HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 114

A phase 1 clinical trial to evaluate the immunogenicity of AIDSVAX B/E bivalent gp120 vaccine and MVA/HIV62B in healthy, HIV-1–uninfected adult participants who previously received MVA/HIV62B in DNA/MVA or MVA/MVA regimens in HVTN 205

DAIDS DOCUMENT ID 12035

BB IND [IND # OR DELETE] HELD BY DAIDS

CLINICAL TRIAL SPONSORED BY

Division of AIDS (DAIDS)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
Department of Health and Human Services (DHHS)
Bethesda, Maryland, USA

STUDY PRODUCT(S) PROVIDED BY

GeoVax, Inc.
Atlanta, Georgia, USA

GSID
South San Francisco, California, USA

June 20, 2016
FINAL
HVTN 114, Version 1.0

Contents

1 Ethical considerations

Multiple candidate HIV vaccines will need to be studied simultaneously in different populations around the world before a successful HIV preventive vaccine is found. It is critical that universally accepted ethical guidelines are followed at all sites involved in the conduct of these clinical trials. The HIV Vaccine Trials Network (HVTN) has addressed ethical concerns in the following ways:

- HVTN trials are designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with Good Clinical Practice (GCP) guidelines.

- HVTN scientists and operational staff incorporate the philosophies underlying major codes [1-3], declarations, and other guidance documents relevant to human subjects research into the design and conduct of HIV vaccine clinical trials.

- HVTN scientists and operational staff are committed to substantive community input—into the planning, conduct, and follow-up of its research—to help ensure that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and supported at all HVTN research sites to ensure community input.

- HVTN clinical trial staff counsel study participants routinely on how to reduce HIV risk. Participants who become HIV-infected during the trial are provided counseling on notifying their partners and about HIV infection according to local guidelines. Staff members will also counsel them about reducing their risk of transmitting HIV to others.

- The HVTN requires that all international HVTN sites lacking national plans for providing antiretroviral therapy (ART) develop plans for the care and treatment of participants who acquire HIV infection during a trial. Each plan is developed in consultation with representatives of host countries, communities from which potential trial participants will be drawn, sponsors, and the HVTN. Participants will be referred to programs for ART provision when the appropriate criteria for starting ART are met. If a program is not available at a site and ART is needed, a privately established fund will be used to pay for access to treatment to the fullest extent possible.

- The HVTN provides training so that all participating sites similarly ensure fair participant selection, protect the privacy of research participants, and obtain meaningful informed consent. During the study, participants will have their wellbeing monitored, and to the fullest extent possible, their privacy protected. Participants may withdraw from the study at any time.

- Prior to implementation, HVTN trials are rigorously reviewed by scientists who are not involved in the conduct of the trials under consideration.

- HVTN trials are reviewed by local and national regulatory bodies and are conducted in compliance with all applicable national and local regulations.

- The HVTN designs its research to minimize risk and maximize benefit to both study participants and their local communities. For example, HVTN protocols provide enhancement of participants' knowledge of HIV and HIV prevention, as well as counseling, guidance, and assistance with any social impacts that may result from research participation. HVTN protocols also include careful medical review of each research participant's health conditions and reactions to study products while in the study.

- HVTN research aims to benefit local communities by directly addressing the health and HIV prevention needs of those communities and by strengthening the capacity of the communities through training, support, shared knowledge, and equipment. Researchers involved in HVTN trials are able to conduct other critical research in their local research settings.

- The HVTN recognizes the importance of institutional review and values the role of in country Institutional Review Boards (IRBs) and Ethics Committees (ECs) as custodians responsible for ensuring the ethical conduct of research in each setting.

2 IRB/EC review considerations

US Food and Drug Administration (FDA) and other US federal regulations require IRBs/ECs to ensure that certain requirements are satisfied on initial and continuing review of research (Title 45, Code of Federal Regulations (CFR), Part 46.111(a) 1-7; 21 CFR 56.111(a) 1-7). The following section highlights how this protocol addresses each of these research requirements. Each HVTN Investigator welcomes IRB/EC questions or concerns regarding these research requirements.

2.1 Minimized risks to participants

45 CFR 46.111 (a) 1 and 21 CFR 56.111 (a) 1: Risks to subjects are minimized.

This protocol minimizes risks to participants by (a) correctly and promptly informing participants about risks so that they can join in partnership with the researcher in recognizing and reporting harms; (b) respecting local/national blood draw limits; (c) performing direct observation of participants postvaccination and collecting information regarding side effects for several days postvaccination; (d) having staff properly trained in administering study procedures that may cause physical harm or psychological distress, such as blood draws, vaccinations, HIV testing and counseling and HIV risk reduction counseling; (e) providing HIV risk reduction counseling and checking on contraception use (for women); and (f) providing safety monitoring.

2.2 Reasonable risk/benefit balance

45 CFR 46.111(a) 2 and 21 CFR 56.111(a) 2: Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result.

In all public health research, the risk-benefit ratio may be difficult to assess because the benefits to a healthy participant are not as apparent as they would be in treatment protocols, where a study participant may be ill and may have exhausted all conventional treatment options. However, this protocol is designed to minimize the risks to participants while maximizing the potential value of the knowledge it is designed to generate.

2.3 Equitable subject selection

45 CFR 46.111 (a) 3 and 21 CFR 56.111 (a) 3: Subject selection is equitable

This protocol has specific inclusion and exclusion criteria for investigators to follow in admitting participants into the protocol. Participants are selected because of these criteria and not because of positions of vulnerability or privilege. Investigators are required to maintain screening and enrollment logs to document volunteers who screened into and out of the protocol and for what reasons.

2.4 Appropriate informed consent

45 CFR 46.111 (a) 4 & 5 and 21 CFR 56.111 (a) 4 & 5: Informed consent is sought from each prospective subject or the subject's legally authorized representative as required by 45 CFR 46.116 and 21 CFR Part 50; informed consent is appropriately documented as required by 45 CFR 46.117 and 21 CFR 50.27

The protocol specifies that informed consent must be obtained before any study procedures are initiated and assessed throughout the trial (Section 9.1). Each site is provided training in informed consent by the HVTN as part of its entering the HVTN. The HVTN requires a signed consent document for documentation, in addition to chart notes or a consent checklist.

2.5 Adequate safety monitoring

45 CFR 46.111 (a) 6 and 21 CFR 56.111 (a) 6: There is adequate provision for monitoring the data collected to ensure the safety of subjects.

This protocol has extensive safety monitoring in place (Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 114 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

2.6 Protect privacy/confidentiality

45 CFR 46.111 (a) 7 and 21 CFR 56.111 (a) 7: There are adequate provisions to protect the privacy of subjects and maintain the confidentiality of data.

Privacy refers to an individual's right to be free from unauthorized or unreasonable intrusion into his/her private life and the right to control access to individually identifiable information about him/her. The term "privacy" concerns research participants or potential research participants as individuals whereas the term "confidentiality" is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (Appendix A). The privacy of participants is protected by assigning unique identifiers in place of the participant's name on study data and specimens. In the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US NIH, which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs an Agreement on Confidentiality and Use of Data/Specimens with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1 clinical trial to evaluate the immunogenicity of AIDSVAX B/E bivalent gp120 vaccine and MVA/HIV62B in healthy, HIV-1–uninfected adult participants who previously received MVA/HIV62B in DNA/MVA or MVA/MVA regimens in HVTN 205

Primary objective(s)

- To evaluate the safety and tolerability of MVA/HIV62B and AIDSVAX B/E given separately or together as boost injections after prolonged immunologic rest, to participants who received MVA/HIV62B vaccinations in DDMM and MMM regimens in HVTN 205

- To compare HIV-specific antibody responses elicited by MVA/HIV62B and AIDSVAX B/E given separately or together as boost injections after prolonged immunologic rest

Study products and routes of administration

- **AIDSVAX B/E**: 300 mcg of subtype B (MN) HIV gp120 glycoprotein and 300 mcg of subtype E (A244) HIV gp120 glycoprotein adsorbed onto 600 mcg of aluminum hydroxide gel adjuvant. Each vial contains 1.2 mL of sterile suspension, to administer 1 mL IM. The vaccine was originally developed and manufactured by Genentech Inc. The development and manufacturing rights were subsequently transferred to VaxGen, Inc. and finally transferred to its current developer, Global Solutions for Infectious Diseases (GSID).

- **MVA/HIV62B** vaccine, also known as MVA62B, MVA/HIV62, or MVA62: a highly attenuated vaccinia virus expressing HIV-1 *gag*, *pol*, and *env* genes. The MVA/HIV62B vaccine has been vialed at 1×10^8 50% tissue culture infective dose ($TCID_{50}$)/mL. A 1×10^8 $TCID_{50}$ dose will be administered as a 1mL IM injection into the deltoid. MVA for this trial has been manufactured by IDT Biologika, Dessau-Rosslau, Germany. The MVA used in HVTN 205 (BB IND 12930) and HVTN 094 (BB IND 14980) was manufactured by BioReliance Ltd, Glasgow, Scotland.

- **Control for AIDSVAX B/E**: Sodium Chloride for Injection USP, 0.9%. The AIDSVAX B/E control will be administered as a 1mL IM injection into the deltoid.

- **Control for MVA/HIV62B**: Sodium Chloride for Injection USP, 0.9%. The MVA/HIV62B control will be administered as a 1mL IM injection into the deltoid.

Table 3-1 Schema

Study arm	n	MVA/HIV62B dose	AIDSVAX B/E dose (B/E/Alum)	Prior HVTN 205 regimen	Boost (deltoid) M0 (Day 0)	Boost (deltoid) M4 (Day 112)
Group 1	20	1×10^8 $TCID_{50}$	0	MMM*	MVA/HIV62B (left) + Control (right)	MVA/HIV62B (left) + Control (right)
Group 2	20	1×10^8 $TCID_{50}$	300/300/600 mcg	MMM	MVA/HIV62B (left) + AIDSVAX B/E (right)	MVA/HIV62B (left) + AIDSVAX B/E (right)
Group 3	20	1×10^8 $TCID_{50}$	0	DDMM**	MVA/HIV62B (left) + Control (right)	MVA/HIV62B (left) + Control (right)
Group 4	20	1×10^8 $TCID_{50}$	300/300/600 mcg	DDMM	MVA/HIV62B (left) + AIDSVAX B/E (right)	MVA/HIV62B (left) + AIDSVAX B/E (right)
Group 5	20	0	300/300/600 mcg	DDMM	Control (left) + AIDSVAX B/E (right)	Control (left) + AIDSVAX B/E (right)
Total	100					

* Denotes a prime-boost vaccine regimen comprising 3 sequential administrations of MVA/HIV62B.

** Denotes a vaccine regimen comprising 2 sequential priming administrations of JS7 DNA plasmid followed by 2 sequential boost administrations of MVA/HIV62B.

Participants

Up to 100 healthy, HIV-1–uninfected volunteers aged 18 to 55 years in the US and Peru who were previously vaccinated in HVTN 205

Design

Multicenter, randomized, double-blind trial

Duration per participant

10 months of scheduled clinic visits (main study) followed by a participant health contact at 2 years following the initial study injection

Estimated total study duration

28 months (includes enrollment, follow-up, and annual health contact)

Investigational New Drug (IND) sponsor

DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)

Study product providers

- **MVA/HIV62B**: GeoVax (Atlanta, Georgia, USA)
- **AIDSVAX B/E**: Global Solutions for Infectious Diseases (South San Francisco, California, USA)

Core operations

HVTN Vaccine Leadership Group/Core Operations Center, Fred Hutchinson Cancer Research Center (FHCRC) (Seattle, Washington, USA)

Statistical and data management center (SDMC)

Statistical Center for HIV/AIDS Research and Prevention (SCHARP), FHCRC (Seattle, Washington, USA)

HIV diagnostic laboratory

University of Washington Virology Specialty Laboratory (UW-VSL) (Seattle, Washington, USA)

Endpoint assay laboratories

- Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)

Study sites

HVTN Clinical Research Sites (HVTN CRSs) to be specified in the Site Announcement Memo

Safety monitoring

HVTN 114 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

Chair	Paul Goepfert University of Alabama, Birmingham 205-975-5667 paulg@uab.edu	*Statistician*	Yunda Huang SCHARP, FHCRC 206-667-5780 yunda@scharp.org
Cochair	Martin Casapia Asociación Civil Selva Amazónica, Iquitos, Peru 51-65-236-277 mcasapia@acsaperu.org	*Medical officer*	Julia Hutter DAIDS, NIAID 240-627-3039 julia.hutter@nih.gov
Protocol Team leader	Marnie Elizaga HVTN Core, FHCRC 650-854-4984 melizaga@hvtn.org	*Laboratory lead*	Georgia Tomaras Duke University, HVTN Laboratory Program 919-681-5598 gdt@duke.edu

Other contributors to the original protocol

Core medical monitor	Marnie Elizaga HVTN Core, FHCRC	*Statistical research associate*	Brittany Sanchez SCHARP, FHCRC
Vaccine developer representatives	Harriet Robinson GeoVax	*Clinical safety specialist*	Megan Jones HVTN Core, FHCRC
	Carter Lee GSID	*Clinical trials manager*	Marianne Hansen HVTN Core, FHCRC
	Vineeta Gulati GSID	*Project manager*	Huguette Redinger SCHARP, FHCRC
Laboratory Program representative	On Ho HVTN Laboratory Program, FHCRC	*HVTN program manager*	Gina Escamilla SCHARP, FHCRC
Regulatory affairs	Rachael McClennen HVTN Core, FHCRC	*Protocol development manager*	Carter Bentley HVTN Core, FHCRC
Clinic coordinators	David Berger Seattle CRS		Meg Trahey HVTN Core, FHCRC
	Lilian Saavedra Asociación Civil Selva Amazónica, Iquitos, Peru	*Community engagement unit representative*	Gail Broder HVTN Core, FHCRC
DAIDS protocol pharmacist	Scharla Estep DAIDS, NIAID 240-627-3046	*Community educator/recruiter*	Patricia Segura Asociación Civil Impacta Salud y Educación, Lima, Peru
Community Advisory Board (CAB) members	Butch McKay Birmingham CAB	*Technical editor*	Erik Schwab HVTN Core, FHCRC
	Nicholas Paulo Boston CAB		

4 Background

4.1 Rationale for trial concept

Despite new treatments and the demonstrated effectiveness of pre-exposure prophylaxis and antiretroviral therapy (ART) in limiting the spread of HIV, the rate of new infections in the United States has been relatively constant for the past 20 years, with 50-55,000 new infections per year [4]. Discouragingly, only 25% of these infections are successfully treated with antiretroviral agents, which could reduce the risk of transmission [5,6]. Particularly discouraging are the increasing rates of infection among youth 13-24, who accounted for 26 percent of new infections in 2010. In men who have sex with men (MSM) in this age group, HIV incidence rose by 132.5% between 2002 and 2011, or around 10.5% per year (Table 4-1). The infection has also become particularly high in certain geographic regions and at risk populations (Figure 4-1). In the Southeastern United States, gay black men have a 60% chance of infection by the time they are 30 years old [7].

