or anal intercourse, douching, or inserting any product into the rectum or vagina for 3 days prior to the mucosal collection. Men will be asked not to masturbate nor ejaculate for 3 days prior to semen collection.

5.6.1.9. Sigmoid Biopsy

Sigmoid biopsies will be performed with a flexible sigmoidoscope. Brief cramping and gas pains may be felt as air is inserted or as the scope advances. The passing of gas is necessary and should be expected after the procedures are terminated. Participants may choose to receive sedation during the procedure in order to ameliorate the discomfort and anxiety they may feel. There may be slight bleeding from the biopsy site, which generally stops spontaneously. In rare cases, an interventional endoscopic technique is needed to stop bleeding. There is a remote possibility that a biopsy may result in significant bleeding or even perforation requiring emergency medical care. A gastroenterologist will perform this procedure in order to minimize these risks. As a precautionary measure, however, all participants will be counseled to avoid receptive anal intercourse for 3 days prior to and 7 days following sigmoid biopsies.

5.6.1.10. Inguinal Lymph Node Biopsy

The excisional inguinal lymph node biopsy will be performed under local anesthesia by a qualified surgeon. As with all surgical procedures, there is a risk of scarring, bruising, bleeding or infection at the surgical site. These risks are minimized by the small size of the incision (approximately 1-2 centimeters), clinical evaluation to determine any additional bleeding risks (such as anticoagulant use or a congenital clotting disorder), and the adherence by the surgeon to aseptic technique. Participants may experience some discomfort following the procedure despite use of local anesthesia; for participants who complain of any subsequent discomfort, additional analgesia will be made available. There is a possibility of seroma formation at the biopsy site, which may require subsequent drainage and further management, or sensory nerve injury during the procedure, which could result in temporary or permanent local reduction in feeling. There is a small risk of chronic swelling of the leg on the side that the lymph node is taken from. For this reason, it is recommended that one participant have no more than 4 inguinal lymph node biopsies, or 2 biopsies per side, done in the subject's entire life, which would include this study, and any other studies, and any medical procedures performed elsewhere for non-research purposes. Finally, there is the unlikely possibility that the procedure will be unsuccessful and no lymph node will be recovered. This procedure will be performed by trained personnel at Chulalongkorn Hospital in Bangkok, which has a long history of safely performing lymph node biopsies on both HIV infected and healthy individuals (Chintanaphol, 2018). Only a single lymph node biopsy will be performed per participant in this study.

5.6.1.11. Leukapheresis

Adverse reactions to leukapheresis procedure are rare and include vaso-vagal episodes related to needle insertions and transient volume shifts, peri-oral paresthesias, chills, nausea, and heartburn caused by the citrate anticoagulant used during the procedure. Vaso-vagal reactions are handled by postural manipulation and fluid administration. Participants will be observed closely by an experienced blood bank technician or trained medical staff during the procedure. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets. Allergic reactions can occur with the use of chemical during this procedure. There is limited data on allergic reactions with individuals undergoing leukapheresis but the possibility of having anaphylactic reactions cannot be completely eliminated or prevented during the procedure. An emergency cart will be on standby to respond to an allergic reaction if it occurs.

5.6.1.12. Social Harm and Discrimination

In the unlikely event of HIV infection during this study, the primary concern is related to ascertaining and providing HIV diagnostic information and, in particular, involuntary disclosure of HIV status to others. These disclosures may result in depression and rarely suicide among individuals learning that they are infected with the HIV virus. Furthermore, involuntary disclosure to others may result in prejudice by the community, family, employers, and psychosocial factors including stigma and discrimination. This risk will be minimized by the fact that all counselors will be trained in pre- and post-test counseling for HIV and will aim at fully informing participants of all activities in the study and attendant risks and benefits. The candidate vaccines may also induce false positivity to standard HIV antibody tests and may result in problems when applying for life or health insurance, international travel, employment or hospitalization. If there are any problems related to the above situations, the investigators will provide HIV testing to confirm HIV status. They will provide this information to the persons involved. Currently, people who have received experimental HIV vaccines are deferred from blood donation even though they do not have HIV infection.

The study staff will take appropriate action to assist participants with any discrimination they may experience by participating in this study. Such measures will be accompanied with appropriate counseling.

5.6.1.13. Unknown Risks

As with all research there is the possibility of risks that are unknown or that cannot be foreseen based on current information. It is currently unknown whether the vaccination will be effective at attenuating or preventing HIV or if it will affect any future HIV vaccinations.

5.6.2. Alternatives to this IND Product or Study

At this time, there are no known alternatives to these vaccines that afford the same potential protection from acquiring HIV.

5.6.3. Potential Benefit for Participants

Study participants will receive no direct benefit from study participation. Female participants who choose to undergo cervical mucosal samplings may benefit from early detection of cervical

cancer by Pap smear. Participants who choose to undergo sigmoid biopsies may benefit from early detection of lower bowel cancer through the evaluation of suspicious polyps that may be found during these biopsies. Findings of medical concern will be referred for appropriate care and treatment. Others may benefit from knowledge gained in this study that may contribute to the development of an HIV vaccine.

5.6.4. Risks to the Study Personnel and the Environment

The principal risk in the clinical setting is in the handling of needles that may be contaminated and the attendant risks including hepatitis, HIV, and other human pathogens. Adherence to standard operating procedures (SOP) for working with infectious agents and universal precautions will reduce the risk of exposure.

There are no known risks to the environment other than those associated with the generation of biohazardous waste attendant to [vaccination] of humans. All biohazardous waste will be disposed of as stipulated by local, state, and Federal regulations and in accordance with study site SOPs.

5.7. Route of Administration, Dosage Regimen, Treatment Period, and Justification

Participants will be randomized to receive either a full dose of IHV01 (approximately 300µg) and A244 (approximately 300µg), a fractional dose of IHV01 (approximately 60µg) and A244 (approximately 60µg), or placebo. Participants in Groups 3 and 4 will receive ALFQ (approximately 0.5ml) as part of the A244 vaccination. Participants will receive 2 IM injections into the quadriceps[1] muscle at Day 0. The same quadriceps muscle will be used for both injections. Participants randomized to receive the vaccines will have one injection of IHV01 and one injection of A244/AHFG at a full or fractional dose with or without ALFQ, whereas participants randomized to receive placebo will get 2 separate injections of Normal Saline. All placebo injection volumes will match the vaccine injection volumes for the group in which a participant has been randomized.

Refer to Section 7.4 and Table 3 for more information.

5.8. Compliance Statement

The study will be conducted according to the protocol and in compliance with ICH GCP, Belmont Principles, and other applicable ethical, regulatory, Federal and DoD requirements. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with the roles and responsibilities descriptions listed in Appendix A.

[1] Although the IHV01 investigator's brochure notes that injection into the non-dominant deltoid will be used in all cases, the product owner has agreed to allow injections into the quadriceps for RV546. We do not anticipate any safety issues with injection into the quadriceps muscle, which was chosen to help participants tolerate the total injection volume.

5.9. Study Population

A total of 80 healthy, HIV-uninfected participants at a low risk for HIV infection, available for 12 months, who were randomized to receive active vaccine in RV306 and completed all vaccinations will be enrolled. A total of 274/300 (91.3%) active recipients from Groups 2, 3, 4a, and 4b received all vaccinations and completed all study visits in RV306.

Each site will recruit participants who participated in RV306 at their respective sites, however recruitment may also include participants who participated in RV306 at the site in Chiang Mai University if the participant is willing to be enrolled and followed at one of the two Bangkok sites. It is anticipated that up to 110 prior RV306 participants will be screened in order to enroll 80 participants into RV546. As much as possible, an equivalent proportion of male and female participants will be enrolled into each arm of the study.

Study staff and participants will be blinded to vaccine/placebo and full dose/fractional dose assignment. As a byproduct of the sequential enrollment of the vaccine/placebo groups, study staff will not be blinded to a participant's receipt of ALFQ. Groups 1 and 2 (without ALFQ adjuvant) will be enrolled first in a randomized, double-blinded manner. Enrollment into Groups 1 and 2 will begin with no more than two participants per site per week for the first two weeks. Once at least 7-days of safety data is available from the first eight participants enrolled across Groups 1 and 2, the Safety Monitoring Committee (SMC) will review 7-days of safety data from these participants to determine whether enrollment can continue. Enrollment into Groups 1 and 2 will be unrestricted after any safety concerns raised by the SMC are addressed and the Sponsor has given their approval for continuation of enrollment. Enrollment into Groups 3 and 4 will commence after enrollment into Group 1 and Group 2 has been completed, the SMC has reviewed 7-days of safety data from all Group 1 and 2 participants, and the Sponsor gives their approval. Enrollment into Groups 3 and 4 will follow a similar approach with no more than two participants per site per week for the first two weeks, followed by an SMC review of 7-days of safety data from the first eight participants enrolled across both Groups, and Sponsor approval before enrollment can continue unrestricted in Groups 3 and 4. The PI, investigators and study participants will remain blinded to vaccine and placebo allocation.

The legal age at which individuals can provide their own consent to participate in research in Thailand is 18 years. Children will not be recruited for RV546 not only because it does not meet the guidelines for inclusion of children in research, but also because participants must have participated in RV306 to be eligible for RV546. Since the minimum age for eligibility in RV306 was 20 years old at the time of enrollment, all potential participants will legally be considered adults. There are no limitations to race, ethnicity or sex. Refer to Section 12.3 for a statistical justification of the sample size.

5.10. Study Site

All clinical study activities, including mucosal secretion collections, will take place at the Vaccine Trial Center, Faculty of Tropical Medicine at Mahidol University and at the Royal Thai Army Clinical Research Center at the Armed Forces Research Institute of Medical Sciences, both in Bangkok.

Leukapheresis and lymph node biopsy procedures will be completed at the Thai Red Cross AIDS Research Center, Institute of HIV Research and Innovation (IHRI) Foundation and the

Chulalongkorn Memorial Hospital, all in Bangkok. Sigmoid biopsy procedures will only be completed at the Chulalongkorn Memorial Hospital.

6. TRIAL OBJECTIVES AND PURPOSE

6.1. Primary Objective

- To evaluate the safety of 3 novel investigational products in a late vaccine boost setting: the candidate IHV01 and A244/AHFG vaccines and the ALFQ adjuvant

6.2. Secondary Objectives

- To evaluate the effect of a full dose of the IHV01 and A244/AHFG vaccines and the ALFQ adjuvant on cellular, humoral, and innate immune responses (peripheral, lymphoid and mucosal).
- To evaluate the effect of fractional dosing of the IHV01 and A244/AHFG vaccines on cellular, humoral, and innate immune responses (peripheral, lymphoid and mucosal).

7. TRIAL DESIGN

7.1. Study Endpoints

7.1.1. Safety Endpoints

Safety will be assessed both by direct physical examination and by diary cards (Appendix O), which serve as memory tools for better identification of reactions. Participants will be assessed at a pre-vaccination baseline and will remain in clinic under direct observation for at least 30 minutes post-vaccination. Participants will return to clinic on Days 1 and 7 post-vaccination as per the Schedule of Evaluations (SOE; Table 3). During these visits, participants will be assessed for symptoms of local (at the injection sites) reactogenicity such as erythema, induration, pain/tenderness, swelling and limitation of leg movement as well as for symptoms of systemic reactogenicity such as fever, tiredness, chills, myalgia, arthralgia, headache, nausea, and rash. After review of the diary card and discussion with the participant, the study staff will document any post-vaccination reaction(s) and all related information (severity, frequency, etc.) concerning such a reaction in the participant's clinic source documents. Results will be expressed as percent of participants with the overall and specific post-vaccination reactions.

AEs and SAEs will be recorded at all visits along with timing and possible attribution to Investigational Product. Because this clinical trial involves an adjuvant, AESIs will also be assessed. Results will be expressed as percent of participants with the AEs and SAEs. Safety laboratory analyses of complete blood count, liver function tests, urinalysis, and pregnancy test in females will also be performed according to the SOE.

7.1.2. Immunogenicity Study Endpoints

Vaccine-induced immune responses will be assessed in study participants as detailed in the SOE (Table 3).

Humoral responses will be assessed by HIV-specific binding antibody assays, HIV-specific neutralizing antibody assays, and non-neutralizing antibody function assays at Days 0, 14, 168, and 336. Humoral mucosal immune responses in the rectal, semen and cervico-vaginal compartments will be assessed using non-invasive sampling methods (sponge, menstrual disc, and masturbation) at the same time points. Cell-mediated immune responses will be assessed utilizing invasive sigmoid and lymph node biopsies that will be performed at Day 14. Results of immunology assessments through invasive collection methods in the mucosal compartment are exploratory and will be mostly descriptive as performed in a limited number of participants.

Measurement of CD4+ and CD8+ T-cell effector function will be evaluated through response by cytokines such as IFN-γ and IL2. The CD4+ function will be assessed by the lymphoproliferation responses as measured by the functional CFSE assay. Innate immunity (e.g., NK cells) will be assessed by quantifying ADCC and ADCP, determining NK cell phenotype using various flow cytometric panels, cytokine array assays to characterize the type of cytokines elicited by this vaccine regimen with or without adjuvants, by the assessment of gene expression by DNA microarray and related techniques, and other related assays. B-cell responses will be characterized in blood and in diverse anatomical compartments (provided by sigmoid biopsies and lymph nodes biopsies) in a subset of participants. Host gene transcription profile and signature to vaccine antigens will be assessed by RNA sequencing and related techniques.

Leukapheresis will be performed at Day 14 in a subset of willing participants for in-depth investigations of the T-cell responses.

In the context of unknown immune correlates of protection, additional assessments may be performed as new scientific technologies and assessment tools are made available.

For further details, see Section 10.1.

7.2. Overall Study Design

The purpose of this Phase I randomized, double-blind, placebo-controlled clinical trial is to define the safety and immunogenicity of IHV01 and A244/AHFG with and without ALFQ at a full dose and at a fractional dose (one-fifth of a full dose) in a late boost setting for participants who had previous received the AIDSVAX®B/E with or without ALVAC in RV306. Blood, lymph nodes, and mucosal specimens will be collected to assess innate, cell-mediated, and humoral immune responses.

A total of 80 healthy, HIV-uninfected previous RV306 participants who were randomized to receive active vaccine with late boosting will be enrolled into this study. Only the RV306 participants who were randomized to receive active investigational product with a late boost in Groups 2, 3, 4a or 4b, and completed all vaccinations, will be eligible for enrollment.

Within each study Group, participants will be randomized to receive 2 IM injections, either IHV01 and A244/AHFG with or without ALFQ or Normal Saline placebo on Day 0 (see Figure 1) at a targeted ratio of 4:1 vaccine to placebo. Half of the IHV01 and A244/AHFG recipients will receive the full dose (Groups 1 and 3) of each vaccine and the other half will receive a fractional dose (Groups 2 and 4) of each vaccine, which is one fifth of the full dose. Groups 3 and 4 will also receive ALFQ. Once the first eight participants are enrolled across Groups 1 and 2 at a pace of no more than two participants per site per week for the first two weeks, the SMC will review 7-days of safety data from these participants to determine whether enrollment can continue. Any safety concerns raised by the SMC will be addressed prior to the continuation of enrollment. Enrollment into Groups 1 and 2 will continue unrestricted after any safety concerns raised by the SMC are addressed and the Sponsor gives their approval for the continuation of enrollment. Enrollment into Groups 3 and 4 will commence after enrollment into Groups 1 and 2 has been completed, the SMC has reviewed 7-days of safety data from all Group 1 and Group 2 participants, and the Sponsor gives their approval. Enrollment into Groups 3 and 4 will follow a similar approach with no more than two participants per site per week for the first two weeks, followed by an SMC review of 7-days of safety data from the first eight participants enrolled across both Groups, and Sponsor approval before enrollment can continue unrestricted in Groups 3 and 4.

At the screening visit, the participant's general health will be evaluated via medical history, physical exam, pregnancy testing, urinalysis, and testing for HIV, syphilis, and Hepatitis B.

Participants will return for follow-up at 1, 7, 14, 168, and 336 days after vaccination.

Safety and tolerability will be assessed with both clinical and laboratory monitoring. Vaccine-related reactions will be observed and solicited for 30 minutes post-vaccination and with the aid of a diary card and interview of participants during the 7 days post-vaccination. The information gained from the review of the diary card and the interview with the participants will be

documented in the clinical study chart. In addition, AEs will be documented at each clinical encounter. AEs will be graded for seriousness, severity and relationship to the investigational product.

A subset of willing participants will undergo longitudinal mucosal collections for semen, rectal secretions, and/or cervico-vaginal secretions at Days 0, 14, 168, and 336 as well as leukapheresis, sigmoid biopsy, and/or lymph node biopsy at Day 14 as described in the SOE (Table 3). Participants will be followed until 12 months after vaccination for safety and immunogenicity analyses.