Table 4-1 HIV diagnosis rate among MSM/100,000 in the US [8]**.** The increasing rate of infection in 13 to 24 year olds is in **bold.**

	No. of HIV Diagnoses Among Males With Infection Attributed to Male-to-Male Sexual Contact by Year of Diagnosis[a]										%	EAPC
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Change	(95% CI)
Age group, y												
13-24	***2976***	***3207***	***3748***	***4107***	***4690***	***5449***	***6208***	***6528***	***6912***	***6919***	***132.5***	***10.5 (10.1 to 10.9)***
25-34	7957	7474	7657	7506	7379	7867	7868	7854	7738	7929	-0.4	0.3 (0 to 0.6)
35-44	9782	9296	9284	8642	8563	8125	7264	6537	5824	5417	-44.6	-6.2 (-6.5 to -5.8)
45-54	3936	3933	4090	4145	4217	4564	4471	4185	4040	4145	5.3	1.6 (0.1 to 1.1)
≥ 55	1370	1342	1462	1439	1465	1609	1655	1581	1521	1623	18.5	2.0 (1.0 to 3.0)
Total	26,021	25,251	26,240	25,838	26,313	27,614	27,466	26,685	26,035	26,033	0	0.3 (0.1 to 0.5)

Abbreviation EAPC, estimated annual percentage change.
[a] The number of HIV diagnoses resulted from statistical adjustment that accounted for missing transmission category.

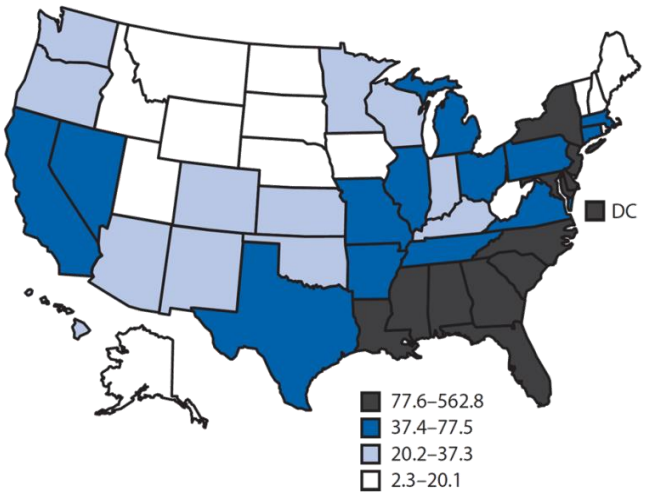


Figure 4-1 Prevalence rates of youth 13-24 living with a diagnosis of HIV in different states. Note the high prevalence rates in the southeastern United States [8].

Clade B HIV is also at epidemic proportions in Peru where 12.5% of MSM are infected and 56% of new infections are in MSM [9], contributing to significant rates of infection in Central and South America (~86,000 in 2012) [10,11]. Clearly, the clade B epidemic continues to be a significant public health problem.

The HIV RV144 vaccine efficacy trial is the only vaccine study to date to demonstrate protection against acquisition of infection [12]. While the 31% efficacy was modest, a particularly important outcome of the study was that post hoc analysis demonstrated significant immune correlates of risk for HIV infection [13]. Specifically, antibodies (Ab) to the V1V2 loop of gp120 correlated with decreased risk of HIV infection. While the neutralizing Ab responses induced by this vaccine regimen were modest, functional Ab responses, such as antibody-dependent cellular cytotoxicity (ADCC), were induced and also correlated with a lower risk of infection. Consistent with the latter, IgG3 also correlated with decreased risk and this immunoglobulin subclass is better at engaging Fc receptors and ADCC [14,15]. Interestingly, certain specificities of serum IgA Ab were associated with decreased vaccine efficacy and *in vitro* experiments demonstrated that IgA Abs were able to partially block the IgG-mediated ADCC activity [16].

HVTN 205 tested the safety and immunogenicity of the recombinant MVA/HIV62 vaccine from GeoVax given alone or following an HIV DNA vaccine priming regimen [17]. GeoVax's prime/boost vaccine regimens present native, fusion-competent Env trimers displayed on virus-like particles (VLPs) to the immune system. As expected from an earlier trial testing the same products [18], the vaccine regimens were safe and immunogenic. T-cell responses were induced by both vaccine regimens, with enhanced CD4+ T cells seen when DNA and MVA were given sequentially (DDMM). In contrast, the magnitude of Ab responses appeared to be enhanced in the individuals receiving 3 vaccinations of MVA alone (MMM); however, this effect was likely due to the extra dose of MVA/HIV62B in addition to the additional spacing of the 2nd and 3rd MVA/HIV62B doses (4 months apart rather than 2 months apart as in the DDMM regimen). More recently, the Ab responses in HVTN 205 have been compared to those induced by the RV144 vaccine regimen, especially with respect to the immune correlates of risk seen in the latter trial. Although Ab in 11% of participants in the DDMM arm and 13% in the MMM arm were seen to bind to the gp70_B.case A _V1V2 loop, the primary inverse correlate of risk identified in the RV144 trial, this was much lower than the 64% of vaccinees showing Ab binding to gp70_B.case A _V1V2 in RV144. Env-specific IgA responses were seen in the plasma of just 12% of the DDMM and 15% of the MMM recipients in HVTN 205, whereas Env-specific IgA responses were observed in 78% of the participants in RV144.

Another difference is, while the RV144 vaccine regimen induced Ab responses restricted to gp120 (gp41 antigen was not included), both the DDMM and MMM regimens in HVTN 205 induced Ab that included gp120 and gp41, but were predominantly focused on gp41.

Note that in HVTN 505, a multiclade DNA/rAd5 vaccine regimen that lacked vaccine efficacy [19], the dominant antibody response was to gp41 Env, as was seen in HVTN 205 [19] (Williams, Tomaras, Haynes, Submitted 2015). However, in contrast to the HVTN 205 vaccine immunogen, an important immunodominant region (IDR) in gp41 was deleted (C-C loop) in the HVTN505 vaccine immunogen. Thus, HVTN505 did not elicit antibodies similar to those elicited by HVTN 205 that target the IDR epitope (Tomaras et al., in prep).

The substantial data demonstrating correlates of risk of HIV infection in the RV144 trial are the only guides that we have amongst the 4 vaccine products that have been tested for clinical efficacy [12,19-21]. While the vaccine regimens in RV144 and HVTN 205 are both immunogenic with Ab and T cells induced in the majority of participants [12,17], the specificity of the responses was clearly different. It is entirely possible that the HVTN 205 regimens could still be efficacious due to other mechanisms of protection. Nevertheless, it would be worthwhile to determine whether responses to gp120 and the V1/V2 region of gp120 could be boosted with a recombinant gp120 glycoprotein. A recombinant protein boost would be expected to enhance the magnitude of Ab responses to gp120 beyond those seen with DNA/MVA or MVA/MVA vaccine regimens alone. Ab responses would be expected to increase, broaden, and possibly include those found to correlate with reduced risk of infection in the RV144 trial. Such responses in addition to those against the dominant epitopes in gp41 could work in concert to enhance protection against HIV. Because AIDSVAX B/E, a bivalent HIV gp120 glycoprotein vaccine, is available for immediate use, we are able to explore these possibilities to better inform selection of vaccine regimens for future vaccine efficacy trials.

In this study, participants previously enrolled into HVTN 205 will be asked to participate in a follow-up study where they will receive another MVA/HIV62B boost, or a boost comprising MVA/HIV62B and AIDSVAX B/E, or AIDSVAX B/E alone. The last vaccination in HVTN 205 was given in January 2012, so these participants will have received their last vaccination around 4 years earlier. As detailed in the protocol schema (Table 3-1), eligible participants will be assigned to one of 5 study groups (20 in each group) depending on their previous vaccine regimen in HVTN 205. Participants previously vaccinated with HIV JS7 DNA as a 2 dose prime followed by a 2 dose MVA/HIV62 boost (DDMM) will either receive MVA/HIV62B alone, AIDSVAX B/E alone, or a combination of MVA/HIV62B and AIDSVAX B/E given concurrently. HVTN 205 participants who initially received 3 doses of MVA/HIV62 (without JS7 DNA priming) are more limited in number, and will be boosted with MVA/HIV62B alone or a combination of MVA/HIV62B and AIDSVAX B/E given concurrently. We will then compare immune responses among the groups at selected timepoints for up to 6 months following the boost regimen.

The primary reason for boosting with concurrent MVA/HIV62B and AIDSVAX B/E is the ability of MVA/HIV62B, but not gp120, to boost gp41 responses. The immunodominant region (IDR) of gp41 is a known target for such Fc-mediated mechanisms of protection as ADCC and virion capture [22,23]. In a preclinical macaque study, concurrent boosting of DNA-primed animals with MVA/HIV62B and a gp120 protein adjuvanted with alum successfully elicited binding Ab to both Con6 gp120 and to gp41. In addition, in the RV305 study, concurrent administration of a viral vector (ALVAC-HIV vCP1521) with AIDSVAX B/E protein elicited higher levels of binding Ab than either the ALVAC vector or AIDSVAX B/E administered alone [24]. In addition, administration of AIDSVAX B/E alone could potentially drive the Ab responses towards IgG4, a non-protective isotype [14,15].

Including groups with each vaccine given alone and with both administered concurrently will allow us to make several comparisons of interest. These include determining whether a concurrent recombinant protein boost given with MVA/HIV62B enhances the magnitude and breadth of the immune response compared to MVA/HIV62B alone. Giving the AIDSVAX B/E boost alone will allow us to determine if there is any difference in the quality of immune responses when compared to the other 2 boost regimens. Giving the MVA/HIV62B alone will allow us to determine the ability of a late

MVA boost, by itself, to enhance the magnitude and quality of the previously elicited immune response, as has been seen in other early phase HIV and malaria vaccine trials [25,26]. The number of participants in each group was determined based on statistical calculations that give a reasonable expectation of detecting differences between the vaccine boost regimens. It also took into consideration the likely availability of participants from HVTN 205 for a follow-up study.

4.2 MVA/HIV62B vaccine

MVA/HIV62B is a highly attenuated vaccinia virus which expresses Env from the HIV-1 primary isolate ADA, Gag sequences from the HXB-2 strain of HIV-1-IIIB, and PR and RT sequences from the BH10 strain of HIV-1-IIIB. The expressed sequences produce non-infectious virus like particles displaying trimeric membrane-bound Env. The MVA/HIV62B vaccine was constructed by introducing a Gag-Pol expression cassette into deletion III of MVA and an Env expression cassette into deletion II (Figure 4-2). The gag-pol gene was truncated so that most of the integrase coding sequences were removed, and amino acids 185, 266, and 478 were mutated to inactivate RT, inhibit strand transfer activity, and inhibit the RNase H activity, respectively. In the clade B CCR5 tropic envelope gene from ADA, TTTTTNT sequences were mutated without changing coding capacity to prevent premature transcription termination; and the cytoplasmic tail was truncated for its 115 C-terminal amino acids to improve surface expression, immunogenicity, and stability of the MVA vector. The Env expression cassette contains an upstream start codon that has the potential for expressing a 33 amino acid fusion protein comprised of 7 amino acid residues encoded by a multiple cloning site and the 26 C-terminal amino acids of Vpu. The upstream start codon attenuates the expression of Env. The sequences in the fusion protein have no matches in the genome database for the 7 amino acid sequence and its fusion outside of the known Vpu match.

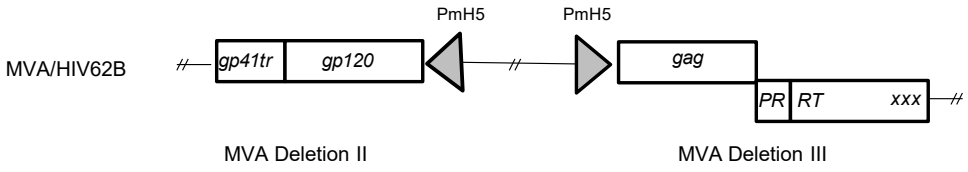


Figure 4-2 Schematic representation of MVA/HIV62B

The MVA/HIV62B was manufactured under cGMP conditions by IDT Biologika, Dessau-Rosslau, Germany, in specific pathogen-free chicken embryo fibroblasts (SPF CEF). This vaccine is the same as used in HVTN 205, but there were some differences in the manufacturing process. Specifically, for HVTN 205, the vaccine was manufactured by BioReliance, the growth in CEF was in roller bottles, and purification was by centrifugation through a sucrose pad. The MVA to be used in this study was produced by IDT, grown in wave bags, and purified by tangential flow filtration. The change from the roller bottle / ultracentrifuge process to the wave bag / tangential flow filtration process eliminated most animal-derived components, and is more consistent and scalable. Release tests for both vaccines were comparable. For additional information, see the Investigator's Brochure (IB).

4.3 AIDSVAX B/E vaccine

AIDSVAX B/E is a bivalent HIV gp120 glycoprotein originally developed and manufactured by Genentech Inc. The development and manufacturing rights were subsequently transferred to VaxGen, Inc. and finally transferred to its current developer, Global Solutions for Infectious Diseases (GSID). It is a purified mixture of gp120 proteins produced by recombinant DNA procedures using Chinese hamster ovary (CHO) cell expression. The sequences of MN gp120/HIV-1 and A244 gp120/HIV-1 are expressed as fusion proteins where a 27 amino acid sequence found in the gD protein of herpes simplex virus type 1 is fused to the amino terminus of each protein. MN and A244 rgp120/HIV-1 are combined to produce the bivalent AIDSVAX B/E vaccine. AIDSVAX B/E encompasses both subtype B (MN) and subtype E (A244) proteins that are adsorbed onto 600 mcg of aluminum hydroxide gel adjuvant.

4.4 Trial design rationale

The overall goal of this study is to determine whether an AIDSVAX B/E protein boost alone or in conjunction with MVA/HIV62B will broaden and increase Ab responses, including but not limited to responses directed against the V1V2 regions of Env as seen in the RV144 trial [13]. To properly test this hypothesis, we will test whether such Ab responses can be induced with the protein boost alone or whether the protein will need to be co-administered with MVA/HIV62B, compared to a boost of MVA/HIV62B alone. These three comparisons will be made in prior DDMM recipients. Ideally, an AIDSVAX B/E alone boost should also be given to prior MMM recipients as is planned for the DDMM recipients; however, only 75 participants received MMM in HVTN 205 compared with 150 who received DDMM. As such it would be difficult to engage 60 of 74 prior MMM recipients to participate in a follow-up study.

An important arm of the proposed study is the co-administration of MVA/HIV62B and AIDSVAX B/E, which, based on data from RV305, is expected to boost a higher magnitude of gp120 Ab compared to protein alone. Data from HVTN 094 (BB IND 14980) demonstrate continued boosting of Env Ab responses with a third MVA/HIV62B following DDMM, so evaluating an MVA/HIV62B-only boost seems reasonable. Furthermore, a plateauing of Ab responses has not been observed following 3 MVA/HIV62B doses, so it is reasonable to determine whether a fourth and fifth boost are able to enhance the magnitude of these responses. Because a single dose of AIDSVAX B/E protein boost may be insufficient to induce high magnitude V1V2 Ab responses, a second boost is also planned.

4.4.1 Dose (amount and number)

The doses selected for MVA/HIV62B and AIDSVAX B/E are based on prior studies by the HVTN and US Military HIV Research Program [12,17,18,27,28].

4.4.2 Schedule

Enrollment of HVTN 205 Part A was completed in May 2010 while Part B completed enrollment in July 2011. Therefore these participants will receive the first boosts around 4-5 years following their final vaccination in that earlier study. HVTN 088 demonstrated that Env-specific Ab responses could be boosted successfully in participants who received their final vaccine 5-17 years prior to the protein boost [29]. A second vaccine

administration is planned at 4 months, an interval based on experience in previous HVTN studies.

4.5 Plans for future product development and testing

Findings from this study are expected to help the HVTN determine whether to move forward to an efficacy study with the GeoVax DDMM or MMM regimens either alone, with an additional MVA/HIV62B boost, or in combination with a recombinant gp120 glycoprotein boost. Hence, the results from this study are expected to inform future product development.

4.6 Preclinical safety studies

The preclinical safety information for each vaccine is summarized below, and in the IB for each product.

4.6.1 Preclinical safety studies of MVA/HIV62B

Preclinical safety studies for MVA/HIV62B are summarized in Table 4-2. For additional information, please see the IB.

Table 4-2 Summary of preclinical safety studies

Study	Vaccine/Control	Animal species	Number of animals/group	Schedule	Laboratory performing study
Safety Pharmacology Study (M11, non-GLP)	SIV239 prototypes of GEO-D03/MVA/HIV62B, JS7/MVA/HIV62B, MVA/HIV62B alone, and Control (no vaccine)	Rhesus macaques (male)	8 DDMM arm 7 DgDgMM arm (1 died) 8 MMM arm 9 Control	DNA Week 0, 8, MVA Week 16, 24 Or MVA at weeks 0, 8, 24	Yerkes National Primate Research Center, Atlanta, GA
9-Week IM Toxicity Study with 2-Week Recovery	MVA/HIV62B 1 × 10^8 pfu & Control	New Zealand White Rabbits	20 (10/sex) MVA/HIV62B 20 (10/sex) Control	Days 1, 22, 43 & 64	Gene Logic, Gaithersburg, MD
13-Week IM Toxicity Study with 2-Week Recovery	JS7 2.9 mg, MVA/HIV62B 1 × $10^{8.3}$ $TCID_{50}$ & Control	New Zealand White Rabbits	20 (10/sex) 20 (10/sex) Control	JS7 days 1, 22, then MVA/HIV62B days 43, 64 & 85	Gene Logic, Gaithersburg, MD

4.6.2 Preclinical safety of AIDSVAX B/E

Data obtained for AIDSVAX IIIB and AIDSVAX MN established the preclinical safety for rgp120/HIV-1 molecules. The acute toxicity and local tolerance of the AIDSVAX MN formulation were evaluated in male guinea pigs treated with single intramuscular (IM) doses of up to 300 mcg per dose (~750 mcg/kg), which were well tolerated. A 6-month chronic study in rats given seven IM doses (approximately every 4 weeks) of either 300 mcg or 600 mcg of AIDSVAX MN showed no adverse events (AEs) attributable to the vaccine. For additional information, please see the IB.

4.7 Preclinical challenge studies

Table 4-3 Summary of preclinical immunogenicity studies

Study	Product	Animal	N	Regimen	Route	Schedule
M11	SIV239 DNA, 3 mg (D), SIV239 MVA, 1×10^8 pfu (M)	Male rhesus	25	DDMM (8) MMM (8) Placebo (9)	Intra-muscular	DNA month 0,2 MVA months 4 and 6 MVA months 0,2,6
M13	SIV239 MVA, 1×10^8 pfu (M)	Male rhesus	14	MMM (6) Placebo 8	Intra-muscular	MVA months 0,2,6
P165	SIV239 DNA, 3 mg (D), SIV239 MVA, 1×10^8 pfu (M)	Male rhesus	25	DDMM (20) Placebo (20)	Intra-muscular	DNA month 0,2; MVA months 4 and 8

4.7.1 M11 study

The M11 study tested the ability of SIV239 DDMM and MMM regimens to protect against 12 repeated rectal challenges with the heterologous SIVsmE660 [30]. All but one control (8/9) animal became infected by the first 4 challenges, which infected only 6 of the 16 DDMM and MMM animals. As the challenges continued, the remaining control monkey became infected and the DDMM and MMM animals continued to accrue infections. At 12 challenges, 2/8 DDMM and 2/8 MMM animals remained uninfected. Both regimens elicited similar 61-64% reductions in the per challenge risk of SIVsmE660 transmission and provided some protection against infection with 25% of the animals in each group resisting the 12 challenges (Figure 4-3). In contrast, 9/9 of the unvaccinated controls became infected. No differences in peak viremia or areas under the curve for plasma viral load for 1 to 24 weeks post infection were noted. Thus, the vaccines provided protection against SIVsmE660 infection, but did not lead to control of postchallenge viremia.

The vaccinations elicited Env-specific Ab, CD4+ and CD8+ T cell responses in all animals. The DDMM regimen elicited higher magnitudes of CD4+ T cells whereas the MMM regimen elicited higher titers and greater avidity Env-specific IgG and higher titer SIV-specific IgA in rectal secretions. Both regimens elicited similar magnitudes of CD8+ T-cell responses. Magnitudes of T-cell responses, specific activities of rectal IgA, and the tested specificities for neutralizing Ab and ADCC did not correlate with risk of infection.

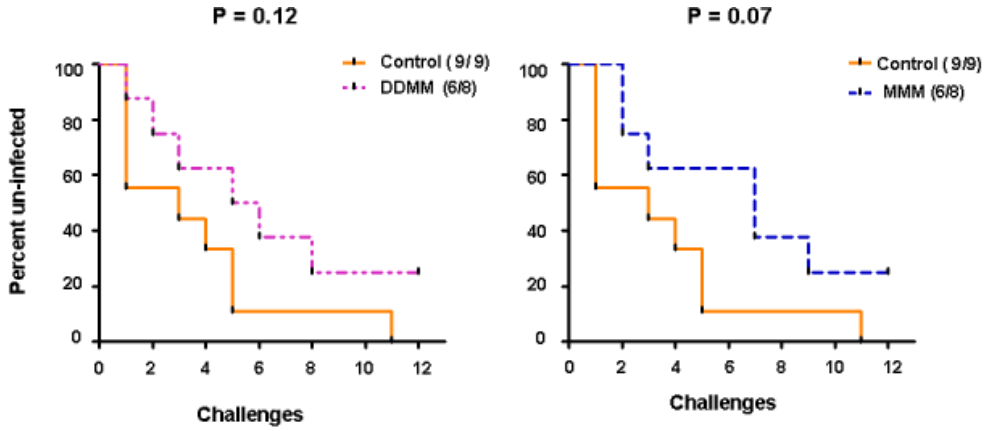


Figure 4-3 Kaplan-Meier curve for uninfected status of macaques in M11 during a weekly intrarectal challenge with 11 MID40 of SIV E660 viral RNA

4.7.2 M13 study

The preclinical study M13 further studied the protective potential of the SIV239 MMM regimen against an SIVsmE660 challenge with an additional 6 male rhesus macaques receiving the MMM regimen and an additional 8 animals serving as unvaccinated controls (manuscript in preparation). Figure 4-4 summarizes the challenge results for M11 and M13 showing protection for the MMM animals from both trials combined (upper panels) and the results according to the TRIM5α restriction phenotype (lower panels). In the total group, 6 of 14 animals were protected against infection as opposed to 0 of 17 controls. Five out of the six animals with aTRIM5α restrictive phenotype were protected, whereas only 1 of 8 with a permissive genotype was protected. The median number of challenges to infection for all vaccinated animals was 8, and for all control animals 2.5 ($p = 0.004$, Gehan-Breslow-Wilcoxon test). For TRIM5α permissive animals the median number of challenges to infection was 3.5 for vaccinated animals and 2.0 for control animals ($p = 0.07$). The results of M13 again showed partial protection for the MMM vaccinated animals with this protection being enhanced by the presence of theTRIM5α restriction (*TFP/TFP* or *TFP/CYPA* genotypes).

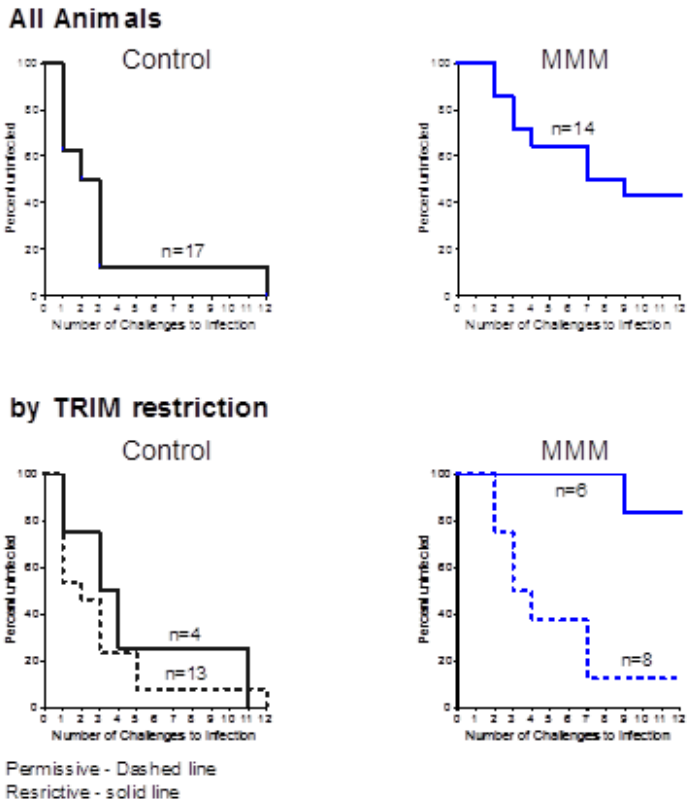


Figure 4-4 Kaplan-Meier curve for uninfected status of macaques in M11 and M13 during a weekly intrarectal challenge with SIVsm E660 viral RNA. Survival data are for all animals in the upper panels and by TRIM5α restriction in the lower panels.