Figure 1: Study Group Design

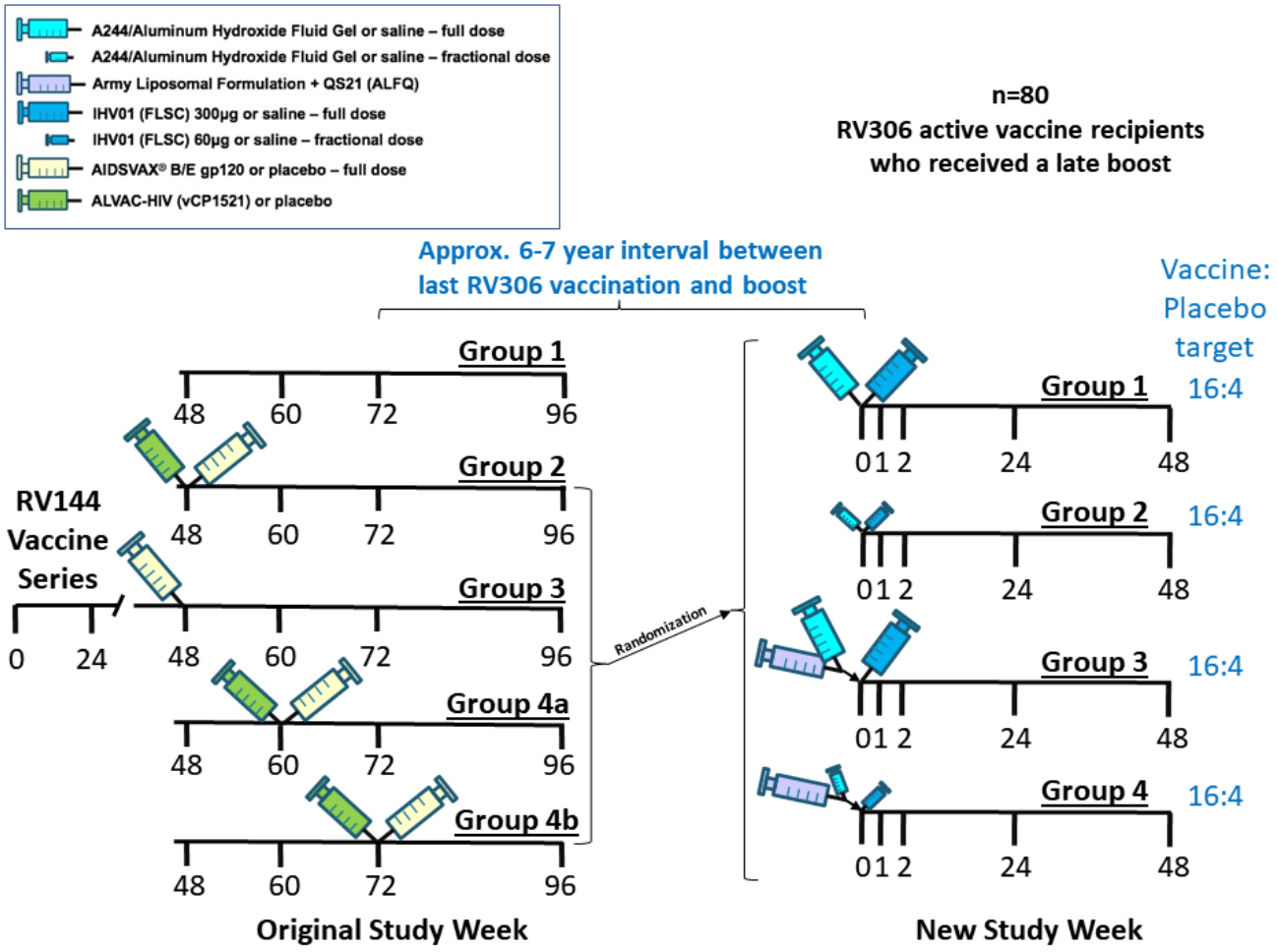


Table 3: Schedule of Evaluations

Visit	S1	1[11]	2	3	4	5	6 (Exit)	7
Visit Day	-45 to -16	0	1	7	14	168	336	post database lock
Visit Week		0	1	1	2	24	48	
Visit Month		0			0.5	6	12	
Visit Window			+2d	-/+2d	-2/+3d	-/+7d	-/+7d	
Clinical								
Informed Consent (Main, Storage/Future Use, Genetic, and Optional Procedures)	X							
Test of Understanding	X							
Enrollment and Randomization		X						
Vaccination		X						
Vital Signs[8] and Physical exam[1]	X	X		X	X	X	X	
Medical History & Concomitant Medications	X	X		X	X	X	X	
Adverse Event Documentation (AE, SAE, AESI)		X	X	X	X	X	X	
Diary Card		X	X	X				
HIV Risk Assessment/Risk Counseling	X	X			X	X	X	
Pregnancy Test & Pre/Post Counseling	X	X			X	X	X	
Urinalysis (blood, protein and glucose)	X	X	X	X	X	X	X	
CBC w/ diff & CD4	2	2	2	2	2	2	2	
Creatinine, ALT/AST	3.5			3.5	3.5	3.5	3.5	
Syphilis and Hepatitis B Serology	3.5							
HIV Testing & Pre/Post Counseling[9]	6				6	6	6	
Optional Procedures								
Mucosal Secretions[2,7]; Plasma LH and FSH (cervical secretions only)		X			X	X	X	
STI Testing[7]	X							
Vaginal[2,7] and/or rectal[3,7] and/or penile[7] swab for microbiome and proteome		X			X	X	X	
Endocervical Cytobrush/swab[2,7]		X			X	X	X	
Pap Smear, if none available in last two years[7]	X							
Sigmoid Biopsy[3,7]					X			

Final Results and Unblinding[5]

Visit	S1	1[11]	2	3	4	5	6 (Exit)	7
Visit Day	-45 to -16	0	1	7	14	168	336	post database lock
Visit Week		0	1	1	2	24	48	
Visit Month		0			0.5	6	12	
Visit Window			+2d	-/+2d	-2/+3d	-/+7d	-/+7d	
Lymph Node Biopsy					X			
Leukapheresis[4]					X (10ml)			
Safety Tests for Biopsy or Leukapheresis (up to max possible volume)[10]					20			
Research								
HIV Binding Antibody		SP			SP	SP	SP	
HIV Neutralizing Antibody Assays		6			6	6	6	
Functional Antibody Assays		SP			SP	SP	SP	
B-Cell Analysis[6]		30		30	30	30	30	
Multiparameter Flow Cytometry		20			20	20	20	
Innate Cell Analysis		20	20	20	20	20	20	
Transcriptomics		20	20	20	20	20	20	
Additional Immunogenicity Testing[6]		30		30	30	30	30	
Daily Volume (mL)	**15**	**128**	**42**	**105.5**	**157.5**	**137.5**	**137.5**	
Daily volume (mL) for participant weighing below 50 kgs	**15**	**93**	**42**	**70.5**	**122.5**	**137.5**	**137.5**	
Cumulative Volume (mL)	**15**	**143**	**185**	**290.5**	**448**	**585.5**	**723**	
Cumulative Volume (mL) for participant weighing below 50 kgs	**15**	**108**	**150**	**220.5**	**343**	**480.5**	**618**	
12-Week Cumulative Volume (mL)[6]					**448**	**137.5**	**137.5**	
12-Week Cumulative Volume (mL) for participant weighing below 50 kgs[6]					**343**	**137.5**	**137.5**	

SP = Assay performed from stored plasma, no additional blood volume required

[1] Full physical examination at screening (Visit S1) and targeted physical examination at all other visits.

[2] FGT questionnaire (Appendix J) will be administered to all participants who choose to undergo mucosal secretion collections and/or endocervical cytobrush/swab for microbiome/proteome assessment and cell collection prior to procedure at each collection visit

[3] Gastrointestinal and dietary questionnaires (Appendix I & Appendix K) will be administered to all participants who choose to undergo a sigmoid biopsy and/or rectal swab for microbiome/proteome assessment prior to the procedure at each collection visit

[4] Participants undergoing leukapheresis at Visit 4 will not have peripheral blood drawn for B-cell analysis, multiparameter flow cytometry, innate cell analysis, transcriptomics, or additional immunogenicity testing. In addition to the blood drawn for the remaining tests on the SOE, 8.5ml of blood will be collected by phlebotomy for PBMC and plasma storage. Red blood cell loss from leukapheresis is estimated to be 10mL.

[5] Participants will be contacted for the unblinding visit after the clinical data is locked.
[6] The blood volumes drawn are in accordance with the Thai National Blood Bank policy: donation every 3 months but no more than 4 times a year; the amount of blood for each donation is according to weight that applies to both male and female: >50 Kg, 450 mL; ≤50 Kg, 350 mL. For participants who weigh **below** 50 Kg, blood for "Additional Immunogenicity Testing" will not be collected at Visits 1, 3 and 4 and only 25 mL of blood will be collected for "B-Cell Analysis" at Visits 1, 3, and 4.
[7] Participants who consent for optional mucosal collections (semen, penile, rectal, and cervico-vaginal secretions, and cytobrush) and sigmoid biopsies will undergo additional testing for gonorrhea/chlamydia genital infections. Female participants who consent for optional mucosal collections will also undergo a pelvic examination that includes a Pap smear (if not done in 2 years prior to the mucosal collection) and visual inspection to evaluate for abnormalities. Female participants who choose to self-insert the menstrual disc collection device will be given instructions for self-insertion (Appendix T). Male participants who consent for optional mucosal collections will undergo a brief visual examination at the screening visit to assess the presence of urethral inflammation and will be given instructions for self-collection (Appendix U). Abnormalities that affect the participant's safety or welfare or those that would increase the risk to a participant will preclude their participation in optional procedures.
[8] Vitals to be collected both pre- and at about 30 minutes post-vaccination at Day 0.
[9] Due to the time required to receive results of HIV testing, post-test counseling will occur at the next study visit after the results are available. HIV testing will be completed as described in Section 11.1.4.4 and management of positive results will be as described in Section 9.5.
[10] Up to 10mL of blood will be drawn for safety tests within 2 days before the biopsy procedures for safety purposes. For the leukapheresis procedure, up to 10mL of blood will be drawn for safety tests within 2 days before and after the procedure for a total of 20mL of blood collected across both blood draws.
[11] Repeat testing that is necessary to establish a participant's eligibility will not affect the post-screening enrollment window unless the repeat testing is necessary to establish a baseline CD4 count, in which case enrollment will occur no sooner than 16 days after the date that blood was drawn for the baseline CD4 count.

7.3. Measures Taken to Minimize/Avoid Bias

7.3.1. Randomization

A randomization schedule will be centrally generated by EMMES. The study will recruit approximately equal numbers of males and females, but formal targets will not be established. Randomization will be performed creating a random ordinal listing. Sets of contiguous recruitment slots will be assigned to each clinic.

The individual site lists will be kept under lock and key by the pharmacy staff at the respective clinical sites. At the end of the study after unblinding of the participants, the lists will be returned to the study sponsor.

7.3.2. Blinding

The PI, study staff, and participants will be blinded as to receipt of active vaccines or placebo but the PI and study staff will not be blinded to group allocation as it pertains to the inclusion of ALFQ. Since the vaccines and placebos are not identical in appearance, to preserve blinding the material inside the syringe will be masked by the pharmacist. In addition, the pharmacy staff preparing the vaccine syringes will not be involved in the clinical assessment of participants and will be instructed not to comment on the appearance of experimental agent to study staff. For all participants, the volume of injections will be consistent.

Pharmacy staff will be trained in GCP and instructed not to discuss the vaccine randomization lists, codes, or participant assignments with study personnel. Pharmacy staff will be required to sign a confidentiality agreement and will be the only person(s) on site who will know the randomization assignment. The randomization assignment will not appear on any label or source document leaving the pharmacy. Samples will be labeled on site using labels containing participant study numbers, protocol number and visit number. The samples will be accompanied by a specimen tracking form that records the study number, date and time of sample collection.

Since HIV-1 binding antibody and Western Blot/immunochromatography results (Section 5.6.1.6) may reflect vaccine or placebo assignments, access to such data will be limited to the laboratory personnel who are performing the tests and managing the data, and the independent statistician. If diagnostic testing of original and verification samples reveals true HIV infection, the participant will be notified by the clinic staff / physician. The PI and Sponsor will be informed of HIV infection by the laboratory. HIV-infected participants will be informed about their HIV infection, but the participant, study site investigators, Sponsor, and manufacturer staff will remain blinded as to their treatment assignment until the study is closed and the database is locked. Diagnostic HIV testing will utilize a sequence of validated tests that will differentiate between vaccine-induced seroreactivity and true HIV infection. Information to the study staff of each vaccine trial site will not include the results of specific tests but will state only HIV "infected" or "not infected", or that repeat testing is needed (as in the case of need for a verification specimen). Report of results will be standardized so that the timing of HIV test reporting does not compromise the double-blind nature of the trial, as a result returned immediately after EIA testing could signal the clinical team that this specimen did not require the additional testing which would be needed if vaccine had induced an antibody response. Some participants may be tempted to know their assignment to vaccine or placebo through voluntary

HIV testing. Participants will be actively discouraged from having HIV testing outside of the trial protocol. If specific needs arise, the research team will provide HIV testing and assist participants who need HIV status determination. Further, participants will be counseled that if they have engaged in behavior that may have increased risk of exposure to HIV, they should have HIV testing done and this should be done through the vaccine trial system.

7.3.3. Unblinding

In case of vaccine-related death or life-threatening SAEs, knowledge of whether a participant received vaccine or placebo can be critical for the interpretation of the significance of clinical findings and thus impact decisions regarding continuation of study participation. In such cases, the PI will consult with a pharmacovigilance (PVG) physician from the USAMRDC ORA Product Safety Surveillance Office (PSSO), the Protocol Chair/Sponsor's Medical Expert for the Trial and/or the HIV Vaccine Product Manager before making the decision to unblind. If the group determines that unblinding is necessary, the PI will request the unblinding of the participant's assignment from the Study Pharmacist.

Any other request for unblinding, with its rationale, must be forwarded through the PI. The PI will evaluate the request and will notify the Protocol Chair/Sponsor's Medical Expert for the Trial and HIV Vaccine Product Manager. They will evaluate the request and will advise the USAMRDC ORA PSSO regarding a course of action. A PVG physician from the USAMRDC ORA PSSO will decide whether to approve the request for unblinding.

In the case of a decision to unblind, the Sponsor will authorize the independent statistician at EMMES to provide this information to the PI, who must notify the IRBs (see Table 5) and provide the study assignment (vaccine vs. placebo) to the site physician. It should be noted that there are very few circumstances in which unblinding will be essential to the medical management of a vaccine (or placebo) recipient. The site investigator will report episodes of accidental unblinding, with an explanation, to Protocol Chair/Sponsor's Medical Expert for the Trial, the IRBs and the USAMRDC ORA PSSO. Follow-up of unblinded participants will continue through the duration of the trial.

After completion of the study and database lock, the Sponsor will authorize the independent statistician to prepare a list containing each participant's unblinded treatment assignment. Participants will be contacted to return to clinic or will be notified via phone or letter (Appendix M) of their unblinded treatment assignment.

7.4. Investigational Product

The investigational products to be utilized in this trial include IHV01, A244/AHFG with and without ALFQ, and a Placebo.

IHV01, an IHV product, consists of the FLSC gp120-CD4 chimera subunit HIV-1 vaccine formulated in Aluminum phosphate adjuvant (Alum). The formulation consists of 0.3 mg/ml of FLSC, 2.4 mg/ml of Alum, 5 mM NaOAc, 40 mg/mL mannitol, pH 6.2.

A244, a Duke University product, consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01AE A244 derived from the CM244 CRF_01AE. The A244 gp120 envelop has an 11 amino N-terminal deletion, similar to the protein used in RV144 (Alam, 2013). The formulation consists of 0.5 mg/mL of the A244 gp120 protein in 20 mM Tris, 123 mM NaCl, pH 7.4. The

AHFG adjuvant that is mixed with A244 consists of Rehydragel HPA that has been diluted with sterile water and has an aluminum concentration of 5 ± 1 mg/mL.

ALFQ, a U.S. Army product, is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (3D-PHAD®) with the addition of QS-21. The liposomal lipid bilayer of ALFQ contains synthetic phospholipids DMPC and DMPG, and synthetic cholesterol (55 mol%). The concentrations of synthetic phospholipid (DMPC + DMPG) and cholesterol in an ALFQ vial are 22.9µM and 27.9µM, respectively. A full dose of ALFQ vaccine formulation contains 200µg of MPLA and 100µg of QS-21.

Normal Saline (0.9% Sodium Chloride for Injection), purchased from a commercial vendor, will serve as a placebo for the trial. All placebo injection volumes will match the study injection volumes for the group to which a participant has been randomized.

7.4.1. Investigational Product Packaging and Labeling

The investigational products IHV01 (FLSC), A244 and ALFQ are covered under an IND application. Each vial label will include a variation of the following statement: “Caution: New Drug – Limited by Federal (or United States) law to investigational use”. Product labels will comply with both US and Thai labeling requirements. The Pharmacy Manual of Procedures (MOP) details specific product labels.