4.7.3 P165 study

Preclinical Trial P165 conducted by the NIH Simian Vaccine Evaluation Unit further tested the protective potential of DNA priming and MVA boosting using a longer rest (4 months) between the MVA boosts than had been used in M11, such that the vaccination

schedule was DNA at months 0, 2 and MVA at months 4 and 8 (work in progress). This trial included 20 rhesus macaques in the DDMM and control groups. Results were also analyzed in the context of the TRIM5α genotype (Figure 4-5). The results of this trial showed the DDMM regimen providing partial protection with the one TRIM5α-restrictive animal resisting the 12 challenges. In terms of all animals by treatment group, 3/20 of the vaccinated animals resisted the 12 infections whereas 2 of 20 of the unvaccinated controls resisted all infections. Infection, however, was delayed in the vaccinated animals. Considering all animals, the median number of challenges to infection was 5.5 for the vaccinated group and 2.0 for the control group (p = 0.03, Gehan-Breslow-Wilcoxon test). For the TRIM5α permissive animals, the median time to infection was 5 for the vaccinated group and 1.5 for the control group (p = 0.004).

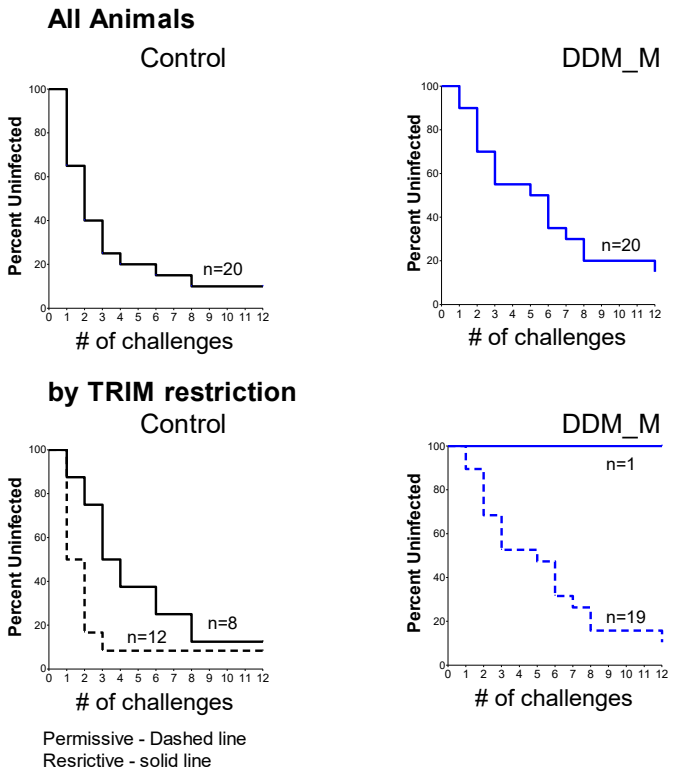


Figure 4-5 Kaplan-Meier curve for uninfected status of macaques in P165 during a weekly intrarectal challenge with SIVsm E660 viral RNA. Survival data are for all animals in the upper panels and by TRIM5α restriction in the lower panels.

Thus both DNA priming and boosting and MVA priming and boosting with SIV239 immunogens have shown partial protection against serial rectal challenges with SIVsmE660 in two independent experiments, with this protection being enhanced by the presence of the TRIM5α restriction.

4.8 Clinical studies

There is extensive published clinical experience with the MVA/HIV62B and AIDSVAX B/E vaccines given separately. The combination of MVA/HIV62B and AIDSVAX B/E vaccines given at the same time has not been tested in humans. However AIDSVAX B/E

vaccine has been given at the same time with another poxvirus vaccine, ALVAC-HIV (vCP1521), in HVTN 097 and RV144. In those studies, the poxvirus and protein vaccines were given in separate deltoids, as proposed here.

4.8.1 Clinical studies of MVA/HIV62B

The MVA/HIV62B vaccine has been evaluated in 345 normal healthy HIV-uninfected adults in 3 studies: HVTN 065, HVTN 205, and HVTN 094. These studies included regimens of MVA/HIV62B alone, or primed with JS7 DNA or GEO-D03 DNA. See the IB for additional clinical safety information.

JS7 DNA is a 9.5 kb plasmid DNA expressing the HIV-1 proteins Gag, PR, RT, Env, Tat, Rev, and Vpu, from a single transcript. GEO-D03 DNA is a 9.9 kb plasmid that is identical to JS7 plasmid DNA vaccine except that GEO-D03 also encodes human GM-CSF in a 435 base pair open reading frame in the position of a deleted *nef* sequence.

Table 4-4 Clinical Trials of MVA/HIV62B in HIV-uninfected healthy subjects

Study	Type of study	Product	MVA recipients	Regimens (N)	Schedule
HVTN 065 [18]	Phase 1	JS7 DNA, (D) MVA 62B (M)	95	DDMM, 0.3 mg DNA and 1×10^7 $TCID_{50}$ MVA (10)	DNA Month 0, 2 MVA Month 4, 6
				DDMM, 3 mg DNA and 1×10^8 $TCID_{50}$ MVA (30)	DNA Month 0, 2 MVA Month 2, 6
				DMM, 3 mg DNA and 1×10^8 $TCID_{50}$ MVA (30)	DNA Month 0 MVA Month 2, 6
				MMM, 1×10^8 $TCID_{50}$ MVA (30)	MVA Month 0, 2, 6
				Placebo (20)	
HVTN 205 [17]	Phase 2a	JS7 DNA (D) MVA 62B (M)	213	DDMM, 3 mg DNA and 1×10^8 $TCID_{50}$ MVA (150)	DNA Month 0, 2 MVA Month 4, 6
				MMM, 1×10^8 $TCID_{50}$ MVA (75)	MVA Month 0, 2, 6
				Placebo (75)	
HVTN 094	Phase 1	GEO-D03 (Dg) MVA 62B (M)	37	DgDgMMM, 0.3 GEO-D03 DNA and 1×10^8 $TCID_{50}$ MVA (10)	DNA Month 0, 2 MVA Month 4, 6, 8
				DgDgMMM, 3 mg GEO-D03 and 1×10^8 $TCID_{50}$ MVA (15)	DNA Month 0, 2 MVA Month 4, 6, 10
				DgDgMM, 3 mg GEO-D03 and 1×10^8 $TCID_{50}$ MVA (15)	DNA Month 0, 2 MVA Month 4, 8

The vaccines were safe and well tolerated at the doses and schedules tested. MVA was associated with mild or moderate local reactogenicity in most participants.

4.8.1.1 Clinical safety experience

In HVTN 065 and HVTN 094, there were no reports of severe local (injection site) reactogenicity. In HVTN 205, 2 participants (1 DDMM, 1 MMM) experienced severe pain and/or tenderness at the injection site, and 4 participants (3 DDMM, 1 MMM) experienced severe systemic reactogenicity symptoms such as malaise and/or fatigue, chills, or fever. In HVTN 094, two participants reported severe malaise and/or fatigue and one participant reported severe headache.

One participant in HVTN 065 developed chest tightness and dyspnea 30 min after vaccination with MVA/HIV62B, which was considered a possible allergic reaction, probably related to vaccination. In HVTN 205, 1 participant experienced an allergic reaction ≤ 15 minutes after the second MVA/HIV62B vaccination, which was considered definitely related to the vaccine. The symptoms resolved within 2 hours.

One individual in HVTN 065 experienced a moderate decrease in neutrophils 14 days following the first DNA vaccination. One person in HVTN 094 experienced a mild decrease in neutrophils 3 days after the first GEO-D03 vaccination. One person in HVTN 205 experienced a severe decrease in lymphocyte count 1 day after receiving MVA/HIV62B. Other AEs related to vaccination were such injection site symptoms such as pain, pruritus, swelling, induration, hematoma/bruise or paresthesia; axillary lymphadenopathy or axillary tenderness, dizziness or lightheadedness, nasal congestion, tremor and dyspnea. All of these AEs resolved without complications. In this study, participants will have already received 2 or 3 injections of MVA/HIV62B previously in HVTN 205.

4.8.1.2 Immunogenicity of MVA/HIV62B

Studies on the immune responses elicited by the MMM and DDMM regimens in HVTN 065 and 205 have been published [17,18]. Similar patterns of responses were observed in both trials with the results of the larger phase 2a trial, which was also analyzed in more detail, being recapped here.

Figure 4-6 shows response rates in HVTN 205 for CD4+ and CD8+ T cells and for IgG and IgA Ab to gp140. The data show the DDMM regimen eliciting higher response rates for CD4+ T cells than the MMM regimen ($p = 0.05$). This was also seen in HVTN 065. The data show both regimens eliciting high response rates for binding Ab to gp140, with IgG binding Ab being present in 93% of DDMM and 95% of MMM participants. In contrast to the high response rates for binding Ab for IgG, low response rates for gp140 binding Ab for IgA were induced. Only 12 to 13% of participants mounted a detectable serum IgA response to gp140.

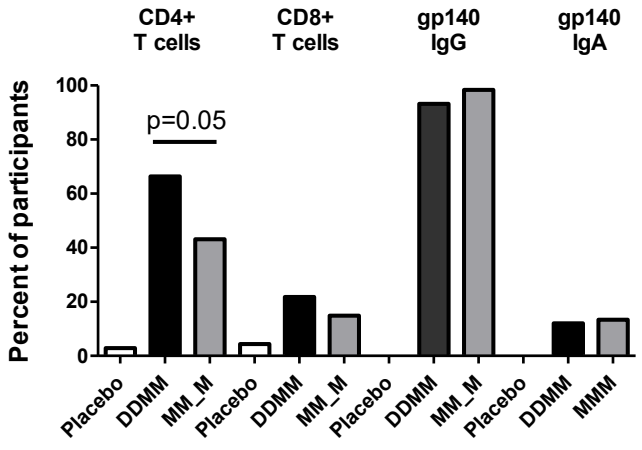


Figure 4-6 Response rates for T cells and Ab in HVTN 205. Study groups are below the graph and the measured characteristic above the graph. T-cell data were obtained using intracellular cytokine staining (ICS) and Ab data using the binding antibody multiplex assays (BAMA) at the HVTN Fred Hutchinson and Duke Laboratories respectively

Analyses for the specificity of the IgG binding Ab response revealed that the MMM regimen had a higher response rate for gp120 than the DDMM regimen (70% vs 47%, respectively; p = 0.004) and that the responses for both regimens were gp41-biased (response rates of 93% vs 98%, respectively) [18]. In both groups, the magnitude of the gp41 response was much higher (~20x higher) than the magnitude of the gp120 response. The IgG responses predominantly comprised IgG1 and IgG3 with very limited IgG2 and IgG4 (data not shown).

Serum IgA responses to gp140 were present not only at low rates (Figure 4-6) but also at low titers. These responses again showed a gp41 bias, being detectable for gp120 only in the MMM group. The median magnitudes of IgA binding activity for gp41 were >20-times lower than the median magnitudes of the IgG binding activity.

Response rates for gp140 declined less than 20% between 2 and 24 weeks after the final MVA boost. Ongoing studies on the durability of different specificities of the responses show that Ab to gp41 and to the IDR of gp41 are much more durable than Ab to gp120 with the former undergoing < 3-fold drops in magnitude between 2 weeks and 24 weeks post the last MVA boost. In contrast, Ab to gp120 drops by about 10-fold in the same period of time.

HVTN 094 demonstrated high response rates for ADCC activity in bound gp120 (Figure 4-7) and bound virion (Figure 4-8), but not the infected cell assay (G. Ferrari, data not shown). HVTN 094 tested the effect of a 3rd MVA boost for a DNA prime that co-expressed VLPs and GM-CSF. The magnitude of ADCC responses was enhanced by the third MVA boost, suggesting the importance of delivering three MVA inoculations. The 3rd MVA boost was undertaken because the 3-dose MMM regimen had consistently elicited higher Ab responses than the DDMM regimen, which includes only two MVA boosts.

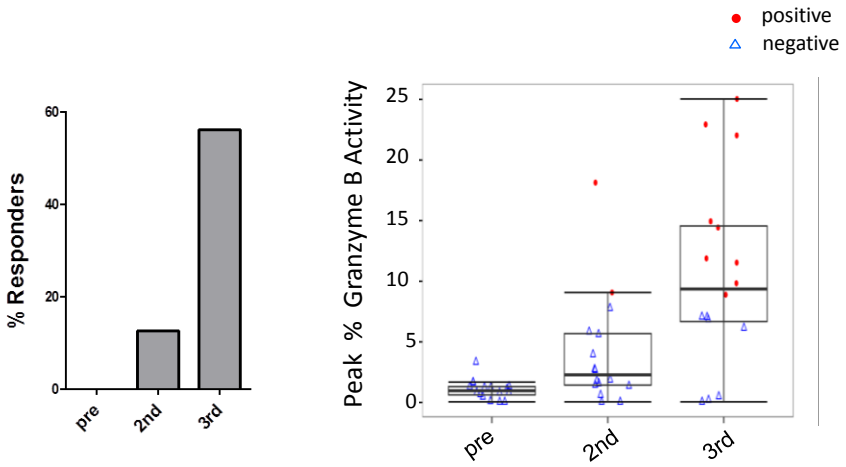


Figure 4-7 ADCC responses in a bound Bal gp120 assay for HVTN 094 at baseline and after 2nd and 3rd MVA boosts. The assay was conducted by Dr. Guido Ferrari at the Duke HVTN lab.