7.4.2. Investigational Product Storage

The investigational products will be stored in a secure pharmacy refrigerator or freezer at the clinical sites. Doses of IHV01 (FLSC), ALFQ, and AHFG must be kept at +2-8°C; A244 at ≤-65°C; and placebo (normal saline) at ≤30°C until preparation. The vaccine/placebo should be given within 2 hours after preparation.

If a deviation in storage temperature occurs outside of the allowable excursions defined in the Pharmacy MOP, the pharmacy staff must report the storage temperature excursion promptly to the Principal Investigator (PI) and to USAMRDC ORA. The excursion must be investigated and action must be taken to restore and maintain the required temperature limits. Vaccine products, adjuvants, and placebo products that have experienced a temperature excursion must be quarantined until USAMRDC ORA approves it for further use or authorizes its destruction (see Section 7.4.4).

7.4.3. Investigational Product Preparation

All investigational product preparation will be performed by the Site Pharmacy personnel on the day of vaccine administration, after confirmation by the clinical staff that the participant is present, is eligible for enrollment into the study and has received their randomization assignment. The pharmacy personnel will perform all manipulations of investigational products in a biological safety cabinet in accordance with GCP guidelines and the RV546 Pharmacy MOP. The pharmacy personnel will wear a lab coat and gloves for all manipulations. The center seals of the vials will be removed and the vial stoppers wiped with an alcohol wipe prior to insertion of a sterile needle attached to a sterile syringe. The RV546 Pharmacy MOP outlines specific product preparations.

7.4.4. Investigational Product Accountability

The site pharmacy personnel will be responsible for and will maintain logs of investigational product receipt, storage, reconstitution, accountability by participant, and investigational product remaining before final disposition. These logs will be securely maintained in the pharmacy at each site.

The empty vials and the unused portion of the vial should be discarded in a biohazard containment bag and incinerated after verification from the monitor. Any unopened vials (past the re-test date or otherwise) that remain will be destroyed at the discretion of the sponsor in accordance with policies that apply to investigational agents in Thailand. Partially used vials or expired prepared doses will not be administered to other participants or used for *in vitro* experimental studies. They will be disposed of in accordance with institutional pharmacy policy. Retest dates shall be updated as per analytical analyses of the IP. No IP shall be administered to a participant beyond the current retest/expiry date until stability testing has been completed and it has been determined that the retest/expiry date can be extended without increasing the risk to participants.

7.5. Duration of Participation

The total duration of study participation is about 12 months from enrollment.

7.6. Dose-adjustment Criteria

No modification of dosage for any of the vaccine products will be allowed for this study.

7.6.1. Safety Criteria for Stopping Doses

Site investigators will notify the PI who will notify the PSRT by email of any safety events that potentially meet the study pause criteria listed below. The PSRT will expedite a review of the pertinent safety data to determine if the following pause criteria has been met. The PSRT may pause vaccination at any time but will use as a guide, based upon an abundance of data supporting safety of these vaccinations, the occurrence of:

- One Grade 3 event judged to be probably or definitely related to vaccination; or
- One Grade 4 or Grade 5 event judged to be possibly, probably or definitely related to vaccination; or
- One SAE judged to be possibly, probably, or definitely related to vaccination; or
- Unexplained decline in CD4 cell count (>30% from baseline) in more than 5 participants in a single Group, as confirmed by a similar result (>30% decline from baseline) from a repeat assay performed at least 4-weeks after the first assay from which a >30% decline in CD4 cell count resulted.
  Note: An unexplained drop in CD4 cell count is defined as a decline that cannot be explained by an intercurrent illness or other event known to cause decreased CD4 cell counts

If immediate action is needed to protect the rights, welfare and safety of a study participant, this must be promptly reported to the IRBs. This information should include participant ID,

diagnosis, severity, relation to product, clinical description of event to the extent possible and actions taken, if any.

7.6.2. Pharmacokinetic Criteria for Dose Adjustment or Stopping Doses

Not applicable.

7.6.3. Study Termination

The PI, Sponsor's Medical Expert/Protocol Chair, sponsor, MoPH EC, RTA IRB, FTM EC, Chulalongkorn IRB, WRAIR IRB, Thai FDA, USAMRDC OHARO, U.S. FDA, or U.S. DoD may stop or suspend the study at any time. The PI will notify the IRBs in writing of any stoppage or cancellation of the study in accordance with Section 11.8.2.2.

7.7. Trial Treatment Randomization Codes

See Section 7.3.1 and Section 7.3.2.

7.8. Identification of Data to be Recorded on the Case Report Forms (CRFs)

Source data will be collected at the study site. For more information on data handling, refer to Section 16.

8. SELECTION AND WITHDRAWAL OF PARTICIPANTS

8.1. Recruitment of Participants

As the study population is a fixed subset of healthy individuals who participated in RV306, a targeted recruitment strategy will be employed. Prior RV306 participants who received all RV306 vaccinations, completed all RV306 study visits, and consented to be contacted for future research will be contacted by phone or through the mail to be invited to attend an optional information session at their convenience. Information sessions will be conducted at the clinical sites by the PI or designee and will contain information about the study, vaccines, and participation requirements. The site staff will make all necessary provisions to assure the privacy of these discussions with all potential participants participating in these information sessions. The briefing session will be followed by an opportunity for questions from the participants. All participants interested in this study will subsequently, individually be administered informed consent.

Refer to Section 5.9 for a detailed description of the participant population.

8.2. Informed Consent Process

At the beginning of the screening visit, written informed consent will be obtained from the participant by the PI or their designee. Separate informed consent forms will be administered for the main study and for the optional procedures. Written informed consent will be obtained from each participant before any study procedures are performed. All informed consent forms will be administered individually, in a private setting, with strict respect of confidentiality. Participants will be given ample time and opportunity to inquire about details of the study, discuss with close family members or friends and ask any questions before dating and signing the consent forms. A copy of the informed consent form, signed by both the participant and the investigator conducting the consent process, will be given to the participant along with a copy of the volunteer event schedule (Appendix P). The consenting process will be documented in the CRFs.

After providing signed informed consent the participant will complete a Test of Understanding (TOU) (Appendix G). Participants are allowed to take the TOU 3 times but must have a passing score (80% or greater) including two compulsory questions to be answered correctly by the third attempt to participate in the study. If after 3 attempts to pass the TOU the participant is unable to do so, the participant will become ineligible for study participation. Participants who successfully pass the TOU will then be screened as per the study protocol.

8.3. Eligibility Screening (Visit S1)

Screening for eligible participants will be performed within 45 days prior to administration of the investigational vaccine. Participants who have passed the TOU and have given written informed consent will undergo a complete medical history, physical examination, and screening laboratory assessments to determine eligibility for study participation. The following screening assessments will be completed after the informed consent process has been completed:

- Medical history, including any medications taken
- Physical exam
- Vital signs

- HIV risk assessment and risk counseling
- HIV testing (including pre/post-test counseling)
- Urine pregnancy testing and counseling
- Urinalysis
- Creatinine
- CBC w/diff
- CD4
- ALT/AST
- Syphilis testing
- Hepatitis B testing
- Optional Procedures:
  - STI testing
  - Pap smear (if none available in the 2 years prior to screening)

Screening evaluations for specific eligibility criteria (see Section 8.3.1 and Section 8.5) must be completed within the screening visit window specified (see Table 3) prior to enrollment but may be repeated within the screening visit window to confirm the results. A second screening visit may be conducted outside of the initial screening visit window for individuals who meet certain criteria (see Section 8.3.1) only if study enrollment is ongoing.

Determination of a participant's risk for HIV infection will be completed at screening as described in Section 9.1.8. Counseling for HIV risk reduction and the potential risks of becoming pregnant during participation will be provided by trained counselors at screening and throughout the study (see Table 3).

Participants agreeing to optional mucosal collections (semen, penile, rectal, cervico-vaginal secretions, and cytobrush) and sigmoid biopsies will undergo additional testing to evaluate for sexually transmitted infections. This includes testing for gonorrhea/chlamydia by urine NAAT and by rectal swabs for individuals practicing receptive anal intercourse in the 6 months prior to testing for those undergoing rectal secretion collection or sigmoid biopsy. Female participants will undergo a pelvic examination that includes Pap smear testing with visual inspection to evaluate for further abnormalities. Individuals with abnormalities noted on examination and laboratory testing not explained by these conditions may undergo additional testing (e.g., wet prep for *Trichomonas vaginalis* in female participants) or be referred for further care and treatment. Abnormalities that affect the participant's safety or welfare or those that would increase the risk to a participant will preclude their participation in optional procedures.

The PI or designee will make the final decision regarding eligibility. Clinical data and specimens collected during screening from participants subsequently found to be ineligible for participation in the study will become part of the study records and specimens will be evaluated in accordance with protocol procedures. Only eligible participants will be given the investigational product at the enrollment visit.

8.3.1. Participant Re-screening

Participants who are determined to be ineligible at screening due to out-of-range laboratory values can be re-screened if, in the medical judgement of the investigator, the out-of-range value is due to a temporary condition. Participants who are ineligible due to a condition that meets

study exclusion criteria can be re-screened if the condition is considered to be temporary. Exclusionary conditions that are considered temporary include but are not limited to syphilis, gonorrhea, and chlamydia. Re-screening will be completed only after resolution of the temporary condition.

Participants who are determined to be ineligible at screening due to their receipt of substances listed in exclusion criterion 4 (see Section 8.5) within the specified windows can be re-screened once the applicable windows have expired.

Participants who are not enrolled prior to expiration of their screening visit window, whether initially determined as eligible or temporarily ineligible, and who remain interested in study participation must repeat the full screening visit in order to ensure their eligibility for enrollment. Re-screening will only be completed if study enrollment is ongoing. Individuals who are re-screened will retain the same participant ID number as was assigned at their first screening visit.

8.4. Inclusion Criteria

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

1. Healthy, HIV-uninfected male and female participants
2. Prior RV306 recipients who were randomized to receive active vaccine with late boosting at month 12, 15, or 18 and who completed all vaccinations
3. Have a Thai identity card
4. Must be at low risk for HIV infection per investigator assessment
5. Must be able to understand and complete the informed consent process
6. Must be capable of reading Thai
7. Must successfully complete a Test of Understanding prior to enrollment
8. Must be in good general health without clinically significant medical history
9. HIV-uninfected per diagnostic algorithm within 45 days of enrollment
10. Laboratory screening analysis:
    a. Hemoglobin: Women ≥11.0 g/dL, Men ≥11.5 g/dL
    b. White cell count: 4,000 to 11,000 cells/mm^3
    c. Platelets: 150,000 to 450,000/mm^3
    d. ALT and AST ≤1.25 institutional upper limit of reference range
    e. Creatinine: ≤1.25 institutional upper limit of reference range
    f. Urinalysis: blood and protein no greater than 1+ and negative glucose

    Note: Each laboratory screening test that is out of acceptable range can be repeated during the screening window to confirm the result. A second screening visit may be conducted outside of the initial screening visit window for individuals who meet certain criteria (see Section 8.3.1) only if study enrollment is ongoing.
11. Female-Specific Criteria:
    a. Not currently pregnant or breastfeeding and not planning to become pregnant during the first 3 months after study vaccine/placebo injections
    b. Negative pregnancy test for women at screening, prior to vaccination (same day), and prior to any of the invasive procedures
    c. Be using an adequate birth control method for 45 days prior to receipt of vaccine/placebo and for at least 3 months after receipt of the vaccine/placebo.

Adequate birth control is defined as follows: Contraceptive medications delivered orally, intramuscularly, vaginally, or implanted underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), condoms, diaphragms, intrauterine device, or abstinence.

12. Male-Specific Criteria
    a. Be using an adequate birth control method for at least 3 months after receipt of the vaccine/placebo. For non-vasectomized male participants with female partners of child-bearing potential this includes the use of condoms or abstinence and/or their partner's use of contraceptive medications delivered orally, intramuscularly, vaginally, or implanted underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), diaphragms, or intrauterine device.

8.5. Exclusion Criteria

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

1. Asplenia: any condition resulting in the absence of a functional spleen
2. Bleeding disorder diagnosed by a medical doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions)
3. History of allergic reaction, anaphylaxis, or other serious adverse reaction to vaccines or components of the vaccines
4. Volunteer has received any of the following substances:
    a. Chronic use of therapies that may modify immune response, such as IV immune globulin and systemic corticosteroids (in doses of $\geq$20 mg/day prednisone equivalent for periods exceeding 10 days)
    Note: The following exceptions are permitted and will not exclude study participation: use of corticosteroid nasal spray for rhinitis, topical corticosteroids for an acute uncomplicated dermatitis; or a short course (duration of 10 days or less, or a single injection) of corticosteroid for a non-chronic condition (based on investigator clinical judgment) at least 14 days prior to enrollment in this study.
    b. Blood products within 120 days prior to HIV screening
    c. Immunoglobulins within 30 days prior to HIV screening
    d. Any licensed vaccine within 14 days prior to study vaccine administration in the present study
    e. Receipt of any investigational HIV vaccine other than RV306 products
    f. Investigational research agents or vaccine within 30 days prior to enrollment in the present study
    g. Receipt of a Coronavirus disease 2019 (COVID-19) vaccine that has been given Emergency Use Authorization (or those that become licensed) by the Thai FDA within 14 days prior to study vaccine administration in the present study
    Note: Volunteers receiving a COVID-19 vaccine that requires 2 doses will not be enrolled until 14 days after the second dose has been administered
    h. Anti-tuberculosis prophylaxis or therapy during the past 90 days prior to enrollment

Note: Participants determined to be ineligible at screening due to receipt of the above listed substances may be re-screened once the applicable window of receipt has expired.

5. Active sexually transmitted infection confirmed by clinical exam and diagnostic test

6. Any medical, psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contradiction to protocol compliance or impairs a volunteer's ability to give informed consent
7. Psychiatric condition that precludes compliance with the protocol; past or present psychoses; past or present bipolar disorder; disorder requiring lithium; or within 5 years prior to enrollment, a history of suicide ideation or attempt
8. Study site employees who are involved in the protocol and/or may have direct access to study related area

8.6. Early Discontinuation or Withdrawal of Study Participants

Each participant has the right to withdraw from the study at any time for any reason without penalty. In those cases where a "withdrawal of consent" is requested by the participant, documentation of the withdrawal of consent and the reason(s) for the request will be captured in the participant's clinical research records and/or on the status change CRF. Although the participant is not obligated to give reason(s) for withdrawing, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights. The participant will also be asked if she/he will agree to complete the clinical assessments listed for the study termination/exit visit (Visit 6).

8.6.1. Investigator Withdrawal of Participants

The PI may withdraw a participant without their consent for the following reasons:

- Failure to comply with study procedures or requirements
- A participant's health or safety may be compromised by further participation
- The PI assesses that it's not in the best interest of the participant to continue participation in the study

If a participant is withdrawn by an investigator, the reason should clearly be stated in the source documents and on the status change CRF.

When a participant withdraws due to an AE or is withdrawn by the PI due to an AE, the sponsor's safety office, the USAMRDC ORA PSSO, must be notified within 5 business days (usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@health.mil). Investigators must follow specific policies regarding the timely reporting of AEs and SAEs to the IRBs (Section 11.8.1.2).

8.6.2. Data Collected for Withdrawn Participants

Only data and samples already collected will be analyzed according to the protocol. The study team will not utilize samples or data from this participant for any future use if he/she has not consented to future use and will discard residual samples when the study is completed as is done for all participants who refuse storage and future use of their samples.

8.6.3. Replacement of Withdrawn Participants

Participants who withdraw or are withdrawn at any time prior to receiving the study injections until 30 days after receiving the injections may be replaced. A participant who has been assessed as eligible for the study through screening will take the place of the withdrawn participant.

Participants who withdraw or are withdrawn within this window will not count toward the total number of participants enrolled in the study.

8.6.4. Follow-up for Withdrawn Participants

After withdrawal, the study team will engage in no further communication with the participant except as directed by an IRB and/or USAMRDC regarding information concerning participant safety.

9. STUDY PROCEDURES

9.1. Vaccination and Follow-up Visits

9.1.1. Day 0 Through Day 336

Day 0 is defined as the day of protocol enrollment and vaccine/placebo injections. At the enrollment visit, screening results will be reviewed and general eligibility will be assessed. All screening results, including results from any repeat testing, must be received prior to enrollment. Repeat testing will not affect the post-screening enrollment window (see Table 3) unless the repeat testing is necessary to establish a baseline CD4 count, in which case enrollment will occur no sooner than 16 days after the date that blood was drawn for the baseline CD4 count.

If clinical assessment on Day 0 suggests significant changes may have occurred since the screening visit, then the physical examination, complete blood count, prothrombin / international normalized ratio of partial thromboplastin time, blood chemistries and urinalysis done at screening must be repeated, as appropriate, and results received before the participant receives the study injections. Pregnancy test results for female participants must be obtained prior to the study injections.