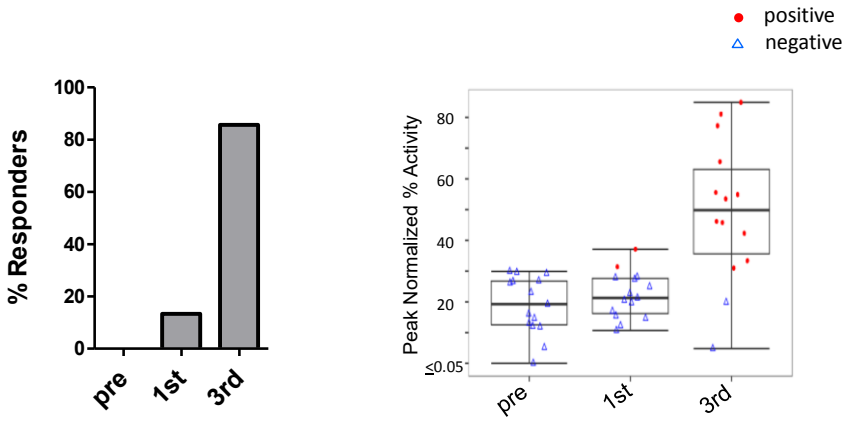


Figure 4-8 ADCC responses in a bound virion (Bal) assay for HVTN 094, at baseline and after 1st and 3rd MVA boosts. The assay was conducted by Dr. George Lewis at the Institute for Human Virology. The magnitudes of ADCC responses were not as high as for infected people (G. Lewis, personal communication).

In summary, MVA/HIV62B has shown good ability to elicit Ab and T cell responses that were still detectable in the majority of responders at 6 months when administered alone or following a DNA prime. The Ab responses have higher binding activity for gp41 than gp120 and much higher levels of serum IgG than serum IgA. The Ab has also had good functional characteristics, with high response rates for ADCC in bound Bal gp120 and bound virion assays and moderate neutralizing activity for laboratory-adapted strains of HIV and Tier 1A patient isolates. Ongoing studies in HVTN 094 indicate that three MVA boosts of a DNA prime are more effective than 2 MVA boosts of a DNA prime. They also indicate that a 3rd MVA dose effectively boosts a 2nd MVA dose and thus justify testing additional MVA doses in the multiple dose MVA/HIV62B regimen.

4.8.2 Clinical studies of AIDSVAX B/E

The AIDSVAX B/E vaccine has been evaluated in more than 10,000 normal healthy HIV-uninfected adults (Table 4-5).

Table 4-5 Selected clinical trials of AIDSVAX B/E in HIV-uninfected healthy subjects

Study number	Product	Type of study	Location	N	Vaccine to Placebo	Dose groups	Route	Schedule
VAX 001 [27]	AIDSVAX B/E	Phase 1/2	Thailand	92 healthy low-risk or IDU	Open label vaccine	100, 300, or 600 mcg of each antigen	IM	Months 0, 1, 6, and 12
VAX 002	AIDSVAX B/B, AIDSVAX B/E	Phase 1/2	US	122 healthy low-risk	Open label	100, 300, or 600 mcg AIDSVAX B/B, or 300 mcg AIDSVAX B/E of each antigen	IM	Months 0, 1, 6, and 12
RV 135 [28]	ALVAC-HIV vCP1521; AIDSVAX B/E	Phase 1/2	Thailand	133	3:1	ALVAC alone, $10^{6.5}$ $TCID^{50}$; ALVAC-HIV and AIDSVAX B/E 100 mcg; ALVAC-HIV and AIDSVAX B/E 300 mcg of each antigen	IM	ALVAC-HIV at weeks 0, 4, 12 and 24 and AIDSVAX B/E at weeks 12 and 24;
VAX 003 [20]	AIDSVAX B/E	Phase 3	Thailand	2546IDU	1:1	AIDSVAX B/E 300 mcg of each antigen	IM	Months 0, 1, 6, 12, 18, 24, and 36
RV144 [12]	ALVAC-HIV vCP1521; AIDSVAX B/E	Phase 3	Thailand	16402	1:1	ALVAC-HIV (vCP1521) 10^6 $CCID^{50}$ and AIDSVAX B/E 300 mcg of each antigen	IM	ALVAC-HIV at weeks 0, 4, 12 and 24 and AIDSVAX B/E at weeks 12 and 24
HVTN 097	ALVAC-HIV vCP1521; AIDSVAX B/E; tetanus toxoid vaccine; HBV vaccine	Phase 1b	South Africa	100	4:1	ALVAC-HIV (vCP1521) $5.2x10^7$ $CCID^{50}$ and AIDSVAX B/E 300 mcg of each antigen	IM	ALVAC at Month 1, 2, 4 7 and AIDSVAX B/E at Month 4, 7
RV 328	AIDSVAX B/E	Phase 1/2	Thailand	40	3:1	AIDSVAX B/E 300 mcg of each antigen	IM	Months 0, 1, 6, and 12

In VAX001, AIDSVAX B/E vaccine was reported as being well tolerated. Seventy-four volunteers (80.4%) reported at least 1 reactogenicity event. The most common symptoms reported were pain and tenderness at injection site (75%), followed by malaise (25%), myalgia (21.7%), fever (9.8%), and local erythema (8.7%). All local and systemic reactogenicity symptoms were mild to moderate in nature and self-limited and resolved within 14 days of onset [27].

In a phase 1/2 study of ALVAC HIV vCP1521 and AIDSVAX B/E in Thailand, immunizations were well tolerated. The majority of AEs were mild to moderate in intensity. Four AEs were considered to be vaccine related: there were 2 cases of myalgia and 1 case of pruritus, and 1 volunteer had an erythematous rash. No serious adverse events (SAE) were related to vaccination [28].

In the VAX003 efficacy trial in Thai injection drug users, tenderness at the injection site was the most common symptom, reported by 902 (71.0%) of vaccine recipients and 830 (65.7%) of placebo recipients. It did not increase with multiple injections. There were no differences between vaccine and placebo recipients in SAEs or deaths [20].

In the RV144 trial, local reactions occurred in 88.0% of vaccine and 61.0% of placebo recipients ($p<0.001$) and were more frequent after ALVAC-HIV than AIDSVAX B/E vaccination. Local and systemic reactions were mostly mild to moderate, resolving within 3 days. The frequency of serious AEs was similar in vaccine (14.3%) and placebo (14.9%) recipients ($p = 0.33$). None of the 160 deaths (85 in vaccine and 75 in placebo recipients, $p = 0.43$) was assessed as related to vaccine [12].

In HVTN 097, 1/78 (1%) of vaccine recipients reported severe local symptoms related to AIDSVAX B/E. For the ALVAC-HIV and AIDSVAX B/E regimen overall, 16% of vaccine recipients reported no local pain or tenderness, 48% reported mild, 28% reported moderate, and 9% reported severe local symptoms. Severe systemic reactogenicity symptoms due to the combined vaccine regimen have been reported by 4/80 (5%) vaccine recipients, and have included myalgia, arthralgia, and headache, compared to 1/20 (5%) placebo recipients who also reported a severe headache. No SAEs have been related to vaccination.

In RV328 an SAE was reported that was deemed related to AIDSVAX. A participant developed a case of severe hypersensitivity reaction after receiving the 4th and final dose of AIDSVAX B/E. Within 3 minutes of receiving the vaccine, the participant developed a generalized pruritic rash, facial swelling, and malaise, but had no difficulty breathing or hypotension. This individual had received 3 prior vaccinations with AIDSVAX B/E with no concomitant symptoms. The participant was treated and recovered completely within a few hours.

4.8.3 Immunogenicity of AIDSVAX alone

The addition of envelope glycoproteins from different strains of HIV-1 was found to expand the breadth of the immune response. In the Thai study, VAX 001, 100% seroconversion to both MN rgp120 and A244 rgp120 was observed in 3 different dose groups at months 1.5, 6.5, 12.5, and 18. Percentage seroconversion to each V2 peptide was high, with 100% of participants in VAX001 seroconverted to neutralizing antibodies to homologous MN HIV-1 isolates at month 6.5, month 12.5 and at month18. Similarly, in VAX002, a phase 1/2 study in the US, 100% seroconversion to MN rgp120 was observed at the same timepoints as seen in VAX001. In the phase 3 VAX 003 study, both antigens were well tolerated and immunogenic, producing both MN neutralizing and CD4 binding Abs. Of the volunteers tested in VAX003, 100% had an immune response, with substantial increases in Ab titers after each dose given (see AIDSVAX IB).

4.8.4 Clinical studies of the proposed product combination

No previous clinical studies have been done of AIDSVAX B/E as a boost following MVA/HIV62B, or of the MVA/HIV62B and AIDSVAX B/E combination proposed for Groups 2 and 4. However the extensive experience with AIDSVAX overall, and more recently clinical experience with the combination of AIDSVAX B/E with ALVAC-HIV (vCP1521) in RV144 and HVTN 097, indicate an acceptable safety profile for these products.

4.9 Potential risks of study products and administration

Table 4-6 summarizes the potential risks associated with administration of the study products.

Table 4-6 Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain, tenderness, erythema, or swelling/induration/edema • Malaise/fatigue, myalgia, or headache in the first few days following injection • A vaccine-induced positive HIV Ab test result • Axillary lymph node swelling, pain, or tenderness
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, nausea, vomiting, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure/tremor • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, other transient lesions, itching, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis • Muscle damage at the injection site
Unknown frequency	• Myo/pericarditis
Theoretical risks	• Autoimmune disease • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV, if the participant is exposed to HIV • Effects on the course of HIV infection/disease, if the participant is infected with HIV • Effects on the fetus and on pregnancy

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Primary objective 1:

To evaluate the safety and tolerability of MVA/HIV62B and AIDSVAX B/E given separately or together as boost injections after prolonged immunologic rest, to participants who received MVA/HIV62B vaccinations in DDMM and MMM regimens in HVTN 205

Primary endpoint 1:

Frequency and severity of local and systemic injection site reactogenicity signs and symptoms, for each boost regimen

Laboratory measures of safety: white blood cells (WBC), neutrophils, lymphocytes, hemoglobin, platelets, ALT, AST, alkaline phosphatase, and creatinine

Frequency of AEs categorized by MedDRA System Organ Class, and MedDRA Preferred Term, severity and assessed relationship to study products; detailed description of all AE meeting DAIDS criteria for expedited reporting

Number of participants with early discontinuation of vaccinations and reason for discontinuation

Primary objective 2:

To compare HIV-specific antibody responses elicited by MVA/HIV62B and AIDSVAX B/E given separately or together as boost injections after prolonged immunologic rest

Primary endpoint 2:

Frequency, magnitudes and isotypes of Env-specific Ab responses for gp120, gp41, V1/V2, and the IDR of gp41 at 2 weeks after each boost

Neutralizing Ab titers and breadth against the Env vaccine strain (ADA) and heterologous tier 1 and tier 2 strains at 2 weeks after each boost

5.2 Secondary objectives and endpoints

Secondary objective 1:

To compare HIV-specific T-cell responses to MVA/HIV62B and AIDSVAX B/E given separately or together as boost injections

Secondary endpoint 1:

Frequency and magnitudes of HIV-1 specific CD4+ and CD8+ T-cell responses as measured by ICS at two weeks after each boost

Secondary objective 2:

To compare durability of HIV-specific Ab and T-cell responses

Secondary endpoint 2:

Frequency, magnitudes and isotypes of Env-specific Ab responses to gp120, gp41, V1/V2, and the IDR of gp41 at 6 months after the final boost

Frequency and magnitudes of HIV-1 specific CD4+ and CD8+ T-cell responses as measured by ICS at 6 months after the final boost

5.3 Exploratory objectives

Exploratory objective 1:

To evaluate ADCC responses

Exploratory objective 2

To evaluate antibody avidity of Env-specific Ab for V2, V3, and IDR of gp41

Exploratory objective 3:

To assess the elicitation of mucosal Ab responses in semen [men], cervicovaginal secretions [women], and rectal secretions

Exploratory objective 4:

To compare durability of DDMM and MMM elicited Ab and T cell responses from HVTN 205 immediately prior to the first boost vaccination in this study

Exploratory objective 5:

To further evaluate the immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed on blood and mucosal samples, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 100 healthy, HIV-uninfected participants previously enrolled into HVTN 205. Eligible participants will be assigned into one of 5 study groups (20 in each group) depending on their previous vaccine regimen received in HVTN 205. Participants previously vaccinated with DDMM will be randomized to receive MVA/HIV62B alone, AIDSVAX B/E alone, or a combination of MVA/HIV62B and AIDSVAX B/E given concurrently. HVTN 205 participants initially vaccinated with MMM will be randomized to be boosted with MVA/HIV62B alone or a combination of MVA/HIV62B and AIDSVAX B/E given concurrently.

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, low cell viability of processed peripheral blood mononuclear cells (PBMCs), or high background. Immunogenicity data from 17 phase 1 and 2 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), indicate that 10% is a reasonable estimate for the rate of missing data. For this reason, the sample size calculations in Section 6.1.2 account for 10% enrolled participants having missing data for the primary immunogenicity endpoints. To maximize the power of evaluating boosting regimens, Groups 1 and 3 with MVA/HIV62B-only boost and Groups 2 and 4 with MVA/HIV62B + AIDSVAX B/E boost will be combined in the primary analyses accounting for the prior priming regimens, in addition to analyses by each individual group. Interaction tests will be used to evaluate whether the effect of boosting regimens differs by the prior prime regimens. However, the study has low power to detect the interaction; therefore we will treat these interaction analyses as exploratory.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect SAEs (Section 9) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine group of the study (n = 20), there is a 90% chance of observing at least 1 event if the true rate of such an event is 10.9% or more; and there is a 90% chance of observing no events if the true rate is 0.5% or less. For vaccine groups 1 and 3 combined and groups 2 and 4 combined (n = 40), there is a 90% chance of observing at least 1 event if the true rate of such an event is 5.6% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less. As a reference, in HVTN vaccine trials from December 2000 through April 2014, about 4% of participants who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events among groups of size 20 and 40 are presented in Table 6-1 for a range of possible true AE rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among groups of size 20 and 40, for different true event rates

True event rate (%)	Pr(0/20)	Pr(1+/20)	Pr(2+/20)	Pr(0/40)	Pr(1+/40)	Pr(2+/40)
1	81.8	18.2	1.7	66.9	33.1	6.1
3	54.4	45.6	12	29.6	70.4	33.8
5	35.8	64.2	26.4	12.9	87.1	60.1
7	23.4	76.6	41.3	5.5	94.5	78
9	15.2	84.8	54.8	2.3	97.7	88.6
10	12.2	87.8	60.8	1.5	98.5	92
20	1.2	98.8	93.1	< 0.1	> 99.9	99.9
30	0.1	99.9	99.2	< 0.1	> 99.9	> 99.9
40	< 0.1	> 99.9	99.9	< 0.1	> 99.9	> 99.9

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval (CI) for the true rate of an AE based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method [31]. If none of the 40 participants receiving a MVA/HIV62B only boost (groups 1 and 3 combined) or MVA/HIV62B + AIDSVAX B/E boost (groups 2 and 4 combined) experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events is 8.8%. For each individual vaccine group (n = 20), the 2-sided upper confidence bound for this rate is 16.1%.

Table 6-2 Two-sided 95% CI based on observing a particular rate of safety endpoints for groups of size 20 and 40

Observed event rate	95% CI (%)
0/20	[0, 16.1]
1/20	[0.9, 23.6]
2/20	[2.8, 30.1]
0/40	[0, 8.8]
1/40	[0.4, 12.9]
2/40	[1.4, 16.5]

6.1.2 Sample size calculations for immunogenicity

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of Ab response rates and magnitudes among participants who received MVA/HIV62B and AIDSVAX B/E alone or together as boost after prolonged immunologic rest. No adjustment for multiple comparisons will be made for the use of multiple assays. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-3. Calculations are done using the score test method [31]. The n = 18 and 36 assume a 10% loss of data.

Table 6-3 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the individual (n=18) or combined groups (n = 36)

No. of responses	Observed response rate (%)	CI
	n = 18	
1/18	5.6	[1, 25.8]
3/18	16.7	[5.8, 39.2]
5/18	27.8	[12.5, 50.9]
7/18	38.9	[20.3, 61.4]
9/18	50	[29, 71]
11/18	61.1	[38.6, 79.7]
13/18	72.2	[49.1, 87.5]
15/18	83.3	[60.8, 94.2]
17/18	94.4	[74.2, 99]
	n = 36	
3/36	8.3	[2.9, 21.8]
5/36	13.9	[6.1, 28.7]
7/36	19.4	[9.8, 35]
11/36	30.6	[18, 46.9]
15/36	41.7	[27.1, 57.8]
19/36	52.8	[37, 68]
23/36	63.9	[47.6, 77.5]
27/36	75	[58.9, 86.2]
33/36	91.7	[78.2, 97.1]

As shown in Table 6-4, there is limited power for a formal comparison of immunogenicity response rates between groups of size n = 18 or combined groups of size 36. For either 80% or 90% power, the sizes of differences that the trial is powered to detect are fairly large. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05.

Table 6-4 Power for comparison of response rates between 2 groups (n1 = 18 and n2 = 18 or n1=36 and n2=36)

True response rate Group 1 (%)	Minimum true response rate in Group 2 in order to detect a difference	
	80% power	90% power
	n1 = 18, n2 = 18	
10	58	65
20	71	77
30	81	87
40	89	94
50	95	99
60	100	-
	n1 = 36, n2 = 36	
10	41	46
20	54	60
30	66	71
40	75	80
50	84	87
60	91	94
70	97	99

Table 6-5 shows the power for comparison of immune response magnitudes between 2 groups for detecting a range of effect sizes. Effect size is the difference of means per one unit of standard deviation (sd). A two-sided two-sample t test is used to compare immune responses between two groups assuming equal variances at significance level of 0.05. The actual difference in group means depends on the standard deviation. For example, the power of detecting effect size of 1 between 2 groups of size 18 is 83%. The actual difference of group means corresponding to this effect size will depend on the variation of the endpoints. The larger the variation, the larger difference of group means can be detected. So for assay with smaller variation sd = 0.2, there is 83% power to detect 0.2 difference in group means, while for assay with larger variation sd = 1.1, the same 83% power can only detect 1.1 difference in group means. For the primary immunogenicity endpoints, binding Ab and neutralizing Ab data, $\log_{10}$ transformation will be done before statistical testing. The HVTN 205 data indicate that the standard deviations of $\log_{10}$ IC_{50} titer, IgG and IgA binding Ab magnitudes from vaccinee groups range from 0.1 to 1.2 depending on antigens.

Table 6-5 Power for comparison of response magnitudes between 2 groups

	Power (%)			Difference in 2 group Means					
Effect Size	n1 = n2 = 18	n1 = n2 = 36	n1 = 18, n2 = 36	sd = 0.2	sd = 0.3	sd = 0.5	sd = 0.7	sd = 0.9	sd = 1.1
0.4	21	39	27	0.08	0.12	0.2	0.28	0.36	0.44
0.5	31	55	40	0.1	0.15	0.25	0.35	0.45	0.55
0.6	42	71	53	0.12	0.18	0.3	0.42	0.54	0.66
0.7	53	83	66	0.14	0.21	0.35	0.49	0.63	0.77
0.8	65	92	78	0.16	0.24	0.4	0.56	0.72	0.88
0.9	75	96	86	0.18	0.27	0.45	0.63	0.81	0.99
1	83	99	92	0.2	0.3	0.5	0.7	0.9	1.1
1.1	89	100	96	0.22	0.33	0.55	0.77	0.99	1.21
1.2	94	100	98	0.24	0.36	0.6	0.84	1.08	1.32

An alternative to formal superiority comparisons of groups is to rank the groups by their response rates to binding Ab assays and neutralizing Ab assays. Ranking will be performed separately for each of these assays. Table 6-6 shows various true response rates for which this study will correctly select the group with the highest response rate with 0.8 or 0.9 probabilities through ranking the groups by their response rates. Each line in the table shows the results based on 40,000 simulated datasets of response rates for 5 or 3 groups generated using 2 different binomial probabilities, with the best response probability used to generate data for one group and the second best response probability used to generate data for the remaining groups. The top panel is the results for ranking all 5 individual groups and the bottom 2 panels are for ranking 3 groups after combining groups 1 and 3, groups 2 and 4 (sizes = 18, 36, 36). The middle panel has the best group of size 18 and the very bottom panel has the best group of size 36. If the difference in response between the best and second best groups is smaller than the assumed difference, the chance of correctly selecting the group with the true highest response will be less than 80% (90%).

Table 6-6 True immunogenicity response rates for which the regimen with the highest response probability will be correctly selected with 0.8 (0.9) probability among 5 groups of size 18 or 3 groups of size 18, 36 and 36 (combined groups 1 and 3, groups 2 and 4)

Second best response probability	Best response probability	Difference
5 groups		
10%	29% (35%)	19% (25%)
20%	43% (49%)	23% (29%)
30%	54% (60%)	24% (30%)
40%	65% (71%)	25% (31%)
50%	74% (79%)	24% (29%)
60%	83% (87%)	23% (27%)
70%	90% (93%)	20% (23%)
80%	96% (99%)	16% (19%)
3 groups (best group =18)		
10%	23% (28%)	13% (18%)
20%	35% (41%)	15% (21%)
30%	46% (52%)	16% (22%)
40%	57% (62%)	17% (22%)
50%	66% (72%)	16% (22%)
60%	75% (80%)	15% (20%)
70%	84% (88%)	14% (18%)
80%	91% (94%)	11% (14%)
3 groups (best group =36)		
10%	21% (26%)	11% (16%)
20%	34% (39%)	14% (19%)
30%	45% (51%)	15% (21%)
40%	56% (61%)	16% (21%)
50%	66% (71%)	16% (21%)
60%	75% (80%)	15% (20%)
70%	84% (88%)	14% (18%)
80%	92% (95%)	12% (15%)

In case the trial cannot be fully enrolled due to unavailability of eligible participants, the power of the trial will be reduced. If 60% of targeted sample size is enrolled, which is n = 12 per group, there is a 90% chance of observing at least one safety event if the true rate is 17.5% (compared with 10.9% for n = 20) or more, and a 90% chance of observing no events if the true rate is 0.8% (compared with 0.5% for n = 20) or less. If no participant experiences a safety event, the 95% 2-sided upper confidence bound for the true rate is 24.2% (compared with 16.1% for n = 20). For the immunogenicity endpoints, assuming 10% loss of data, with n = 11 the trial will have 80% power to detect significant difference in response rates between 2 groups if the true rate in one group is 40% and the minimum true rate in the other group is 99% (compared with 89% for n = 18). For the magnitude responses, the power of detecting effect size of 1 between 2 groups of size 11 reduces to 61% from 83% when n = 18.

6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through a Web-based randomization system. The randomization will be stratified by the initial HVTN 205 vaccine regimens and be done in blocks to ensure balance across groups. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments.

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment group assignments. Study product assignments are accessible to those HVTN CRS pharmacists, DAIDS protocol pharmacists and contract monitors, and SDMC staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff is prohibited. The HVTN SMB members also are unblinded to treatment assignment in order to conduct review of trial safety.

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until all participants are unblinded to learn his or her treatment assignment.

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 114 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analyses

This section describes the final study analysis, unblinded as to treatment group assignment. In addition to analysis of each individual group, the primary analyses will be based on combined groups across different prior priming regimens to maximize the statistical power in the evaluation of the effect of boosting regimens. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety data. Analyses are modified

intent-to-treat in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

Analyses for primary endpoints will be performed using SAS and R. All other descriptive and inferential statistical analyses will be performed using SAS, StatXact, or R statistical software.

No formal multiple comparison adjustments will be employed for multiple safety endpoints, multiple primary immunogenicity endpoints, or secondary endpoints. However, multiplicity adjustments will be made for certain immunogenicity assays, as discussed below, when the assay endpoint is viewed as a collection of hypotheses (eg, testing multiple peptide pools to determine a positive response).

6.4.1 Analysis variables

The analysis variables consist of baseline participant characteristics, safety, and immunogenicity for primary- and secondary-objective analyses.

6.4.2 Baseline comparability

Treatment groups will be compared for baseline participant characteristics using descriptive statistics.

6.4.3 Safety/tolerability analysis

Since enrollment is concurrent with receiving the first vaccination, all participants will have received at least 1 vaccination and therefore will provide some safety data.

6.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment group and the percentages displayed graphically by group. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated. Kruskal-Wallis tests will be used to test for differences in severity between groups.

6.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment group the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing groups is not planned since interpretation of differences must rely heavily upon clinical judgment.

A listing of SAEs reported to the DAIDS Regulatory Support Center (RSC) Safety Office will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received.

6.4.3.3 Local laboratory values

Box plots of local laboratory values will be generated for baseline values and for values measured during the course of the study by treatment group and visit. Each box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

For each local laboratory measure, summary statistics will be presented by treatment group and timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the DAIDS AE Grading Table (Section 11.2.2) will be tabulated by treatment group for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.4.3.4 Reasons for vaccination discontinuation and early study termination

The number and percentage of participants who discontinue vaccination and who terminate the study early will be tabulated by reason and treatment group.

6.4.4 Immunogenicity analysis

6.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants postinfection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment group at each timepoint for which an assessment is performed. Response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method [31].

To compare the response rates of any 2 groups, a significant difference will be declared if the 2-sided p-value from either a (stratified when appropriate) Barnard's or Fisher's exact test is ≤ 0.05. To compare the response rates among more than 2 groups, first an overall test for any difference in response rate among all groups will be conducted, using a Freeman-Halton exact test (or a Chi-square test, depending on whether there are numbers less than 5 in a given cell of the contingency table). When all pair-wise comparisons between the multiple vaccine groups are of interest, a Tukey-like procedure for proportions as described in [32] will be used. Parametric regression models may also be used to adjust for covariates of interest. More details will be provided in the SAP.

For quantitative assay data (eg, IC_{50} titer from the neutralizing assay), graphical and tabular summaries of the distributions by antigen, treatment group, and timepoint will be made. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study groups. Typically the results will be shown for each vaccine group and for the combined groups.

To compare the response magnitude at a specific time-point between any 2 groups, a nonparametric (stratified, if appropriate) Wilcoxon rank sum test will be used if the data are not normally distributed and with a (stratified, if appropriate) 2-sample t-test if the data appear to be normally distributed. To test for magnitude differences at a specific time-point among more than 2 groups, first a Kruskal-Wallis rank test or an F-test (depending on the normality assumption) will be used to test for overall differences. Secondly, if the overall test is significant at the 2-sided 0.05 level, then individual tests comparing pairs of vaccine groups will be done unless pre-specified. When all pair-wise comparisons between the multiple vaccine groups are of interest, the Tukey procedure [32] will be used. Parametric regression models may also be used to adjust for covariates of interest. More details will be provided in the SAP. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity.

More sophisticated analyses employing repeated measures methodology (eg, linear mixed models or marginal mean models fit by generalized estimating equations) may be utilized to incorporate immune responses over several timepoints and to test for differences over time. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

Based upon previous HVTN trials, missing 10% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed PBMCs. To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right-

censored, then the linear mixed effects models of Hughes [33] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted generalized estimating equation [34] methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

6.4.4.2 Analyses of neutralization assay data

Primary analyses: The area-under-the-magnitude-breadth curve (AUC-MB) to a panel of isolates will be computed for each participant with evaluable neutralization data at 2 weeks post boost.