After Day 0, deviations from the visit windows in completing study visits are discouraged and will be recorded as protocol deviations, but are permitted, at the discretion of the PI (or designee), in consultation with the sponsor, in the interest of obtaining participant safety and immunogenicity evaluations following study injections.

Study visit procedures and tests through Day 336 include the following as indicated in the SOE (Table 3):

- Clinical evaluations: targeted physical examination and vital signs (both pre- and at about 30 minutes post-vaccination on Day 0)
- Interim medical history, including any new medications taken
- Adverse event documentation
- Study vaccine/placebo injections
- Diary Card: Baseline in the evening on the day of the injections; 7-day diary card for self-assessment by participant
- HIV risk assessment and risk counseling
- HIV testing (including pre/post-test counseling)
- Urine pregnancy testing and counseling
- Urinalysis
- CBC w/ diff
- CD4
- Optional Procedures:
  - Leukapheresis (with safety tests before and after procedure) (Day 14)
  - Sigmoid Biopsy (with GI and dietary questionnaires and safety tests prior to biopsy) (Day 14)
  - Lymph node biopsy (with safety tests prior to biopsy) (Day 14)

- Mucosal secretion collection, plasma LH and FSH (cervical secretions only) and Female Genital Tract (FGT) Questionnaire (Days 0, 14, 168 and 336)
- Endocervical cytobrush/swab and FGT Questionnaire (Days 0, 14, 168 and 336)

At intervals specified in the SOE, blood will be drawn for safety and immunologic assays. For participants who weigh >50 kg, the total blood volume drawn from each participant will not exceed 450 mL in any 12-week period. For participants who weigh ≤50 kg, the total blood volume drawn from each participant will not exceed 350 mL in any 12-week period.

9.1.2. Post-Database Lock

Upon study completion and locking of the database, participants will return for a final visit to receive their final clinical laboratory tests and unblinding assignment. Participants who received active investigational product will be counseled that these remain experimental candidate vaccines that have no proven ability to protect against subsequent HIV infection, so continued appropriate precautions for HIV behavioral prevention must be exercised. In the event that an individual participant has undergone vaccine-induced seroreactivity, they will be informed that they may face discrimination if undergoing a HIV testing outside the clinic facility, as disclosed in the informed consent. These participants will be offered the opportunity to return to the clinic for routine monitoring of seroreactive status every 6 months as a clinical service even after study closure until it disappears.

9.1.3. Mucosal Secretion Collection

Mucosal secretion collection (cervico-vaginal, semen, rectal secretions, vaginal swab, endocervical cytobrush/swab, penile swab and rectal swab) will be performed at Visits 1, 4, 5, and 6 on participants who provide separate informed consent for these procedures.

Consenting women will have a pelvic examination done by an experienced doctor, who will inspect the tissue of the vagina and cervix and will collect endocervical cells using an endocervical cytobrush/swab as well as collecting a vaginal swab to assess the microbiome/proteome. Participants will also complete the FGT questionnaire. After the examination, the participant will be instructed on how to use a menstrual disc device to collect cervical and vaginal secretions by either self-insertion the night before the visit or the morning of the visit, or with assistance by trained clinic staff during the visit. Participants who choose self-insertion will be given a menstrual disc at a prior visit and instructions (Appendix T) on how to insert the menstrual disc on the night before or the morning of the collection visit. The menstrual disc will remain in place for 4 to 12 hours. Female participants will be asked to avoid receptive vaginal intercourse or insertion of objects intravaginally (tampons, douching, etc.) for the 3 days prior to specimen collection. Pregnant women and those with a history of toxic shock syndrome will be excluded. Collection will not take place if the woman is menstruating or has symptoms of active inflammation or infection of the vagina or cervix. For participants who choose to self-insert the menstrual disc, study staff will call the participant the day before their study visit to remind them about inserting the menstrual disc and to ask about pregnancy, menstruation, and any abnormalities that may preclude them from using the menstrual disc. Participants will be advised to not self-insert the menstrual disc as necessary. For menstruating females, mucosal collection window can be extended to -3/+14 days. The date of last menstrual period will be recorded at each collection visit and blood samples for hormone levels will be evaluated. If,

during the course of the study, a woman becomes pregnant, she will be excluded from further genital secretion collections.

Consenting men will have a brief visual examination at the screening visit which will be completed by an experienced doctor who will inspect the participant's penis for signs or symptoms of urethral inflammation. The participant will be given instructions (Appendix U) on how to self-collect the swabs and will be asked about any signs or symptoms of urethral inflammation prior to the collection of penile swabs and/or a semen sample at follow-up visits. Both penile swabs and semen collections will be deferred if there are signs or symptoms of urethral inflammation. Penile swab will be collected by full circumference of the penis at the coronal sulcus, the junction between the glans and the shaft of the penis. This procedure can be completed by the participant after receiving instruction from study staff or if the participant prefers, the study staff can complete the collection. For the semen collection, the participant will be asked to ejaculate into a sterile container. A private room in clinic will be provided for both procedures. Male participants providing semen will be asked to avoid ejaculation for the 3 days prior to specimen collection.

Both male and female participants may elect to provide rectal secretions and rectal swabs for microbiome/proteome assessment. Participants providing rectal secretions will be asked to avoid receptive anal intercourse or insertion of objects into the anus (enema, etc.) for the 3 days before specimen collection. The anal rectal sponge will be inserted and removed by trained medical staff and will be placed in the rectum for a maximum of 5 minutes. Examination and specimen collections take up to 30 minutes to complete. Rectal sponge and swab collections will be deferred if there are signs or symptoms of perianal inflammation. Gastrointestinal and dietary questionnaires will be administered to all participants who choose to collect rectal secretion/swab collection.

9.1.4. Sigmoid Biopsy

Sigmoid biopsies will be performed only at Visit 4 on participants who provide separate informed consent for the procedure. The procedure will be performed by trained personnel at the King Chulalongkorn Memorial Hospital in Bangkok, Thailand.

Prior to undergoing a sigmoid biopsy, participants will be tested for COVID-19 (if required by institutional procedures) and a pregnancy test will be performed on female participants. All participants tested for COVID-19 must have a negative test result to proceed with the biopsy. Female participants must also have a negative pregnancy test to proceed. Participants who test positive for COVID-19 will be referred for care and treatment if required by institutional procedures.

Gastrointestinal and dietary questionnaires will be administered to all participants who choose to undergo a sigmoid biopsy prior to the procedure.

Using a GI endoscope, a gastroenterologist will collect about 16 to 30 tissue samples from the participant's gut. This procedure carries the possibility of pain, so prior to and during the sigmoid biopsy a licensed physician will offer participant intravenous sedative and pain medications. Medication will be provided to the participant free of charge by the study team. The use of aspirin and non-steroid anti-inflammatory drugs by participants will be prohibited 7 days before and 1 day after the procedure. Participants who undergo sigmoid biopsies will also be

asked to avoid receptive anal intercourse 3 days before and 7 days after the biopsy. The sigmoid biopsy procedure will take up to 1.5 hours to complete and participants will be observed for about 3 hours post-procedure.

9.1.5. Leukapheresis

Leukapheresis will be performed only at Visit 4 on participants who provide separate informed consent for the procedure. The procedure will be performed by trained personnel at the King Chulalongkorn Memorial Hospital, the Thai Red Cross AIDS Research Centre, or the Institute of HIV Research and Innovation (IHRI) Foundation.

For the leukapheresis procedure, participants will be connected to a cell separator machine via IV, which will separate white blood cells for laboratory testing while returning red blood cells either through the same needle or through a second needle placed in the opposite arm. When the procedure is completed, the maximum total red blood cell loss is about 10 mL (less than one tablespoon). Participants who undergo the leukapheresis procedure will not have blood drawn for immunogenicity assays at Visit 4. In addition to the white blood cells that are collected, approximately 150 mL of plasma will be removed at the end of each leukapheresis procedure for storage. Participants who undergo leukapheresis will have blood collected by phlebotomy in accordance with the SOE (Table 3), however they be exempted from the immunogenicity blood draw scheduled at the same visit. An additional 8.5ml of blood will be collected from these participants for PBMC and plasma storage in order to have comparator cells from each visit. Leukapheresis will be performed on participants whose platelet counts or hemoglobin counts meet the protocol inclusion criteria.

The entire procedure will take up to 2-3 hours, during which time the participant will be under constant observation by trained leukapheresis staff. If tingling in the face, lips or hands is experienced, study staff may offer calcium carbonate tablets to correct the calcium loss. Leukapheresis can occasionally cause nausea and may temporarily lower platelet count. More rarely, vomiting, fainting, seizures, dizziness, muscle cramps, itching, flushing, or burning sensation, rash, swelling of hands and feet, headache, nasal congestion, sneezing, wheezing, or chest tightness can occur. After the procedure is complete, participants will be observed for 30 minutes.

9.1.6. Inguinal Lymph Node Biopsy

Inguinal lymph node biopsies will be performed only at Visit 4 on participants who provide separate informed consent for the procedure. The procedure will be performed by trained personnel at the King Chulalongkorn Memorial Hospital, the Thai Red Cross AIDS Research Centre, or the Institute of HIV Research and Innovation (IHRI) Foundation.

Inguinal lymph node biopsy will be performed by a qualified surgeon. Participants will be placed under local anesthesia and an inguinal lymph node will be removed via a surgical incision. The procedure will last approximately 30 to 40 minutes, however, participants will also be asked to rest under observation for up to 4 hours post-procedure. The use of aspirin and non-steroid anti-inflammatory drugs by participants will be prohibited 7 days before the procedure so as to decrease the risk of bleeding.

Participants agreeing to inguinal lymph node biopsy may be asked to undergo photography of the biopsy wound immediately after the procedure and again 6 months later in order to provide an

objective reference for assessment of wound healing and for possible use as participants' educational material. Participants are free to refuse photography of their wound at any point should they not be amenable for any reason.

Transportation will be organized by the study staff and provided at no cost to the participant. Accommodations will be offered for the participant's convenience and not for medical need as all procedures are outpatient. The sigmoid biopsies, leukapheresis and Inguinal Lymph Node biopsies will be performed at the Thai Red Cross AIDS Research Centre, King Chulalongkorn Memorial Hospital, Bangkok, and/or Institute of HIV Research and Innovation (IHRI) Foundation.

9.1.7. Future/Genetic Use of Study Samples

Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations as noted in Table 3. Research samples will be processed and stored at the AFRIMS Department of Retrovirology Specimen Processing Laboratory (SPL).

Stored samples with consent for genetic testing (Appendix V) may be used to elucidate genetic factors associated with immune response to a vaccine and to further evaluate responses to the vaccine. Results from genetic testing, whether for this study or from future genetic testing, will not be provided to participants as the results are not validated for clinical use. Any remnant tissue, cells, serum, or plasma will be stored for future exploratory virological and immunological assays as per individual participant's consent for future use (Appendix E). Any future use of stored samples will require IRB review and approval prior to use. At the time of protocol termination, samples will remain at the AFRIMS Department of Retrovirology SPL or, after IRB approval, transferred to another repository. At this time, a list of participants who refused future use of their samples will be generated from the study database and the corresponding samples will be destroyed.

9.1.8. HIV Risk Assessment and Risk Counseling

Participants must be considered at low risk for HIV infection in order to meet eligibility criteria. This determination was also required for participation in the RV306 trial. Given that all participants in this study will be recruited from the pool of RV306 participants who completed all vaccinations, these participant's risk level for HIV infection have already been determined as low. Despite their prior risk level assessment determining that they were at low risk, each participants risk level will be re-assessed in this study.

Investigators will determine a participant's level of risk for HIV infection based on various self-reported metrics, including but not limited to medical history as it pertains to STIs, use of condoms, use of drugs and alcohol, sexual preference, number of sexual partners, and other self-reported measures with consideration of the low risk for HIV infection assessment given prior to participation in RV306. Information about a participant's level of risk for HIV infection that is not already collected via questionnaire or CRF will be recorded in the visit notes, which are kept in the participants study file. Counseling for HIV risk reduction will be provided by trained counselors throughout the study (see Table 3).

9.2. Concomitant Medications

Information pertaining to receipt of non-study vaccines, research agents, immunoglobulin preparations, immunosuppressive medication, antiretroviral drugs, any blood products, and any other medications that a participant uses will be elicited at study visits and recorded in source documents.

To ensure appropriate medical follow-up for study participants, information regarding concomitant medications used in association with an AE will be collected and recorded in the source documents.

9.3. Management of Participants Who Become Pregnant

Pregnant women and women who plan to become pregnant during the first 3 months after study vaccine administration are excluded from enrollment. If a participant becomes pregnant during the course of the study, they will continue to be followed for the remainder of the study period per the SOE, however the participant will be excluded from optional invasive procedures and blood draws will be conducted for safety purposes only. Every effort will be made to remain in contact with the pregnant participant until birth or termination of the pregnancy in order to record the outcome of the pregnancy.

Pregnancy outcomes will be recorded via a standardized pregnancy CRF. Information documented on this form will include date of last menstrual period, date pregnancy confirmed, history of complications during prior pregnancies (such as congenital abnormalities or spontaneous abortions) and pregnancy outcome information including date of termination or delivery, any complications of pregnancy, the gender, weight, and presence of any congenital abnormalities.

9.4. Management of Participants Who Become Incarcerated

Participation of prisoners is not planned, and any participant will be suspended from study visits while incarcerated, except visits or telephone contact for the purpose of ensuring participant safety. No study product will be administered to a participant who is incarcerated. The IRBs (see Table 5) will be notified of the period of incarceration. Data and samples that were collected before the period of incarceration may still be stored and used for analyses. After release from incarceration, the participant may either return to participation in study visits according to the SOE or study participation may be terminated at the discretion of the PI.

Participants who have returned to the clinic after a period of incarceration will be counseled again about the potential risks of being a participant in the study. A note to the effect that the counseling was done will be written in the progress notes in the participant's binder.

Any participant who is incarcerated for more than 6 months will be re-consented to include taking and passing the TOU.

9.5. Management of Clinical Test Results and Participant Referrals

Only results from approved clinical tests will be shared with participants, specifically: HIV test results, complete blood count, liver function tests and creatinine, urinalysis, pregnancy tests, gonorrhea/chlamydia test results, syphilis and hepatitis B serology. Results will be shared with

participants at a subsequent study visit unless it is of medical concern. For any results that are of medical concern, the participant will be contacted and notified as soon as possible and will be referred for care and treatment. At no point during the study will individual research results be shared with participants.

Participants found to be infected with HIV or syphilis at screening will be counseled appropriately by a study physician/associate investigator or designee, and then referred to a local health care facility for care and treatment.

Participants who are found to have signs or symptoms of reportable communicable diseases (see Section 15.2.1) will be referred to a local health care facility for immediate care.

9.6. Procedures for Monitoring Participant Compliance

All vaccinations and study procedures will be conducted under the direct supervision of the investigational staff. Study staff will be trained to perform study procedures in accordance with the protocol and Clinical MOP. Source documents, such as visit checklists will be kept to ensure that the appropriate procedures are completed at each visit. Participant charts will undergo regular quality control checks by study staff in order to confirm that all study procedures are being completed accurately and thoroughly. In addition to the internal QC processes, the study sponsor will perform periodic monitoring and quality assurance checks (see Section 14).

10. IMMUNOGENICITY ASSESSMENTS

10.1. Specification of Immunogenicity Endpoints

Table 4: Immunology Assays

Humoral Assays	Sample Type	Function Measurement
ADCC, ADCP, and other non-neutralizing antibody functions	Plasma/ Serum	Measures lysis of HIV expressing targets mediated by HIV specific antibodies
HIV-specific binding	Plasma/ Serum	Binding antibody to vaccine antigens
HIV-specific neutralizing antibodies	Serum	Neutralizing activity against luciferase reporter gene expression
Cellular and Innate Assays	**Sample Type**	**Function Measurement**
Cellular response by cytokines such as IFN-γ and IL2 after stimulation with HIV-specific antigens	PBMC	1. CD4+ and CD8+ antigen-specific response 2. Cellular functionality (CD4+; CD8+ only if sufficient CD8+ univariate responses) 3. Cellular polyfunctionality (CD4+; CD8+ only if sufficient CD8+ univariate responses)
Lymphocyte proliferation	PBMC, sigmoid biopsies, or lymph node biopsies	Characterize the function of proliferating cells in response to HIV antigens
B-cell ELISPOT	PBMC, sigmoid biopsies, or lymph node biopsies	Measures cytokine secretion from B cells in response to HIV antigens
Flow cytometry for innate immune cell phenotyping and a cytokine array assay	Plasma/ Serum, sigmoid biopsies, or lymph node biopsies	Phenotype NK and other innate cells and characterize the cytokines elicited by the different vaccine regimens
DNA Microarray: gene expression to vaccine antigens	PBMC, sigmoid biopsies, or lymph node biopsies	Host gene expression profile and signature to vaccine antigens
RNA sequencing: gene transcription to vaccine antigens	PBMC, sigmoid biopsies, or lymph node biopsies	Host gene transcription profile and signature to vaccine antigens
Mucosal Assays	**Mucosal Secretions**	**Function Measurement**
HIV-specific binding antibodies	Semen, rectal secretions, cervico-vaginal secretions	Binding antibody to vaccine antigens
CD4+, CD8+, and other T cell characterization	Cells from lymph nodes biopsy, sigmoid biopsy, and cytobrush	Defines phenotypic characteristics of T cells recognizing the antigen by multicolor flow cytometry
Microbiome/Proteome	Vaginal swab or cytobrush, penile swabs, rectal swabs/sponge, sigmoid biopsies	Assessing the microbiome/proteome

10.2. Immunogenicity Endpoints

Immunogenicity will be assessed using the following assays at time points defined in Section 7.1.2.