Secondary Analyses: The analyses of magnitude-breadth described above are based on the endpoint area-under-the-curve, which is interpreted as the average $\log_{10}$ IC50 to the set of isolates in the test panel. Use of this endpoint is maximally statistically powerful if one vaccine group has greater magnitude and breadth than the comparator vaccine group, but may miss an effect wherein one vaccine group has greater magnitude and the comparator vaccine group has greater breadth. Therefore, a secondary analysis may compare the distribution of magnitude-breadth curves among vaccine groups using the test statistic $\max|B_d^G|$ from Huang, et al [35], which is designed to detect general differences in magnitude-breadth curve distributions.

6.4.4.3 Analysis of binding Ab data

When a small panel of antigens (eg, ≤ 5) is being assessed in a multiplexed immunoassay, the response magnitudes and rates at 2 weeks post boost will be evaluated and compared for each antigen. When a larger panel is being assessed, in addition to assess each antigen separately, a summary measure across a group of similar antigens will be calculated, for example, a weighted-average may be constructed to account for the correlations between antigens as an integrate magnitude of responses to multiple antigens. Then this summary measure will be used in the multivariate linear model as the outcome variable to assess if this overall response measures differ by boost groups.

6.4.5 Analyses prior to end of scheduled follow-up visits

Any analyses conducted prior to the end of the scheduled follow-up visits should 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 should be made available on a need to know basis only.

6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study, as defined in Section 3, for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 114 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after at least one of the primary immunogenicity endpoints of the same class (humoral, cell-mediated, innate or mucosal) or, if no primary endpoint of the same class, at least one of the primary immunogenicity endpoints reaches the aforementioned threshold. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits.

7 Selection and withdrawal of participants

Participants will be healthy, HIV uninfected (seronegative) adults who comprehend the purpose of the study and have provided written informed consent. Volunteers will be recruited and screened; those determined to be eligible, based on the inclusion and exclusion criteria, will be enrolled in the study. Final eligibility determination will depend on results of laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

Investigators should always use good clinical judgment in considering a volunteer's overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or immunogenicity difficult, and some volunteers may be poor candidates for retention.

Although myo/pericarditis has not been documented to occur with MVA, it has been an adverse effect associated with other vaccinia vaccines. This protocol includes cardiac surveillance measures to ascertain any association of myo/pericarditis with MVA vaccination. The eligibility criteria exclude potential participants with preexisting cardiac conditions, an accumulation of cardiac risk factors, baseline elevations in troponin, or clinically significant ECG findings that could complicate the conduct of the trial or compromise the detection of myo/pericarditis. Study clinicians are asked to carefully screen participants for signs and symptoms such as palpitations or poor exercise tolerance that might indicate preexisting cardiac conditions that could become symptomatic during the trial and be misattributed as effects of the study vaccines.

Determination of eligibility, taking into account all inclusion and exclusion criteria, must be made within 56 days prior to enrollment unless otherwise noted in Sections 7.1 and 7.2.

7.1 Inclusion criteria

General and Demographic Criteria

1. **Age** of 18 to 55 years

2. **Prior participation in HVTN 205** with assignment to treatment (not placebo) arm:

    a. Assigned to HVTN 205 Group 1 or Group 3, and received all 4 scheduled vaccinations (2 injections of pGA2/JS7 DNA (months 0, 2) and 2 injections of MVA/HIV62 (months 4, 6); OR

    b. Assigned to HVTN 205 Group 4, and received at least vaccinations 1, 2 and 4 (3 injections of MVA/HIV62 at months 0, 2 and 6).

3. **Access to a participating HVTN CRS** and willingness to be followed for the planned duration of the study

4. Ability and willingness to provide **informed consent**

5. **Assessment of understanding**: volunteer demonstrates understanding of this study; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

6. **Willing to be contacted** 2 years following initial study injection.

7. **Agrees not to enroll in another study** of an investigational research agent

8. **Good general health** as shown by medical history, physical exam, and screening laboratory tests

HIV-Related Criteria:

9. Willingness to receive **HIV test results**

10. Willingness to discuss HIV infection risks and amenable to HIV risk reduction counseling.

11. Assessed by the clinic staff as being at **"low risk" for HIV infection** and committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit.

Laboratory Inclusion Values

Hemogram/CBC

12. **Hemoglobin** ≥ 11.0 g/dL for volunteers who were born female, ≥ 13.0 g/dL for volunteers who were born male

13. **White blood cell count** = 3,300 to 12,000 cells/mm^3

14. **Total lymphocyte count** ≥ 800 cells/mm^3

15. **Remaining differential** either within institutional normal range or with site physician approval

16. **Platelets** = 125,000 to 550,000/mm^3

Chemistry

17. **Chemistry panel**: ALT, AST, and alkaline phosphatase < 1.25 times the institutional upper limit of normal; creatinine ≤ institutional upper limit of normal.

18. **Cardiac Troponin T or I** (cTnT or cTnI) does not exceed the institutional upper limit of normal

Virology

19. **Negative HIV-1 and -2 blood test**: Participants must have a negative test result for HIV infection following the HVTN Lab Program's in-study HIV testing algorithm, prior to initial enrollment.

20. **Negative Hepatitis B surface antigen (HBsAg)**

21. **Negative anti-Hepatitis C virus Ab (anti-HCV)**, or negative HCV polymerase chain reaction (PCR) if the anti-HCV is positive

Urine

22. **Normal urine**:

- Negative urine glucose, and
- Negative or trace urine protein, and
- Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis with red blood cells levels within institutional normal range).

Reproductive Status

23. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

24. **Reproductive status**: A volunteer who was born female must:

- Agree to consistently use effective contraception (Appendix A) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit. Effective contraception is defined as using the following methods:
  - Condoms (male or female) with or without a spermicide,
  - Diaphragm or cervical cap with spermicide,
  - Intrauterine device (IUD),
  - Hormonal contraception, or
  - Any other contraceptive method approved by the HVTN 114 PSRT
  - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy);
- Or not be of reproductive potential, such as having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation;
- Or be sexually abstinent.

25. **Volunteers who were born female must also agree not to seek pregnancy through alternative methods**, such as artificial insemination or *in vitro* fertilization until after the last required protocol clinic visit

7.2 Exclusion criteria

General

1. **Blood products** received within 120 days before first vaccination

2. **Investigational research agents** received within 30 days before first vaccination

3. **Body mass index (BMI)** ≥ 40

4. **Volunteer has 2 or more of the following cardiac risk factors:**

   - Participant report of history of elevated blood cholesterol defined as fasting LDL >160 mg/dL;
   - First degree relative (eg, mother, father, brother, or sister) who had coronary artery disease before the age of 50 years;
   - Current smoker; or
   - BMI ≥ 35

5. **Pregnant or breastfeeding**

6. **Active duty and reserve US military personnel**

Vaccines and other Injections

7. **Any clinically significant AE related to vaccination in HVTN 205**, for which revaccination would be a safety concern such as any grade 3 or 4 related AE

8. **Smallpox vaccine** received within the last 5 years

9. **HIV vaccine(s)** received in a prior HIV vaccine trial other than HVTN 205. For HVTN 205 participants who have subsequently received control/placebo in another HIV vaccine trial, the HVTN 114 PSRT will determine eligibility on a case-by-case basis.

10. **Non-HIV experimental vaccine(s) received within the last 5 yea**rs in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 114 PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) greater than 5 years ago, eligibility for enrollment will be determined by the HVTN 114 PSRT on a case-by-case basis.

11. **Live attenuated vaccines** other than influenza vaccine received within 30 days before first vaccination or scheduled within 14 days after injection (eg, measles, mumps, and rubella [MMR]; oral polio vaccine [OPV]; varicella; yellow fever)

12. **Influenza vaccine or any vaccines that are not live attenuated vaccines** and were received within 14 days prior to first vaccination (eg, tetanus, pneumococcal, Hepatitis A or B)

13. **Allergy treatment with antigen injections** within 30 days before first vaccination or that are scheduled within 14 days after first vaccination

Immune System

14. **Immunosuppressive medications** received within 168 days before first vaccination. (Not exclusionary: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatitis; or [4] a single course of oral/parenteral corticosteroids at doses < 2 mg/kg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment.)

15. **Serious adverse reactions to vaccines or to vaccine components,** including history of anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not excluded from participation: a volunteer who had a nonanaphylactic adverse reaction to pertussis vaccine as a child.)

16. **Hypersensitivity to eggs or egg products**

17. **Immunoglobulin** received within 60 days before first vaccination

18. **Autoimmune disease** (eg, myositis)

19. **Immunodeficiency**

Cardiac

20. **History of myocarditis, pericarditis, cardiomyopathy, congestive heart failure with permanent sequelae, clinically significant arrhythmia** (including any arrhythmia requiring medication, treatment, or clinical follow-up)

21. **ECG with clinically significant findings**, or features that would interfere with the assessment of myo/pericarditis, as determined by a contract ECG Lab or cardiologist, including any of the following: (1) conduction disturbance (complete left or complete right bundle branch block or nonspecific intraventricular conduction disturbance with QRS ≥ 120 ms, PR interval ≥ 220ms, any 2nd or 3rd degree AV block, or QTc prolongation (> 450ms)); (2) repolarization (ST segment or T wave) abnormality that will interfere with the assessment of myo/pericarditis; (3) significant atrial or ventricular arrhythmia; (4) frequent atrial or ventricular ectopy (eg, frequent premature atrial contractions, 2 premature ventricular contractions in a row); (5) ST elevation consistent with ischemia; (6) evidence of past or evolving myocardial infarction

Clinically significant medical conditions

22. **Untreated or incompletely treated syphilis infection**

23. **Clinically significant medical condition**, physical examination findings, clinically significant abnormal laboratory results, or past medical history with clinically significant implications for current health. A clinically significant condition or process includes but is not limited to:

    - A process that would affect the immune response,
    - A process that would require medication that affects the immune response,
    - Any contraindication to repeated injections or blood draws,
    - A condition that requires active medical intervention or monitoring to avert grave danger to the volunteer's health or well-being during the study period,
    - A condition or process for which signs or symptoms could be confused with reactions to vaccine, or
    - Any condition specifically listed among the exclusion criteria below.

24. **Any medical, psychiatric, occupational, or other condition** that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a volunteer's ability to give informed consent

25. **Psychiatric condition that precludes compliance with the protocol**. Specifically excluded are persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years.

26. **Current anti-tuberculosis (TB) prophylaxis or therapy**

27. **Asthma** other than mild, well-controlled asthma. (Symptoms of asthma severity as defined in the most recent National Asthma Education and Prevention Program (NAEPP) Expert Panel report).

    Exclude a volunteer who:

    - Uses a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
    - Uses moderate/high dose inhaled corticosteroids, or
    - In the past year has either of the following:
        - Greater than 1 exacerbation of symptoms treated with oral/parenteral corticosteroids;
        - Needed emergency care, urgent care, hospitalization, or intubation for asthma.

28. **Diabetes mellitus** type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

29. **Thyroidectomy, or thyroid disease** requiring medication during the last 12 months

30. **Hypertension**:

- If a person has been found to have elevated blood pressure or hypertension during screening or previously, exclude for blood pressure that is not well controlled. Well-controlled blood pressure is defined as consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm Hg systolic and ≤ 100 mm Hg diastolic. For these volunteers, blood pressure must be ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic at enrollment.

- If a person has NOT been found to have elevated blood pressure or hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 100 mm Hg at enrollment.

31. **Bleeding disorder** diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

32. **Malignancy** (Not excluded from participation: Volunteer who has had malignancy excised surgically and who, in the investigator's estimation, has a reasonable assurance of sustained cure. or who is unlikely to experience recurrence of malignancy during the period of the study)

33. **Seizure disorder:** History of seizure(s) within past three years. Also exclude if volunteer has used medications in order to prevent or treat seizure(s) at any time within the past 3 years.

34. **Asplenia**: any condition resulting in the absence of a functional spleen

35. History of hereditary **angioedema**, acquired angioedema, or idiopathic angioedema.

7.3 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the vaccination schedule. Pause rules for the trial as a whole are described in Section 11.3.

7.3.1 Delaying vaccinations for a participant

Under certain circumstances, a participant's scheduled vaccination will be delayed. The factors to be considered in such a decision include but are not limited to the following:

- Within 45 days prior to any study injection
  - Receipt of blood products or immunoglobulin

- Within 30 days prior to any study injection
  - Receipt of live attenuated vaccines other than influenza vaccine
  - Receipt of allergy treatment with antigen injections

- Within 14 days prior to any study injection
  - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)

- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.

Vaccinations should not be administered outside the visit window period specified in the HVTN 114 Study Specific Procedures.

In order to avoid vaccination delays and missed vaccinations, participants who plan to receive licensed vaccines or allergy treatments should be counseled to schedule receipt of these substances, when possible, outside the intervals indicated above. The effects of these substances on safety and immunogenicity assessments and their interactions with study vaccines are unknown. Therefore, if circumstances allow, these substances should also be avoided in the 2 week interval between a study vaccination and completion of the 2 weeks postvaccination follow-up visit.

7.3.2 Participant departure from vaccination schedule

Every effort should be made to follow the vaccination schedule per the protocol. If a participant misses a vaccination and the visit window period for the vaccination has passed, that vaccination cannot be given. The participant should be asked to continue study visits.

7.3.3 Discontinuing vaccination for a participant

Under certain circumstances, an individual participant's vaccinations will be permanently discontinued. Specific events that will result in stopping a participant's vaccination schedule include:

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 114 PSRT).