10.2.1. Humoral Immune Responses

HIV-specific Binding Antibody ELISA Assays will be performed to detect serum or plasma IgG and IgA binding antibodies to HIV-1 gp120, in particular to the V1V2 loops. Capture antigens will include V1V2 sequences from both subtype AE and Subtype B HIV-1 Env (gp70V1V2 92TH023 and gp70V1V2 Case A2), and HIV-1 Env gp120 proteins matched to sequences in AIDSVAX® B/E without the gD tag and with an 11 amino acid N-terminal deletion, represented as gp120 A244gD- D11 and gp120 MNgD- D11. Durability of IgG response will be assessed for each participant by estimating the decline in $\log_{10}$ IgG from peak to 6- and 12-months post vaccination.

Neutralizing Antibody Assays will be measured as a function of reductions in luciferase (Luc) reporter gene expression after a single round of infection in TZM-bl cells using high throughput analysis. Stocks of molecularly cloned envelope-pseudotyped viruses have been prepared by transfection in 293T/17 cells (American Type Culture Collection) and titrated in TZM-bl cells. This assay has been formally optimized and validated and was performed in compliance with Good Clinical Laboratory Practices, including participation in a formal proficiency testing program. Tier 2 neutralization was assessed using a panel of 11 pseudoviruses, and a global panel.

Antibody Profiling will be conducted to assess antibody breadth and correlation of Fc receptor usage with antibody function. The current capacity for Antibody Profiling is the assessment of over 300 samples in one experiment. The beads presenting the HIV antigens will be distributed robotically in a high-throughput manner into 384 well plates. Similarly, the samples will be diluted robotically and incubated with the beads. Detection reagents will be added to the wells after washing. These detection reagents are inclusive of subclass, isotype and Fc receptor usage. Therefore, in one well, we will be able to capture 900 to 1000 data points from a single sample, and over 250,000 data points per experiment that will allow evaluation of the humoral immune response to these vaccine strategies.

Antibody-dependent cell-mediated cytotoxicity and antibody-dependent cellular phagocytosis will evaluated using an amine reactive staining (Aqua) to identify killed target cells in addition to the loss of CFSE in the traditional RFADCC. This Aqua-based assay is high throughput, has a better signal to noise ratio and a lower coefficient of variation than the conventional CFSE-based RFADCC assay. The ADCP phagocytic score will also be calculated and compared with Antibody profiling data to evaluate the impacts on IgG subclass and Fc receptor usage on non-neutralizing function between groups.

FLSC-Specific Antibody Assays. Sera collected at the various times pre- and post- vaccination are subjected to anti-FLSC, anti-gp120, and CD4i competitive ELISAs. FLSC and gp120 (BaL) assays are performed as a solid-phase ELISA using FLSC or gp120 as the antigens. The CD4i assay is a competitive solid-phase ELISA that analyzes the ability of the test samples to compete for biotinylated human monoclonal antibodies (e.g., A32).

10.2.2. Cellular Immune Responses

Intra-cellular cytokine staining (ICS) assay. PBMCs will be stimulated with HIV-specific antigens and gated on cell surface markers such as CD4 and CD8 to determine antigen-specific cellular responses by individual cytokines, such as IFNγ and IL2, as well as a composite, calculated functional and polyfunctional response using the COMPASS analysis method.

Cellular Proliferation. The proliferative responses of participant's PBMC and/or tissue to HIV-specific antigens and mitogens will be measured by the CFSE assay to characterize the function of proliferating cells.

B-cell Responses. A highly sensitive B-cell ELISPOT system was developed, which allows the enumeration of antibod-secreting cells from diverse anatomical compartments directed against different structural determinants of HIV envelope. Both plasmablast and memory B cell responses will be quantified to detect gp120 A244-specific IgG producing plasmablasts and long-lived memory B cells using PBMCs and/or tissue.

10.2.3. Mucosal Immune Responses

Genital secretion samples will be assayed for cytokines and inflammatory markers using a Luminex® multiplex assay. IgG and IgA antibodies from anogenital secretions directed against HIV antigens such as gp120 and V1V2 will also be quantified by Luminex®.

Vaginal swab, penile swab and rectal swab samples, and if available CVM and rectal sponge, will be assayed for microbiome and/or host proteome. Next-generation sequencing (NGS) has enabled the profiling of the microbiota within a single sample allowing for a high-throughput and cost saving approach using microbial 16S ribosomal RNA gene analysis. In addition, to understand how the microbiome and host immune functions are interacting in the context of vaccine responses the potential use of a novel proteomics approach will be employed. The proteome could be analyzed by mass spectrometry (MS), and protein identification and expression levels will be performed. This will provide information not only on microbial composition, but also on functional aspects of the microbiome and host immunological pathways.

Sigmoid biopsy samples processing will include but is not limited to isolation of mucosal mononuclear cells for immediate assessment (due to poor recovery of cryopreserved sigmoid samples) of HIV-specific immune responses and cell phenotype including the percentage of CD4+ CCR5+ activated target cells. In addition, samples will be stored for evaluating the gut-associated microbiome, immunohistochemical staining, and transcriptome analysis, and related studies.

Lymph node biopsy samples will be processed by 2 methods. Part of the lymph node will be formalin fixed and embedded in paraffin for immunohistochemical staining for cellular phenotype and markers of immune activation. The other part will be processed for quantification of the frequency of antigen specific CD4 and CD8 T cells in responses to HIV immunostimulatory peptides similar to the ICS assay above, quantified by flow cytometry.

10.2.4. Innate Immunity

Characterization of Innate Immunity. Flow cytometric panels will be used to phenotype the different types of NK cells, while functional ICS will be used to characterize their function.

Luminex® multiplex assays will be performed to compare soluble cytokines and other factors in plasma/serum/tissue across vaccine regimens, to further characterize innate biologic pathways engaged.

Innate and Early Adaptive Responses by Gene Activation Assessment. DNA microarrays will explore the innate and early adaptive immune responses to vaccination by screening genomic expression profile in PBMCs and/or tissue.

Transcriptomics. Transcriptome data will be generated using RNASeq to investigate gene expression in correlation with increased immunogenicity to a particular vaccine combination.

10.3. QA/QC for Immunogenicity Assays

For all assays, standardized SOPs are used and technicians are trained theoretically and practically prior to conducting the assays, with all training documented. Cell viability and percent recovery of cryopreserved PBMC are monitored at USAMD-AFRIMS by a quarterly Immunology Quality Assessment (IQA) program (4 vials per interval prepared by 2 technologists) conducted by Duke University School of Medicine. The ICS assays used by the laboratories in the US and Thailand are qualified and both laboratories are participating in the External Quality Assurance Program Oversight Laboratory (EQAPOL) from the Duke Human Vaccine Institute to allow external quality control of those assays.

10.4. Specimen Archiving and Transfer

Biological samples such as serum, plasma, PBMCs, whole blood, mucosal secretion (cervico-vaginal secretion, penile swabs, semen, rectal secretion), sigmoid biopsies and lymph node biopsies remaining after all assays described in this protocol have been completed will be barcoded and archived using electronic specimen storage and tracking system. PBMCs will be stored at -125ºC or lower and plasma/sera will be stored at -70ºC or lower. Samples from participants who have provided consent for storage and future use will be archived in the AFRIMS Department of Retrovirology, SPL, Thailand for 5 years from protocol closure with possible extension after request to IRBs and Ethics Committees. If no archiving renewal is requested or approved, these specimens will be destroyed per applicable SOP's of the archiving institutions. Samples from participants who have not provided consent for storage and future use will be destroyed at the end of the study in accordance with the archiving institutions SOP. The PI, the Sponsor, IRBs/ECs, SMCs, and authorized regulatory bodies may have access to the data regarding the archived specimens.

Archived samples will only be used for research and will not be sold; nor will study participants receive payment, should samples lead to the development of new products in the future. Any future study requesting these samples must first be reviewed and approved by the IRB of each applicable institute. With concurrence from these parties, archived specimens will be shipped to the MHRP and collaborating laboratories for assays.

After completion of laboratory work to meet with the study objectives, some of the leftover samples, including plasma, serum and/or PBMCs, will be provided to the Bio-Medical Resources Center, Department of Medical Sciences, Thai MoPH. As per the MoPH guidelines, the volume of blood and white blood cells that will be sent for storage at the Bio-Medical Resources Center is up to the researcher's consideration, but it should be as much as possible to benefit Thailand.

The samples will only include the blood products that are collected at any timepoint after the participant received the vaccines or placebo injections. As this is for the benefit of Thailand, samples from all participants will be sent to the repository as availability permits.

11. PHARMACOVIGILANCE, SAFETY AND ADVERSE EVENT REPORTING

11.1. Specification of Safety Endpoints

11.1.1. Demographic/Medical History

Gender, date of birth, level of education, occupation and baseline medical history of participants will be recorded.

11.1.2. Vital Signs

Body temperature, pulse, respiratory rate, and blood pressure will be measured both pre- and at about 30 minutes post-vaccination.

11.1.3. Physical Examination

For the purpose of assessing AEs, symptoms and directed medical examination will be performed based on the medical judgment of the investigator. In addition, participants will be asked to record their temperature and complete a diary card at home in the evening after the vaccination and each day for the next 7 days. Study staff will provide both thermometers and rulers to participants and will train participants on how to correctly use the thermometer, read the temperature, and measure any swelling or redness.

11.1.4. Laboratory Assessments

11.1.4.1. Hematology

Complete blood cell count (with differential) will be performed on whole blood as per the SOE (Table 3).

11.1.4.2. Blood Chemistry

Creatinine and alanine amino-transferase will be tested on plasma or serum according to the SOE.

11.1.4.3. Urinalysis

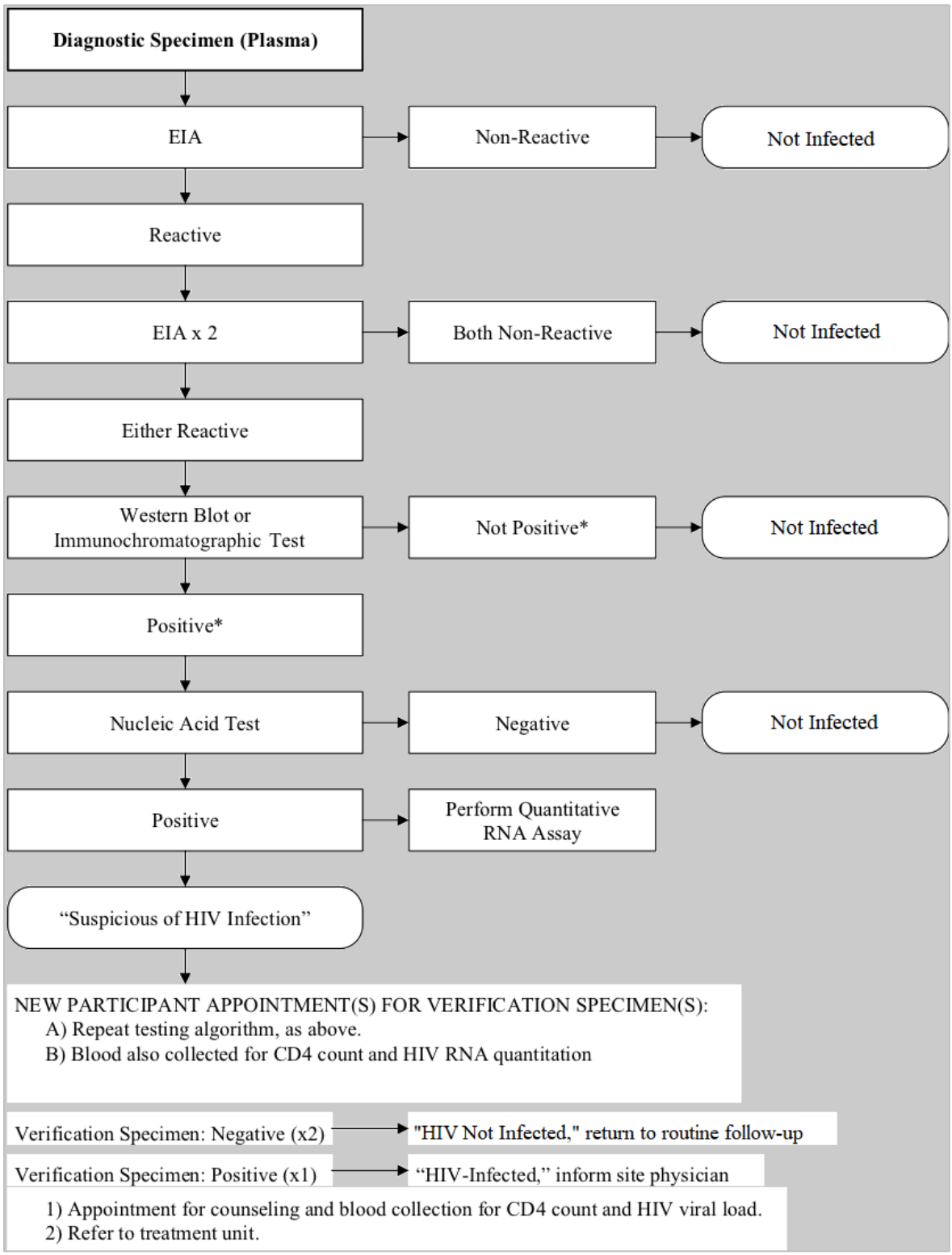
Urinalysis by dipstick for blood, protein, and glucose will be performed as per the SOE. If dipstick testing indicates a hematuria level of 1+ at screening or enrollment visits or ≥1+ at any post-injection follow-up visits where dipstick testing is performed, the urine sample will undergo microscopic analysis. Results from microscopic analysis will be used to confirm a participant's eligibility and to allow for the assessment of severity (see Section 11.3.1), if the result indicate an adverse event. The results will also allow for the identification of non-exclusionary, pre-existing conditions at baseline in comparison to post-injection laboratory results for the assessment the product safety. Per Section 11.2.1, any post-injection changes in laboratory results (i.e., a worsening of a pre-existing condition post-injection) or a clinically significant change in lab values will be considered an adverse event.

11.1.4.4. HIV Diagnostic Algorithm

Diagnostic HIV testing will utilize a sequence of validated tests that will differentiate between vaccine induced seroreactivity and true HIV infection. Information to the study staff of each vaccine trial site will not include the results of specific tests but will state only HIV "infected" or "not infected", or that repeat testing is needed (as in the case of need for a verification specimen). Report of results will be delayed to at least 10 days from blood collection so that the timing of HIV test reporting does not compromise the double-blind nature of the trial, as a result returned immediately after EIA testing could signal the clinical team that this specimen did not require the Western blot or immunochromatographic testing which would be needed if vaccine had induced an antibody response.

An HIV-1 EIA assay will be utilized throughout the course of the study. If the EIA is reactive, the test will be repeated in duplicate. If repeatedly reactive, an HIV Western blot or immunochromatographic test will be performed. If the Western blot or immunochromatographic test is positive, HIV nucleic acid testing (NAAT) will be performed on the plasma specimen stored at –70°C or lower. If the NAAT is positive, a diagnosis of HIV infection is suspected. Notification from the lab that a verification specimen is required is sent to the CRC at the research site and the participant is called back for counseling and repeat blood draw. A second blood specimen (verification specimen) will be obtained for complete repeat HIV diagnostics. If the second plasma specimen is positive both serologically and by NAAT, a diagnosis of HIV infection is considered established. If the verification specimen is not positive (negative or indeterminate), the participant will be counseled that one additional blood collection (second verification specimen) for retesting will be necessary. If results of this repeat verification specimen are positive, infection is established; if not positive, the participant will be informed that he/she is not HIV infected and will return to the protocol’s regular visit schedule. This process is outlined below in the HIV Testing Algorithm in Figure 2.

Figure 2: HIV Testing Algorithm



*If the first verification specimen is found to be not HIV positive (negative or indeterminate), a second verification specimen will be collected and fully tested. If this repeat verification specimen is also negative, the participant is diagnosed as HIV not infected; if positive, the diagnosis of HIV infection is considered established.

11.1.4.4.1. Laboratory assays

HIV EIA and HIV Western blot or immunochromatographic test: Either Thai or U.S. FDA-approved kits maybe used.

HIV nucleic acid tests: plasma collected in a suitable anti-coagulant (EDTA or ACD) will be assayed for HIV NAAT using at least one nucleic acid test platform. Supplemental nucleic acid tests may also be incorporated.

Samples that are Western Blot or immunochromatographic test indeterminate may also be subject to supplemental Nucleic Acid testing at the discretion of the PI or designee.

11.1.4.5. Pregnancy Screen

Pregnancy tests will be performed on all female participants at screening; prior to the vaccination; at Visits 4, 5, and 6; and prior to optional procedures (mucosal secretion collection, leukapheresis, sigmoid biopsy, and inguinal lymph node biopsy) as indicated in the SOE. No vaccine or optional procedures will be performed if the pregnancy test is positive.

11.2. Adverse Event Reporting

The following terms, as defined by 21 CFR 312.32, apply to IND safety reporting.

11.2.1. Adverse Event and Suspected Adverse Reaction

An AE is any untoward medical occurrence in a clinical investigation participant administered a pharmaceutical product and does not necessarily have a causal relationship with this product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. (International Conference on Harmonization (ICH) E6)

An AE is considered to be any adverse change or exacerbation from a baseline condition that occurs following the administration of an investigational product whether or not the event is considered related to the investigational product. Examples of this include but are not limited to the following:

- Adverse changes including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition including the increased frequency of an event or an increased intensity of a condition.
- Concomitant disease with onset or increased severity after the start of investigational product administration.
- A new pattern in a pre-existing condition, occurring after the receipt of investigational product that may signal a clinically meaningful change.
- Clinically significant changes in laboratory values.

A suspected adverse reaction is any AE for which there is a reasonable possibility that the investigational product caused the AE, with “reasonable possibility” meaning there is evidence to suggest a causal relationship between the investigational product and the AE. This implies a

lesser degree of certainty about causality than adverse reaction, which means any AE caused by an investigational product.

11.2.2. Solicited Adverse Event

A solicited AE is a predetermined event, identified in the Investigator's Brochure, which may reflect safety concerns related to the investigational product. AEs that will be solicited and assessed for this study include localized post-vaccination reactions such as erythema, induration, pain and tenderness, swelling and limitation of leg movement, and systemic reactions such as fever, tiredness, chills, myalgia, arthralgia, headache, nausea, and rash. These reactogenicity events will be recorded 30 minutes post-vaccination and during the 7 days following the vaccination. Any local or systemic reactogenicity events beyond 7 days will be reported as an AE.

11.2.3. Serious Adverse Event or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32, an AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect.

An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

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 participant 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.

11.2.4. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed. An unexpected AE for this study refers to an adverse vaccine experience that has not been previously observed (not included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the vaccine. Expected Events are described in the Investigator Brochure and the Clinical MOP. All other events would be considered as unexpected.

11.2.5. Other Adverse Events

All other AEs (those that do not fall under the categories of solicited, an SAE or unexpected) that are identified by site staff, study physicians or the PI, will be documented in the participant's clinic records and entered in the study CRFs, including AESIs as listed in Appendix H.

11.3. Relationship to Investigational Product

The investigator must assign a relationship of each AE to the receipt of the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The relationship of the vaccination to an AE will be determined based on the following definitions:

Not related: No relationship to investigational product. Applies to those events for which evidence exists that there is an alternate etiology.

Unlikely: Likely unrelated to the investigational product. Likely to be related to factors other than investigational product but cannot be ruled out with certainty.

Possible: An association between the event and the administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the participant's clinical status or underlying factors including other therapy.

Probable: There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the participant's clinical state or factors including other therapy.

Definite: An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out.

11.3.1. Severity Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or potentially life-threatening. Refer to the DAIDS grading scale in Appendix B for further guidance in the assignment of severity. The criteria below may be used for any symptom not included in the grading scale. Any grade 4 (potentially life-threatening) or grade 5 (death) AE must be reported as an SAE.

The severity reported on the AEs form will reflect only the highest severity for continuous days an event occurred.

Mild (Grade 1): Does not interfere with routine activities; minimal level of discomfort.

Moderate (Grade 2): Interferes with routine activities; moderate level of discomfort.

Severe (Grade 3): Unable to perform routine activities; significant level of discomfort.

Potentially life-threatening (Grade 4): Hospitalization, ER visit, or urgent intervention indicated for potentially life-threatening event.

Death (Grade 5): Death.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is **not** the same as "serious", which is based on participant/event **outcome** or **action** criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

11.4. Independent Safety Monitor

The Independent Safety Monitor is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion in order to assure the safety of research participants, regulatory compliance, and data integrity. The Independent Safety Monitor is required to review and provide an unbiased written report for all SAEs and participant deaths to the ORA PSSO within 24 hours of their awareness of the event. The report provided must include, at a minimum, a brief summary of the Independent Safety Monitor's review of the event and event outcome, relationship of the event to the investigational product, and alternate etiology if the event is deemed unrelated to the investigational product.

11.5. Protocol Safety Review Team

The Protocol Safety Review Team (PSRT) will review all AEs (including reportable AEs) on a regular and expedited basis, as needed. In addition, the PSRT will review aggregate safety data reports on a weekly basis until 4 weeks after all participants have completed the vaccination, then the safety reviews will occur monthly. The PSRT will review safety events that potentially meet study pause criteria on an expedited basis and will decide if vaccinations should be paused for participant safety purposes. The PSRT may pause vaccination at any time but will use as a guide, based upon an abundance of data supporting safety of these vaccinations, the study pause criteria listed in Section 7.6.1. The PSRT will review the events that led to a study pause and will make the decision to consult the SMC, resume, amend or close the study. Vaccinations will resume only if PSRT and SMC (if consulted) reviews of the events that led to the pause result in a recommendation to permit further vaccinations. Screening and follow-up visits will continue uninterrupted during a vaccination pause.

The PSRT will include the following:

- Sponsor's Medical Expert/Protocol Chair or designee
- Site PIs or designees
- Sponsor Representative or designee
- ORA PVG Physician (only required for study pause reviews)
- Independent Safety Monitor

Additional PSRT participants may include, as needed, the following:

- Associate Investigators
- Clinical research nursing staff

- Laboratory directors
- Data management and regulatory staff
- Site investigators

PSRT conference calls or meetings will require, at a minimum, the participation of the Sponsor's Medical Expert/Protocol Chair or designee, at least one site PI or designee, a representative of the sponsor (may include ORA PVG Physician as necessary), an ORA PVG Physician (only required for study pause reviews), and the Independent Safety Monitor. If the Independent Safety Monitor is not available for the PSRT conference call/meeting, they must provide input by email and then be immediately emailed the outcome of the PSRT discussion. The IRBs will be notified of the decisions taken by the PSRT if they involve suspensions, clinical holds (voluntary or involuntary), or terminations of this research in accordance with Section 11.8.2.2. The USAMRDC ORA will be responsible for safety reporting to the U.S. FDA.

11.6. Safety Monitoring Committee Reviews

The SMC for this study will be comprised of an independent group of experts who review safety data during the clinical trial. The SMC will meet annually to review blinded study data, after the first eight participants are enrolled across Groups 1 and 2 to determine whether it is safe to continue enrollment into those groups, after all participants are enrolled in Groups 1 and 2 to determine whether it is safe to start enrollment into Groups 3 and 4, and after the first eight participants are enrolled across Groups 3 and 4 to determine whether it is safe to continue enrollment into those groups. Enrollment will continue unrestricted after any safety concerns raised by the SMC are addressed and the Sponsor gives their approval for the continuation of enrollment. Screening and follow-up visits will continue uninterrupted during a vaccination pause. In addition to the scheduled meetings, the SMC will meet at the request of the PSRT to evaluate the next steps during study safety pauses and/or to provide other recommendations regarding the safe conduct of the study.

After all SMC reviews, the SMC Administrator will provide the SMC recommendations to the Sponsor's Representative. The Sponsor's Representative will then decide whether to continue, modify, or suspend the study. The USAMRDC ORA PSSO will communicate the final decision to the investigators, who in turn will notify the IRBs and regulatory authorities as appropriate. The sponsor will notify the FDA of the final decision, as appropriate.

11.7. Recording Adverse Events

Section 7.3.3 presents procedures to request unblinding of a participant's randomization code due to a medical emergency or serious medical condition.

11.7.1. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs, solicited AEs, and SAEs will be assessed at all study visits, documented in the source records, and recorded on the CRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the CRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to investigational product, severity, and other possible etiologies.

The timeframe for the collection of AEs and SAEs begins at the time of administration of investigational product and persists through the end of the study. When an event has not resolved by study closure, it will be documented on the AE CRF as "not recovered/not resolved".

11.7.2. Duration of Follow-Up of Participants After Adverse Events

Non-clinically significant AEs still ongoing at the end of the study will be listed as "not recovered/not resolved". SAEs ongoing at the end of the study will be followed to resolution (return to baseline status) or stabilization of the condition with the probability that it will become chronic. All SAE outcomes will be reported to the USAMRDC ORA PSSO using the SAE Report Form. This form should include both a start date and, when available, a resolution date to indicate when the AE met serious criteria and when it resolved, stabilized, or was determined chronic.

Investigators are not obligated to actively seek SAEs in former participants; however, if a SAE, considered to be related to the investigational product is brought to the attention of the investigator *at any time* following completion of the study, the event will be reported to the sponsor's safety office as defined in Section 11.8.1.1.

11.8. Reporting Adverse Events

The PI, co-investigators, site investigators and site staff will exercise due diligence in ascertaining, accurately recording and promptly entering data on the CRF for all AEs for all study participants. As data becomes available from the participant, the clinics and the laboratories, AEs will be recorded and entered on the CRF by the site staff. Site investigators will review this AE source data in a timely manner and will determine the severity of the events and their relation to the study vaccines. Site investigators are encouraged to contact the USAMRDC ORA PSSO for consultation regarding AEs.

The PI will report all AEs to the USAMRDC ORA PSSO and the IRBs (see Table 5) in the appropriate safety, annual, and/or final reports. The study site will provide data files to the sponsor's representative for preparation of annual and final reports to the U.S. FDA.

11.8.1. Reporting Serious and Unexpected Adverse Events

Contact information for reporting SAEs is provided in Table 5.

Table 5: Study Contacts for Reporting Serious and Unexpected Adverse Events

Sponsor's Safety Office	US Army Medical Research & Development Command Office of Regulated Activities Product Safety Surveillance Office ATTN: FCMR-ORA 1430 Veterans Drive Fort Detrick, MD 21702-5009 Fax: +1 301 619 7790 Telephone: +1 301 619 1005 Email: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@health.mil

Institutional Review Board	Ministry of Public Health Ethical Committee Office of the Secretary, the Ethical Review Committee of Research in Human Subjects 3rd Floor, Department of Medicine Services Building Tiwanon Road, Nonthaburi 11000, Thailand Telephone: +66 2 591 8251, +66 2 590 6171-2 Fax: +66 2 591 8251 Email: ecmoph@gmail.com Royal Thai Army Medical Department IRB 5th floor, Phramongkutklaovejvithaya Building Phramongkutklao Medical School 315 Rajvithi Road, Bangkok 10400, Thailand Telephone: +66 2 354 7600, Ext 94270; +66 2 763 4297 Fax: +66 2 354 9011 Email: irbrta@yahoo.com Ethics Committee of the Faculty of Tropical Medicine Mahidol University c/o Research and Academic Services 4th Floor, The 60th Anniversary of His Majesty the King's Accession of the Throne Building 420/6 Rajvithi Road, Bangkok 10400, Thailand Telephone: +66 2 354 9100-4 extension 1349, 1525 Fax: +66 2 306 9126 Email: tmectropmed@mahidol.ac.th The Institutional Review Board, Faculty of Medicine, Chulalongkorn University 1873, 3rd Floor, Ananthamahidol building, Rama IV Rd., Patumwan, Bangkok, Thailand Telephone: +66 2 256 4493 Fax: +66 2 255 4493 Email: medchulairb@chula.ac.th Walter Reed Army Institute of Research IRB 503 Robert Grant Avenue Silver Spring, MD 20910, USA Telephone: +1 301 319 9940 Email: usarmy.detrick.medcom-wrair.mbx.hspb@health.mil
Thai FDA	IND Section, Medicine Regulation Division (Bureau of Drug Control), Food and Drug Administration Ministry of Public Health 88/24 Tiwanond Road, Nonthaburi 11000, Thailand Telephone: +66 2 590 7061

	Note: No email address. The report will be submitted via http://thaihpvc.fda.moph.go.th or hard copy
USAMRDC Office of Human and Animal Research Oversight	Office of Human Research Oversight Office of Human and Animal Research Oversight (OHARO) US Army Medical Research and Development Command, ATTN: FCMR-RPH 504 Scott Street Fort Detrick, MD 21702-5012 Fax: +1 301 619 7803 Telephone: +1 301 619 2165 Email: usarmy.detrick.medcom-usamrmc.other.hrpo@health.mil

11.8.1.1. Reporting to the Sponsor

The investigator will report SAEs to the USAMRDC ORA PSSO according to the requirements in Table 6. Further, the investigator should comply with relevant study site SOPs on reporting AEs judged to be related to the investigational product and SAEs.

The information that the investigator will provide to the USAMRDC ORA PSSO includes, but is not limited to the following:

- Investigation new drug application number, sponsor study number
- Name of the investigational product
- Investigator name and contact number
- Subject identification number
- Serious adverse event term, description
- Onset date
- Date(s) of investigational product administration
- Severity
- Relationship to the investigational product
- Participant's current status
- Admission/Discharge Summary
- Consultations
- Medical record progress notes including pertinent laboratory/diagnostic test results

When reporting via email, the subject line should be formatted as follows:

Initial: SAFETY REPORT – IND # 27011, Sponsor Study #S-19-01, Subject# _____, Event term: _____

Follow-Up: SAE Case Number provided by PSSO (e.g., US-000123), SAFETY REPORT – IND # 27011, Sponsor Study #S-19-01, Subject# _____, Event term: _____

Table 6: Sponsor's AE Reporting Requirements

Event	Report	Notification Time	Notification Method
SAEs	SAE Report*	Within 24 hours of event awareness	Email: usarmy.detrick.medcom-
Pregnancy	Pregnancy Report	Within 24 hours of event awareness	

Events of Special Interest	SAE/Events of Special Interest Report*	Within 24 hours of event awareness	usamrmc.mbx.sae-reporting@health.mil If email is not available, the information can be faxed or called in: Fax: +1 301 619 0197 Phone: +1 301 619 1005
AE-related Withdrawals	Contact USAMRDC ORA PSSO	Within 5 business days of event awareness	
Safety-related Deviations	Contact USAMRDC ORA PSSO	Within 5 business days of event awareness	

*Submission of the event report to USAMRDC ORA PSSO must not be delayed

In order to comply with regulations mandating sponsor notification of specified SAEs to the U.S. FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor's representative will report unexpected SAEs associated with the use of the drug to the U.S. FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy regarding the timely reporting of SAEs to the USAMRDC OHARO.

There may be instances when copies of medical records for certain cases are requested by the sponsor's safety office. In this case, all participant identifiers, with the exception of the subject number, must be redacted on the copies of the medical records before submission to the sponsor's safety office.

Reporting to the sponsor's safety office does not fulfill the investigator's duty to report all SAEs to the IRBs. The PI is responsible for notifying the IRBs (see Table 5) and the USAMRDC OHARO.

11.8.1.2. Reporting to the IRBs

All SAEs related to participation in the study and all deaths will be promptly (within 48 hours) reported to the IRBs and written reports will be submitted to the IRBs within 10 working days. The WRAIR HSPB will report all related SAEs and all participant deaths to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21. Follow up reports will be submitted as additional information becomes available.

A summary of the non-serious AEs and SAEs (both related and unrelated) that occurred during the reporting period will be included in the continuing review report (CRR) to the IRBs/ECs.

Investigators are required to forward safety information provided by the sponsor's representative to the IRBs in a timely manner.

11.8.2. Additional Immediately Reportable Events

11.8.2.1. Unanticipated Problems Involving Risks to Subjects or Others

Unanticipated problems involving risks to subjects or others (UPIRTSO) are any incident, experience, or outcome that meets all of the following criteria:

- Is unexpected (in terms of nature, severity, or frequency) given
  - the procedures that are described in the protocol, investigator's brochure, or informed consent document; and
  - the characteristics of the subject population
- Is related or possibly related to participation in the study

- Suggests that the protocol places participants or others at a greater risk of harm than was previously known or recognized

These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of participant data or the investigational product; adverse psychological reactions; or breach of confidentiality.

The principal investigator will determine whether a given incident, experience or outcome constitutes a UPIRTSO and will promptly (within 48 hours) report UPIRTSOs to the IRBs and to the sponsor. The Principal Investigator will then submit a written report within 10 working days to the IRBs.

Follow up reports should be submitted as soon as additional information becomes available. A summary of UPIRTSOs will also be included in the continuing review report submitted to the IRBs. The WRAIR HSPB will report UPIRTSOs to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

11.8.2.2. Study Pause or Termination

The IRBs will be immediately notified of any suspensions, clinical holds (voluntary or involuntary), or terminations of this research by any of the IRBs, the PSRT, the institution, the sponsor, or regulatory agencies. WRAIR HSPB will forward the notification to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

11.8.2.3. Pregnancy

Each pregnancy must be reported to the USAMRDC ORA PSSO within 24 hours of identification by completing and submitting the Pregnancy Report Form by email or fax. Pregnancies must be reported within 48 hours of becoming aware of the event to the IRBs. WRAIR HSPB will forward the notification to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

Participants who become pregnant after Day 0 will be followed to term, and the following information will be reported on the Pregnancy Report: type and date of delivery, Apgar scores, and health status of the mother and child including the child's gender, head circumference, gestational age at delivery, height and weight.

Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale. Otherwise, pregnancy will not be categorized as an adverse event.

11.8.2.4. Participant Status Change: Incarceration

When a previously enrolled participant becomes a prisoner and the relevant research protocol was not reviewed and approved by the IRB in accordance with the requirements of DODI 3216.02 section 3.9.c, to include prisoners as research participants, the PI shall promptly notify the IRBs. WRAIR HSPB will forward the notification to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

11.8.2.5. AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported by email or fax to USAMRDC ORA PSSO within 5 business days of event awareness. The IRBs will be notified of the withdrawal in a follow-up report as per Section 11.8.1.2.

11.8.2.6. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the U.S. FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, U.S. FDA Form 483, warning letters, or actions taken by any regulatory agency including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the IRBs and the sponsor's representative. The WRAIR HSPB will report knowledge of any pending inspections/audits by regulatory agencies to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

11.8.3. IND Annual Report to the U.S. FDA

The sponsor's representative will notify the PI of the due date of the annual report with sufficient time for the PI to assemble and submit all of the required clinical study information to the sponsor's representative.

The Sponsor and Sponsor's representative (USAMRDC) will be responsible for the preparation of the detailed annual synopsis of clinical activity, including AEs, for submission to the U.S. FDA. Each annual report will summarize IND activity for one year beginning approximately 3 months before the IND U.S. FDA anniversary date. Similar information will also be submitted to Thai FDA on an annual basis.

11.8.4. Final Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" and ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the sponsor's representative for review and approval. The sponsor's representative will use this report to prepare the final clinical study report for submission to the U.S. FDA.

12. STATISTICS

12.1. Analysis Overview

This study intends to descriptively characterize safety and immune responses following delayed boosting of individuals previously vaccinated with a late boost of AIDSVAX®B/E with or without ALVAC in the RV306 protocol. Statistical analysis considers targeted enrollment into the following 5 groups:

- Placebo (n=16)
- Group 1: IHV01 and A244/AHFG Full Dose (n=16)
- Group 2: IHV01 and A244/AHFG Fractional Dose (n=16)
- Group 3: IHV01 and A244/AHFG Full Dose + ALFQ (n=16)
- Group 4: IHV01 and A244/AHFG Fractional Dose + ALFQ (n=16)

Collapsed comparisons of interest include full dose vs. fractional dose (Groups 1+3 vs. Groups 2+4) and effect of ALFQ (Groups 1+2 vs. Groups 3+4). The placebo group is included primarily for purposes of blinding safety and immunogenicity assessments, including blinded samples for the set of immunology assays. The main interest is in determining the safety of IHV01 (FLSC), A244/AHFG and ALFQ as well as whether immunologic differences can be detected between the 4 antigen-containing regimens. Response rates and, where applicable, titer level distributions with appropriate summary statistics will be estimated for each immunologic assay performed. The study will evaluate a series of cellular, humoral and mucosal immune assays. In many cases, trial specimens will be used to further evaluate the practicality of sampling, specimen processing and storage procedures combined with assay performance characteristics. As this study will enroll only participants who had completed the RV306 study, participant data from RV306 will be used in conjunction with data collected in this study to provide a more in-depth analysis of the effect of delayed boosting.

12.2. Final Analysis Plan

12.2.1. Analysis Populations

All participants who received the active vaccination or placebo, and for whom any post-dose data is available, will be included in the safety population. The immunogenicity population will consist of all participants who received the vaccination or placebo and have at least one measured post dose blood sample collected.

12.2.2. Demographics and Baseline Characteristics

Participant demographics and baseline characteristics will be summarized. The number of participants who enroll in the trial, and the number and percentage of participants who complete each assessment, will be presented. The percentage of participants who withdraw from the trial or discontinue the study drug, and reasons for withdrawal or discontinuation, will be summarized.

12.2.3. Safety Analysis

AEs will be listed individually and summarized by body system and preferred terms within a body system for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. For the tabulation of the AEs by body system, a participant will be counted only once in a given body system. For example, a participant reporting nausea and diarrhea will be reported as one participant, but the symptoms will be listed as 2 separate AEs within the class. Therefore, the total number of AEs reported within a body system may exceed the number of participants within the body system reporting AEs. AEs will also be described by time period (i.e., overall and within 28 days of vaccination).

The occurrence of local and systemic reactogenicity symptoms, AEs and SAEs will be assessed as the proportions of participants experiencing such safety events along with 95% CIs. A key comparison of interest is between participants receiving vaccination with and without ALFQ (i.e., Groups 1+2 vs. Groups 3+4). Between group and collapsed comparisons will be performed using a chi-square (or exact test as appropriate).

12.2.4. Immunogenicity Analysis

The primary immunogenicity endpoint is the magnitude of immune response to the endpoint titer of plasma IgG, the same variable assessed in RV306. Immune response over time (durability) will be assessed by the positive incremental area under the curve (AUC) based on a graph with log endpoint titer on the y-axis and visit day on the x-axis. Summary statistics at specific time points of interest will also be provided using the mean and standard deviation (or median and IQR as appropriate). Specific comparisons of interest include overall effect of the vaccine (all active vs. placebo), the effect of ALFQ (Groups 1 + 2 vs. Groups 3 + 4), and the effect of fractional dosing (Groups 1+3 vs. Groups 2 + 4). Primary comparisons will use a 2-sample t-test with an unadjusted alpha of 0.05. Further exploratory comparisons may be performed and adjusted for multiplicity as appropriate.

Secondary immunogenicity endpoints will entail primarily descriptive analyses that mirror similar assessments being performed in the RV306 trial, as described in Section 10. In general, continuous outcomes will be assessed using summary statistics such as the mean, median, standard deviation and IQR. Binary responses will be described using frequencies and percentages. Changes from baseline may be assessed within groups using paired t-tests (or Wilcoxon signed rank as appropriate) or McNemar's test for binary outcomes. Changes over time between groups may be evaluated using longitudinal models with unstructured covariance matrices where possible or covariance strictures selected as appropriate for the observed data.

12.2.5. Missing Values and Outliers

All attempts will be made to collect all data per protocol. No imputation will be performed for missing values. Outliers will not be excluded from the primary analyses. Outliers identified during the analysis will be discussed in the analysis report.

12.2.6. Subgroup Analysis

Gender is a key covariate of interest. While the study is not specifically powered to detect gender differences, safety and immunogenicity analyses may also be described by gender to evaluate potential gender specific differences.

12.2.7. Clinical Laboratory Data Analyses

Clinical laboratory values, including change from baseline, will be summarized by dose cohort and control group. The values will be graded according to the toxicity scale (Appendix B) and, if clinically significant, reported as AEs.

Descriptive summary statistics (mean, SD, median, minimum, and maximum) for clinical laboratory data at admission and each applicable post-dosing visit, including changes from the baseline value collected at admission, will be calculated. If multiple baseline values are obtained, only the most recent value will be analyzed. For change-from-screening summaries, participants with an undefined change from screening, due to missing data, will be excluded. Clinical significance of abnormalities will be indicated in the listings.

12.3. Planned Enrollment and Sample Size Consideration

The primary purpose of the study is to evaluate the safety of a single dose of IHV01 and A244/AHFG with or without ALFQ. Table 7 depicts adverse event rates and associated 95% exact Clopper-Pearson confidence intervals in each potential grouping of interest, given 0, 1, 5, 10, or 15 participants with observed events. If we were to observe zero events within a single active study group, (n=16), the upper limit of a 2-sided exact 95% CI would be 20.6% and true event rates above this could be ruled out at the α=0.025 level.

Table 7: Exact 95% Clopper-Pearson CI for the AE Rate in Each Study Group

Group	Sample Size	Number of Participants with an Event(s) Observed				
		0	1	5	10	15
All Active Groups	64	0% (0%, 5.6%)	1.6% (0%, 8.4%)	7.8% (2.6%, 17.3%)	15.6% (7.8%, 26.9%)	23.4% (13.8%, 35.7%)
Fractional Dose (Groups 2+4)	32	0% (0%, 10.9%)	3.1% (0.1%, 16.2%)	15.6% (5.3%, 32.8%)	31.2% (16.1%, 50%)	46.9% (29.1%, 65.3%)
Full Dose (Groups 1+3)						
ALFQ (Groups 3+4)						
No ALFQ (Groups 1+2)						
Any Individual Analysis Group	16	0% (0%, 20.6%)	6.2% (0.2%, 30.2%)	31.2% (11%, 58.7%)	62.5% (35.4%, 84.8%)	93.8% (69.8%, 99.8%)

While not primary, assessment of immunogenicity is a secondary objective. Table 8 depicts the minimum detectable differences in standard deviations for a 2-sample t-test of the IgG antibody measures at an alpha of 0.05 for key comparisons of interest. We assume that the immunogenicity measures will be normally distributed after log transformation, similar to RV306. As per the table below, collapsed comparisons provide adequate power to quantify our primary endpoints. For example, we would have an 80% power to detect a 1.02 standard

deviation difference between the mean log IgG titer or mean AUC of individual active groups at the α=0.05 level with a 2-sample t-test. Based on the relatively tight standard deviations observed in RV306, we can reasonably estimate that the log IgG endpoint titer in Group 1 will have a standard deviation ranging from about 0.5 to 1.0 depending on the time point.

Table 8: Minimal Detectable Differences, in Standard Deviations, for a 2-Sample T-Test (2 sided, α=0.05)

Comparison	Sample sizes for comparison groups		Power		
	Group 1	Group 2	80%	85%	90%
All Active vs. Placebo	64	16	0.79	0.85	0.92
Fractional Dose (Groups 2+4) vs. Full Dose (Groups 1+3)	32	32	0.71	0.76	0.82
ALFQ (Groups 3+4) vs. No ALFQ (Groups 1+2)					
Between Individual Analysis Groups	16	16	1.02	1.09	1.18

12.4. Level of Significance to be Used

Five percent level two-sided tests will be used when comparing safety and primary immunogenicity outcomes. Secondary and exploratory outcomes will control for multiplicity as appropriate for each analysis.

12.5. Interim Analyses

Interim analyses may be completed to assess early effects on cytokine production prior to introduction into other trials and to ensure the safety of study participants. These data will be assigned a blinded unique identifier separate from subject ID to ensure maintenance of blinding. The PI will ensure concurrence from USAMRDC ORA as outlined in Section 17 prior to presentation or publication of data from interim analyses. Any analyses conducted prior to the end of the scheduled follow-up visits will not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments. In particular, early unblinded analyses by treatment assignment require careful consideration and will be made available on a need to know basis only.

12.6. Statistical Criteria for the Termination of the Trial

There are no statistical criteria for the purpose of study termination in this clinical trial.

12.7. Procedures for Reporting Deviations from the Statistical Plan

Any deviation(s) from the statistical plan as indicated in the protocol will be described in an amendment to the protocol or the clinical study report. The protocol amendment will be submitted to all IRBs as per Section 15.1.2.

12.8. Accounting for Missing, Unused, and Spurious Data

Non-analyzable data will be documented in the deviations.

13. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Participants will be identified on eCRFs and source documents by a unique participant identification number. No personal identifier will be used in any publication or communication used to support this research study. The participant identification number will be used if it becomes necessary to identify data specific to a single participant. Clinical and research records may be reviewed by representatives of USAMRDC OHARO OHRO, WRAIR IRB, MoPH EC, RTA IRB, Chulalongkorn IRB, Mahidol FTM EC, U.S. FDA, Thai FDA, the US DoD, the sponsor, the sponsor's representative and other regulatory agencies as part of their responsibilities for insuring the protection of research participants. Personal identifiers will be removed from photocopied medical and research records.

13.1. Study Monitoring

Study monitoring will be executed under contract by experienced, independent study monitors with monitoring oversight and regulatory compliance falling under the responsibility of the USAMRDC ORA. Prior to initiating the monitoring services, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records. As needed, the clinical monitor may witness the informed consent process or other applicable study procedures to assure the safety of participants and the investigators' compliance with the protocol and GCPs.

Monitoring visits by the independent study monitors will be scheduled to take place during the study at appropriate intervals, and after the last participant has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions), USAMRDC ORA, and the product manager.

13.2. Audits and Inspections

Authorized representatives of the sponsor, the U.S. FDA, Thai FDA, the independent ethics committee or institutional review board may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guideline of the ICH, and any applicable regulatory requirements.

The investigator should contact the sponsor's representative, WRAIR HSPB/IRB and OHARO OHRO immediately if contacted by a regulatory agency about an inspection.

13.3. Institutional Review Board

The PI must obtain approval from all IRBs (Table 5) prior to initiation of the study. Initial IRB approval, and all materials approved by the IRBs, including the participant consent form and recruitment materials, must be maintained by the investigator and made available for inspection.

The PI will be responsible for preparing and submitting CRRs per institution and IRB requirements (see Section 15.1.4). The PI or a designee will transmit the approved final study report to the IRBs as soon as the documents are available.

14. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor's representative may conduct quality assurance audits. Refer to Section 13.2 for more details regarding the audit process.

Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies and procedures. Auditing will be undertaken, as needed, by independent personnel designated by USAMRDC ORA. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

15. ETHICS

15.1. Ethics Review

The study is based on suitably performed laboratory and animal experimentation and will be conducted under a protocol reviewed by all applicable IRBs (see Table 5) and USAMRDC OHARO OHRO. The study is to be conducted by scientifically and medically qualified persons. The IRB will determine whether the benefits of the study are in proportion to the risks. The rights and welfare of the participants will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; participants will give their informed consent and will be competent to do so and not under duress; and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

15.1.1. Review/Approval of Study Protocol

Before this clinical study can be initiated, the study protocol and other required documents will be submitted to the following departments in the order listed for review and/or approval, with the concurrent reviews by the U.S. FDA and Thai FDA:

- Integrated Product Team
- AFRIMS Scientific Review Committee
- Sponsor's Protocol Review Board (PRB)
- WRAIR IRB, RTA IRB, Faculty of Tropical Medicine Ethical Review Board, Mahidol University, Chulalongkorn IRB, MoPH EC, and TSHAVD
- Office of Human and Animal Research Oversight, Office of Human Research Oversight (OHARO OHRO)
- Commander, WRAIR, if applicable
- Sponsor's Representative (acting for The Office of the Surgeon General of the Army)
- USAMRDC Commanding General, if applicable

Enrollment in this protocol may not begin until all approvals have been obtained and the formal authorization letter is received by the PI from the sponsor's representative.

15.1.2. Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, recruitment materials, etc.) must be reviewed and approved prior to implementation. Any protocol amendment will be agreed upon and approved by the sponsor's representative, via submission to the PRB, prior to submission to the IRBs (see Table 5) and prior to implementation of said change or modification. Any modification that could potentially increase risk to participants must be submitted to the U.S. FDA prior to implementation.

Substantive modifications to the protocol and any modifications that could potentially increase risk to participants must also be submitted to the USAMRDC OHARO OHRO for approval prior to issuance of the WRAIR Commander Approval Authorization. The USAMRDC OHARO OHRO defines a substantive modification as a change in PI, change or addition of an institution, elimination or alteration of the consent process, change in the IRB of Record, change to the study population that has regulatory implications (e.g., adding children, adding active duty population,

etc.), significant change in study design (i.e., would prompt additional scientific review) or a change that could potentially increase risks to participants. Any changes of the IRB used to review and approve the research will be promptly reported to the USAMRDC OHARO OHRO and ORA PRB. The WRAIR HSPB will submit protocol amendments and modifications to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

The Informed Consent Form must be revised to concur with any significant amendment that directly affects participants and must also be reviewed and approved with the amendment. New participants enrolled in the study will be consented with the most recent approved consent form. Participants already enrolled in the study will be informed about the revision and asked to re-consent. This may be accomplished by repeating the consent process with the revised consent form with attention given to the changes, or it may be done using an addendum consent that states the revision or new information. The new document must be signed, placed in the study record, and a copy given to the participant.

Modifications or updates to the investigational brochures (IBs) will also be submitted as protocol amendments to the IRBs for review and approval during the data collection phase of the study and for review and acknowledgment during the data analysis phase of the study.

15.1.3. Protocol Deviation Procedures

A protocol deviation is defined as an isolated occurrence involving a procedure that did not follow the study protocol.

The timeline for reporting protocol deviations to the IRBs is determined by the categorization of the deviation: (1) emergent/significant or (2) non-emergent/minor. Protocol deviations arising from or leading to unanticipated problems should be reported in the appropriate timeframe according to the seriousness of the event as a significant deviation or a minor deviation. The unanticipated problem will be submitted as described in Section 11.8.1.2.

Emergent/significant deviations are departures from the protocol that have a significant impact on the welfare or safety of a participant or on the integrity of the study data. Examples: providing the wrong lab result to a participant or failure to obtain a scheduled blood draw for multiple participants. Changes in protocol procedures may be initiated without prior IRB and sponsor approval, only in cases where the change(s) is/are necessary to eliminate an immediate apparent hazard. Emergent/significant deviations should be reported promptly (within 48 hours) to the IRBs and the sponsor, upon becoming aware of the event, by telephone or email. A written report is required to be submitted by the PI to the IRBs and sponsor within 10 working days of knowledge of the significant deviation. Deviations will be reported by the WRAIR HSPB to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

Non-emergent/minor deviations are routine departures that typically involve a participant's failure to comply with the protocol. Examples include missing scheduled visits and failing to complete a required questionnaire. Minor deviations will be reported to the sponsor and the WRAIR IRB in a summary report with the CRRs and the closeout report.

15.1.4. Continuing Review and Closeout Reports

The PI is responsible for submitting the required CRRs and associated documents to the IRBs for review and approval, allowing sufficient time for review and continuation determination prior to

the established continuing review date. Summaries of enrollment and safety reports will be provided in the CRR, as they are made available by the sponsor.

After all study related activities, including data analysis are completed, a closeout report will be submitted as required to the same bodies.

A closeout report will be submitted after 5 years or upon completion of the study, whichever occurs first. The WRAIR HSPB will forward CRRs and closeout report to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

15.2. Ethical Conduct of the Study

This study will be conducted in accordance with all applicable IRB/EC, Federal, and DoD human research protections requirements; all state and local laws; and the Belmont Principles of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline, the applicable ethical principles, local regulatory requirements and the CFR. The PI confirms this by signing this study protocol and U.S. FDA Form 1572.

15.2.1. Confidentiality

The PIs will maintain participant research records at the sites for this study. All participants will receive study numbers that are known only to the investigators and study staff. All samples and documents (with the exception of the consent forms) will be labeled only with a participant's study number and not personal information. The link between a participant ID number and participant identifiable information will be maintained in a secure, locked file cabinet in a locked room, which is accessible only to the PI/designees. Clinical and research records may be reviewed by representatives of USAMRDC OHARO OHRO, WRAIR IRB, MoPH EC, RTA IRB, Chulalongkorn IRB, Mahidol FTM EC, U.S. FDA, Thai FDA, the US DoD, the sponsor, the sponsor's representative, and other regulatory agencies as part of their responsibilities for insuring the protection of research participants.

Every effort will be made to keep the records as confidential as possible within the limits of the law. All data and medical information obtained about participants as individuals will be considered privileged and held in confidence. By Thai law, participant health information must be reported to the Thai government authority if they are diagnosed with any of the following communicable diseases: Plague, Smallpox, Crimean-Congo hemorrhagic fever, West Nile fever, Yellow fever, Lassa fever, Nipah virus disease, Marburg virus disease, Ebola virus disease, Hendra virus disease, SARS, MERS, extensively drug-resistant tuberculosis, and COVID-19. This study does not test for these diseases, however knowledge of these diagnoses will prompt study staff to file a report to the Thai government authority. If a participant shows signs or symptoms of these diseases, then they will be referred for immediate care.

Research and clinical information relating to participants will be shared with other investigators and the scientific community through presentation or publication; however, participants will NOT be identified by name or Thai National ID number. Electronic data will be stored for at least 2 years after the IND is inactivated.

15.2.2. Compensation for Participation

Participants will receive 1,000 Baht for food, time, inconvenience and transportation costs associated with each scheduled visit, including post-test counseling visits. Participants will receive 1,000 Baht for each unscheduled visit that is necessary to address safety concerns, at the Investigator's or designee's discretion.

In addition to the compensation provided for scheduled and unscheduled study visits, participants will receive additional compensation for food, time, inconvenience and transportation costs associated with the optional procedure(s) that they choose to undergo as shown in Table 9 below:

Table 9: Compensation for Participation

Optional procedure	Amount
Semen collection	1000 Baht
Rectal sponges/swab	1000 Baht
Cervico-vaginal menstrual disc	1000 Baht
Vaginal/endocervical cytobrush/swab	1000 Baht
Penile swab	1000 Baht
Leukapheresis	2000 Baht
Sigmoid biopsy	2000 Baht
Lymph node biopsy	2000 Baht

15.2.3. Medical Care for Research-Related Injury

Participants who experience illness or injury arising from participation in the study should contact the study site immediately. The site will either treat and care for the participant directly or refer the participant to an appropriate medical facility for care and treatment. Regardless of where the participant is treated, the study team will continue to provide health care counseling and guidance regarding the appropriate treatment. The cost of medical care for such illness or injury will be paid for by a limited set-aside fund and a clinical trials medical insurance policy. While it is anticipated that the combination of the set-aside fund and the insurance policy is more than enough to pay for the research related injury medical care cost associated with this study, there is a limit to the amount of coverage available. The participant's personal insurance coverage schemes will not be used to cover the cost of medical treatment. Other than medical care and other payments as stated in this protocol, there is no other compensation available to the study participant from this research study; however, participants may pursue their legal right in accordance with Thai law to ask for other compensation relating to research related injury.

15.3. Written Informed Consent

The informed consent process and document will be reviewed and approved by the IRBs and sponsor's representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the participant permits witnessing of applicable study procedures by the sponsor's representative, as well as access to relevant medical records by the sponsor's representative and by representatives of the U.S. and Thai FDAs. The sponsor's representative

will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the U.S. FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRBs.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, 45 CFR 46, and the Belmont Principles will be signed by the participant before any study-related procedures are initiated for that participant. This consent document must be retained by the investigator as part of the study records. Each participant will receive a copy of the signed informed consent document. The investigators or their designees will present the protocol in lay terms to individual participants. Questions on the purpose of the protocol, protocol procedures, and risks to the participants will then be solicited. Any question that cannot be answered will be referred to the PI. No participant should grant consent until questions have been answered to his/her satisfaction. The participant should understand that the study product is an investigational drug and is not licensed by the U.S. and Thai FDAs for commercial use, but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the participant be informed about the principal potential risks and benefits. This information will allow the participant to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary,
- Participants may withdraw from participation at any time,
- Refusal to participate involves no penalty, and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

Should the protocol be modified, the participant consent document must be revised to reflect the changes to the protocol. If a previously enrolled participant is directly affected by the change, the participant will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the participant. A description of this clinical trial will be available on http://www.ClinicalTrials.gov. The data stored in this website will be an aggregate summary of the trials results and will not contain individual demographic information or personal identifiers.

16. DATA HANDLING AND RECORDKEEPING

The primary source document for this study will be the participant's study folder. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. The source documents will be retained at each site. During the study, all hard copy data collection forms will be locked in cabinets accessible only by appropriate study staff when not in use.

For this study, an EDC database system will be used for the collection of the study data in an electronic format. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts 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. No source data will be recorded directly in the eCRF without a prior written record of the data. The investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the study clinical monitor and BIOPHICS. During the study, de-identified data will be periodically transferred in a secure manner from BIOPHICS to EMMES/DCAC for interim blinded analyses and PSRT/SMC safety review. After database lock, data will be transferred from BIOPHICS to EMMES/DCAC, the PI and the Sponsor in a secure manner.

A detailed data management plan will be written and approved by the study team and the Protocol Chair prior to study start, with approval by the sponsor's representative in the USAMRDC ORA. All updates to the data management plan must be approved before study close-out and database lock.

16.1. Inspection of Records

The sponsor's representative or designee will be allowed visit the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, investigational product stocks, drug accountability records, participant charts, study source documents, and other records relative to study conduct.

Participants' health information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that by signature, the participant permits access to relevant medical records by the sponsor's representative and by representatives of the US and Thai regulatory authorities.

16.1.1. Retention of Records

For this study, an eCRF will be used for study data collection and monitoring. Completed, monitored eCRFs will be electronically stored with password protection in a secure location by the sponsor's representative or designee. A copy of each completed eCRF will be retained by the investigator. If it becomes necessary for the sponsor's representative or designee or the U.S. or Thai FDAs to review any documentation relating to the study, the investigator must permit access to such records. The clinical trial information will be entered into a database maintained by the National Institutes of Health/National Library of Medicine (NIH/NLM).

Federal regulations require that the PI retain a copy of all records that support eCRFs for this study (i.e., ICFs, clinical laboratory results, source documents, IP dispensing records) for whichever of the following is the shortest:

- Two years following the date of approval by the U.S. FDA of the IP for the purposes that were the subject of the investigation; or
- Five years following the date on which the results of the investigation were submitted to the U.S. FDA in support of, or as part of, an application for a research or marketing permit for the IP for the purposes that were the subject of the investigation.

If the investigation does not result in the submission of data in the support of, or as part of, an application for a research or marketing permit, records must be retained for 2 years following notification by the Office of the Surgeon General, or his representative, that the entire clinical investigation (not merely the PI's portion) is completed, terminated or discontinued; or for 2 years following the withdrawal of an IND Application.

If a PI retires, relocates or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept this responsibility. The Office of the Surgeon General, or his representative, must be notified in writing of the name and address of the new custodian.

The PI will be responsible for retaining sufficient information about each participant (i.e., name, address, telephone number, and participant identifier) in the study, so that the sponsor's representative, the WRAIR IRB, MoPH EC, RTA IRB, Chulalongkorn IRB, Mahidol FTM EC, the U.S. FDA, Thai FDA, sponsor, employees of USAMRDC, or other regulatory authorities may have access to this information should the need arise.

It is the policy of the USAMRDC that data sheets are to be completed for all participants participating in research (Volunteer Registry Data Sheet). The data sheets will be entered into this Command's Volunteer Registry Database. The information to be entered into this confidential database includes:

- Names (first and last name)
- ID Number
- Date of birth
- Contact Information, both permanent and local
- Study name and study dates, and dates of individual's participation
- Serious AE and unexpected AEs related to the vaccines experienced during the time of trial participation
- Details of the product received

The intent of this data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRDC; and second, to ensure that USAMRDC can exercise its obligation to ensure research participants are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored in Thailand for 75 years under the responsibility of Armed Forces Research

Institute of Medical Sciences, Thailand in coordination with the Faculty of Tropical Medicine, Mahidol University. The database will be kept according to Personal Data Protection Act (PDPA) 2019. However, name, surname and ID number will be stored confidentially and separately from the volunteer registry database. This database will not be used for research.

17. PUBLICATION POLICY

All data collected during this study will be used to support this IND. All data may be published in the open medical or military literature with the identity of the participants protected. Participant confidentiality will be maintained by exclusion of personally identifiable information from the research database and from any publications that result. Anyone desiring to publish or present data obtained during the conduct of the study will conform to study site policies and then forward the publication for review to the USAMRDC ORA or designee and usarmy.detrick.medcom-usamrmc.list.clearances@health.mil prior to submission.

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19. APPENDICES

APPENDIX A. STUDY PERSONNEL ROLES AND RESPONSIBILITIES

Protocol Chair/Sponsor's Medical Expert: Responsible for overseeing the direction and the management of the study and will provide consultative medical and technical expertise for the overall conduct of the clinical trial.

Site-Principal Investigator: Responsible for the study design and will serve as a liaison between the sites, vaccine developer and Sponsor, and contribute support to overall project management and the analysis and reporting of the study data. The PI will act as the qualified physician responsible for all trial site related medical decisions and will take all necessary precautions to ensure that the study obtains the proper clearance for all publication and abstracts, and maintain a study regulatory file as instructed by the study IND sponsor.

Associate Investigators: Will oversee the conduct of the study at the clinic, and are responsible for local IRB submission and approval, study conduct, and reporting all unanticipated problems and AEs to the protocol team and the IRBs. They may conduct the participant study visits, assist with AE assessment and reporting, report any findings and study status directly to the study PI, and coordinate with the PI in the planning, design, and execution of the study.

Community Representative: The Community Advisory Board Chairperson will liaise between the PI and communities where the participants are recruited and conveys questions, concerns, advises and recommendations regarding participants' participation in the study.

U.S. Army HIV Vaccine Product Manager: Responsibilities include providing input on protocol development and oversight of study operations. The U.S. Army HIV Vaccine Product Manager will not have contact with study participants or personal identifiers.

U.S. MHRP HIV Vaccine Product Manager: Responsible for supervising the manufacturing, QC/QA, and stability testing of the vaccine candidates; overseeing the shipment of study products to the study sites; providing input on protocol development; and will serve as a liaison between MHRP and the study pharmacist. The U.S. MHRP HIV Vaccine Product Manager will not have contact with study participants or personal identifiers.

Laboratory Investigator: Responsible for supervising clinical and research laboratory activities for the study. The laboratory investigator will not have contact with study participants or personal identifiers.

Independent Safety Monitor: Responsible for providing independent safety monitoring in order to ensure the safety of research participants, regulatory compliance, and data integrity. The Independent Safety monitor will provide unbiased written reports to the ORA PSSO for all SAEs and participant deaths. The Independent Safety Monitor may also provide assessments on safety data reviews and study pauses resulting from safety events that meet halting criteria.

Study Pharmacist: Responsible for overseeing the import, distribution, inventory, and accountability of vaccine, communication with sponsor / MHRP regarding pharmacy related issues, training and consultation with the study site pharmacy personnel.

Counselors: Counselors can be a physician, nurse or any specifically trained study personnel who performs HIV pre- and post-test HIV counseling at clinical study site.

Consultants: Protocol consultants are responsible for providing input for the study design, protocol development, and serve as technical advisors and subject matter experts for study execution. Consultants will not have contact with study participants or personal identifiers.

Data Manager: Responsible for overall data management, periodically providing de-identified data to Emmes/DCAC for PSRT and SMC safety reviews, and providing a final data transfer of all clinical trial data to Emmes/DCAC, the PI, and the Sponsor no later than 20 business days following final database closure including, but not limited to, the data dictionary, formats, assignments, and any accompanying memorandums. The media format and transfer specifications will be agreed upon with the Sponsor prior to closure.

Statistician: Will receive coded data from the data manager and use those data to perform all investigational data analyses in collaboration with MHRP and the study team. The statistician will not have contact with study participants or personal identifiers.

USAMRDC ORA Product Safety Surveillance Office: The USAMRDC ORA PSSO will be responsible for coordinating and integrating the review of safety data regarding OTSG-sponsored products. The USAMRDC ORA PSSO will review each SAE report and other immediately reportable event reports for medical consistency, accuracy, and completeness and will follow each event until it is satisfactorily resolved. The USAMRDC ORA PSSO will also be involved in the decision to unblind as may be necessary for participant safety.

APPENDIX B. DIVISION OF AIDS (DAIDS) TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS

APPENDIX C. MAIN INFORMED CONSENT FORM

APPENDIX D. OPTIONAL PROCEDURES INFORMED CONSENT FORMS

APPENDIX E. STORAGE AND FUTURE USE INFORMED CONSENT FORM

APPENDIX F. WITHDRAWAL OF CONSENT FOR SAMPLE STORAGE FORM

APPENDIX G. TEST OF UNDERSTANDING AND ANSWER KEY

APPENDIX H. ADVERSE EVENTS OF SPECIAL INTEREST (AESI) LIST

APPENDIX I. GASTROINTESTINAL QUESTIONNAIRES FOR MICROBIOME ANALYSIS

APPENDIX J. FEMALE GENITAL TRACT (FGT) QUESTIONNAIRE (FOR FEMALE PARTICIPANTS)

APPENDIX K. DIETARY QUESTIONNAIRES FOR MICROBIOME ANALYSIS

APPENDIX L. EXTERNAL COLLABORATOR LIST

APPENDIX M. UNBLINDING LETTER

APPENDIX N. VACCINE-INDUCED SEROREACTIVITY LETTER

APPENDIX O. DIARY CARD

APPENDIX P. VOLUNTEER VISIT SCHEDULE

APPENDIX Q. APPOINTMENT/EMERGENCY CONTACT CARD

APPENDIX R. BRIEFING SLIDES

APPENDIX S. VOLUNTEER REGISTRY DATA SHEET

APPENDIX T. MENSTRUAL DISC SELF-INSERTION INSTRUCTIONS

APPENDIX U. PENILE SWAB SELF-COLLECTION INSTRUCTIONS

APPENDIX V. INFORMED CONSENT FORM FOR GENETIC TESTING