- Clinically significant condition (ie, a condition that affects the immune system or for which continued vaccinations and/or blood draws may pose additional risk), including but not limited to the following:
  - Pregnancy (regardless of outcome);
  - Any grade 4 local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to vaccination;
  - Any grade 3 lab abnormality or other clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to vaccination; or
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 114 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination; or

- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions).

Such participants should be counseled on the importance of continuing with the study and strongly encouraged to participate in follow-up visits and protocol-related procedures per the protocol for the remainder of the trial, unless medically contraindicated.

In addition, vaccinations will be stopped for participants diagnosed with HIV infection. HIV-infected participants will not continue in the trial (Sections 7.3.4 and 9.7.1).

7.3.4 Participant termination from the study

Under certain circumstances, an individual participant may be terminated from participation in this study. Specific events that will result in early termination include:

- Participant refuses further participation,
- Participant relocates and remote follow-up or transfer to another HVTN CRS is not possible,
- HVTN CRS determines that the participant is lost to follow-up,
- Participant becomes HIV infected, or
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff).
- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

CRS pharmacists should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations. The protocol schema is shown in Table 3-1. See the Investigator's Brochures for further information about study products.

8.1 Vaccine regimen

The schedule of vaccination is shown in Section 3 and additional information is given below.

Group 1

Treatment 1 (T1):

MVA/HIV62B vaccine 1×10^8 $TCID_{50}$ to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 4

AND

Control for AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 4.

Group 2

Treatment 2 (T2):

MVA/HIV62B vaccine 1×10^8 $TCID_{50}$ to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 4

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 4.

Group 3

Treatment 3 (T3):

MVA/HIV62B vaccine 1×10^8 $TCID_{50}$ to be administered as 1mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 4

AND

Control for AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 4.

Treatment 4 (T4):

MVA/HIV62B vaccine 1×10^8 $TCID_{50}$ to be administered as 1mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 4

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 4.

Group 5

Treatment 5 (T5):

Control for MVA/HIV62B vaccine (Sodium Chloride for Injection USP, 0.9%) to be administered as 1mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 4

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 4.

8.2 Study product formulation

8.2.1 MVA/HIV62B vaccine (labeled as MVA/HIV62B 10^8 $TCID_{50}$/mL. GeoVax™, Inc.)

MVA/HIV62B is supplied as an opalescent to cloudy, colorless to off-white or light yellow suspension in single-dose vials containing a volume to deliver 1 mL of 1×10^8 $TC1D_{50}$/mL. The aqueous suspension may contain small visible particles. The vials containing study product must be stored frozen at -70°C or colder.

8.2.2 Control for MVA/HIV62B vaccine (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be used as the Control for MVA/HIV62B and must be stored as directed by the manufacturer.

8.2.3 AIDSVAX® B/E (labeled as AIDSVAX® B/E active (MN/A244 rgp 120/HIV-1)

AIDSVAX® B/E is supplied as a sterile suspension in single-use glass vials containing a volume to deliver 1 mL (300mcg/mL) of each rgp120/HIV-1 protein adsorbed onto a total of 600 mcg aluminum hydroxide gel adjuvant. The product must be stored upright and kept refrigerated (2° to 8°C). DO NOT FREEZE. DO NOT SHAKE.

8.2.4 Control for AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be used as the Control for the AIDSVAX® B/E. Product must be stored as directed by the manufacturer.

8.3 Preparation of study products

8.3.1 MVA/HIV62B

One vial of MVA/HIV62B (labeled as MVA/HIV62B 10^8 $TCID_{50}$/mL) will be needed to prepare this dose. Prior to injection, the pharmacist will remove the vial from the freezer and allow it to thaw in a 2° to 8° C refrigerator for 2 to 18 hours. During this 2 to 18 hour period, if the vaccine is not completely thawed and a volunteer is ready to be vaccinated, the pharmacist may hold the vial in his/her hand to complete the thawing process. The thawed MVA/HIV62B vaccine can be held at 2° to 8° C for up to 5 days (120 hours) after removal from the freezer. Do not STORE at room temperature.

Immediately prior to withdrawing a 1 mL dose into a 3 or 5 mL syringe, the vial should be removed from the refrigerator and inverted gently several times. DO NOT SHAKE. Using aseptic technique, the pharmacist should withdraw the entire contents of the vial, up to 1 mL, into a 3 or 5 mL syringe. The syringe containing the product must not be held at room temperature. Once drawn into the syringe, the vaccine should be kept cold (2° to 8°C) until it is ready to be injected into the participant. The syringe should be labeled as "MVA/HIV62B vaccine 1×10^8 $TCID_{50}$ or Control" and must also be labeled for administration in LEFT deltoid. The syringe should also be labeled with a 24 hour expiration date from the time product was withdrawn from the thawed vial into the syringe. (Note: If the vaccine is not withdrawn from the vial until 96 hours or more after removal from the freezer, then the actual expiration date/time should be used). The syringe must have an overlay to maintain blinding.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.2 Control for MVA/HIV62B (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be needed to prepare this dose. Prior to injection, the Pharmacist will remove the product from storage and place it in a 2° to 8°C refrigerator for 2 to 18 hours.

Immediately prior to withdrawing a 1 mL dose into a 3 or 5 mL syringe, the vial should be removed from the refrigerator. Using aseptic technique, the pharmacist should withdraw 1 mL, into a 3 or 5 mL syringe. The syringe containing the product must not be held at room temperature. Once drawn into the syringe, the control should be kept cold (2° to 8°C) until it is ready to be injected into the participant. The syringe should be labeled as "MVA/HIV62B vaccine 1×10^8 $TCID_{50}$ or Control" and must also be labeled for administration in LEFT deltoid. The syringe should also be labeled with a 24 hour expiration date from the time product was withdrawn from the refrigerated vial into the syringe. (Note: If the placebo is not withdrawn from the vial until 96 hours or more after removal from storage, then the actual expiration date/time should be used). The syringe must have an overlay to maintain blinding.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.3 AIDSVAX® B/E

One vial of AIDSVAX® B/E will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the refrigerator to allow the vial to equilibrate to room temperature for at least 20 minutes. The pharmacist must then:

1) Gently roll the vial horizontally between the palms or on a hard surface for approximately 10 seconds to wet the interior wall of the vial and to suspend the material.

2) Grasp the vial by the sides. Firmly and forcefully tap the bottom of the vial on a solid surface such that the vial hits squarely on a solid surface, in a rapid series of three (3) taps. Tap with enough force to dislodge any material that may be on the sides of the vial while using care not to chip or break the vial.

3) Repeat the gentle rolling and forceful tapping two more times, resulting in one (1) cycle of 9 taps.

4) Visually inspect the neck of the vial for presence or absence of product-related material (grayish film) as well as the sides of the vial. Vaccine that is stored at 2°C to 8°C can form an appearance of a cloudy ring or 'halo' on the vial neck and/or have product related material adhering on the sidewalls. If either condition is observed, the pharmacist should continue to follow the steps as directed below to dislodge and uniformly resuspend such product-related material.

5) If the product-related material still persists at the neck/sides of the vial at the end of the first cycle, repeat steps 1 to 4, for up to two (2) more cycles.

6) If the product-related material is still visible at the end of the third repeated cycle DO NOT use the vial.

After completing these steps, the pharmacist, using aseptic technique, will gently roll the mixture in the vial one more time and then withdraw 1 mL into a 3 or 5 mL syringe using a 21 or 23 gauge needle. The syringe should be labeled as "AIDSVAX B/E 600 mcg or Control 1 mL". The syringe must also be labeled for administration in RIGHT deltoid. The syringe must have an overlay to maintain blinding.

The study product should be administered as soon as possible after being drawn into the syringe.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.4 Control for AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be needed to prepare the dose. The pharmacist using aseptic technique, will withdraw 1 mL of Sodium Chloride for Injection USP, 0.9% into a 3 or 5 mL syringe using a 21 or 23 gauge needle. An overlay must be applied to the syringe.

The syringe should be labeled as “AIDSVAX B/E 600 mcg or Control 1 mL”. The syringe must also be labeled for administration in RIGHT deltoid. The syringe must have an overlay to maintain blinding.

The study product should be administered as soon as possible after being drawn into the syringe.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.4 Administration

All injections are to be given using standard IM injection technique.

For all MVA/HIV62B or Control (for MVA/HIV62B) injections, prior to administration, the pharmacist prepared participant specific syringe containing study product should be removed from the refrigerator. It may be warmed to room temperature or warmer by holding in one’s hand for several minutes at the participant’s side immediately prior to administration

For all AIDSVAX B/E or Control (for AIDSVAX B/E) injections, the person administering the injection should gently roll the syringe prior to administration of the study product. For these injections, a 21 or 23 gauge needle must be used for administration.

If an injection is administered in the contralateral deltoid due to a medical contraindication, the appropriate study staff should document this clearly. Under this circumstance, this is NOT a protocol violation.

When preparing a dose in a syringe and administering the dose, consideration should be given to the volume of solution in the needle before and after the dose is administered. Particularly, if the needle used to withdraw the product is replaced prior to vaccine administration, consideration should be given to conserving the full dose of product. The pharmacy and clinic staff members are encouraged to work together to administer the dose specified in the protocol.

At sites where registered pharmacists are legally authorized to administer injections, the HVTN CRS may choose to have the HVTN CRS pharmacist administer vaccinations.

8.5 Acquisition of study products

MVA/HIV62 will be provided by GeoVax, Inc.

AIDSVAX B/E will be provided by GSID.

Control for all study products (MVA/HIV62B and AIDSVAX B/E (Sodium Chloride for Injection USP, 0.9%)) will not be provided through the protocol and must be obtained by the site.

Once an HVTN CRS is protocol registered, the pharmacist can obtain study products from the NIAID Clinical Research Products Management Center (CRPMC) by following the ordering procedures given in Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

8.6 Pharmacy records

The HVTN CRS pharmacist is required to maintain complete records of all study products. The pharmacist of record is responsible for maintaining randomization codes and randomization confirmation notices for each participant in a secure manner.

8.7 Final disposition of study products

All unused study products must be returned to the CRPMC after the study is completed or terminated unless otherwise instructed by the CRPMC. The procedures and relevant form are included in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

9 Clinical procedures

The schedule of clinical procedures is shown in Appendix G .

9.1 Informed consent

Informed consent is the process of working with participants so that they fully understand what will and may happen to them while participating in a research study. The HVTN informed consent form documents that a participant (1) has been informed about the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in an HVTN study. Informed consent encompasses all written or verbal study information HVTN CRS staff provide to the participant, before and during the trial. HVTN CRS staff will obtain informed consent of participants according to HVTN policies and procedures.

The informed consent process continues throughout the study. Key study concepts should be reviewed periodically with the participant and the review should be documented. At each study visit, HVTN CRS staff should consider reviewing the procedures and requirements for that visit and for the remaining visits. Additionally, if any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign revised informed consent forms.

An HVTN CRS may employ recruitment efforts prior to the participant consenting. For example, some HVTN CRSs use a telephone script to prescreen people before they come to the clinic for a full screening visit. Participants must sign a screening or protocol-specific consent before any procedures are performed to determine eligibility. HVTN CRSs must submit recruitment and prescreening materials to IRB/EC and any applicable Regulatory Entity (RE) for human subjects protection review and approval.

Note: As defined in the DAIDS Protocol Registration Manual, an RE is "Any group other than the local IRB/EC responsible for reviewing and/or approving a clinical research protocol and site-specific ICFs [informed consent forms] prior to implementation at a site." CRSs are responsible for knowing the requirements of their applicable REs.

9.1.1 Screening consent form

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS RSC Protocol Registration Office.

Some HVTN CRSs have approval from their IRB/EC and any applicable RE to use a general screening consent form that allows screening for an unspecified HIV vaccine trial. In this way, HVTN CRS staff can continually screen potential participants and, when needed, proceed quickly to obtain protocol-specific enrollment consent. Sites conducting general screening or prescreening approved by their IRB/EC and any applicable RE may use the results from this screening to determine eligibility for this protocol, provided the tests are conducted within the time periods specified in the eligibility criteria.

9.1.2 Protocol-specific consent forms

The protocol-specific consent forms describe the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. A sample protocol-specific consent form for the main study is located in Appendix A. A separate sample consent form for other uses of specimens is located in Appendix D.

Each HVTN CRS is responsible for developing a protocol-specific consent form(s) for local use, based on the sample protocol-specific consent forms in Appendix A and Appendix D. The consent form(s) must be developed in accordance with requirements of the following:

- CRS's IRB/EC,
- CRS's institution and any applicable REs, and
- Elements of informed consent as described in Title 45, CFR Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6, Good Clinical Practice: Consolidated Guidance 4.8.

Study sites are strongly encouraged to have their local CABs review their sites-specific consent forms. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the level of understandability.

The sample informed consent form includes instructions throughout the document for developing specific content.

Sites should follow the instructions in the Protocol-specific Official Memo distributed along with this protocol regarding when they may begin using their site-specific protocol consent forms.

Regarding protocol registration, sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

9.1.3 Assessment of Understanding

Study staff are responsible for ensuring that participants fully understand the study before enrolling them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely.

An Assessment of Understanding is used to document the participant's understanding of key concepts in this HIV vaccine trial. The participant must complete the Assessment of Understanding before enrollment. Staff may provide assistance in reading and understanding the questions and responses, if necessary. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

IRB/EC and any applicable RE may require that a participant has signed either a screening or protocol-specific consent document prior to administering the Assessment of Understanding. The consent process (including the use of the Assessment of

Understanding) should be explained thoroughly to the IRB/EC and any applicable RE, whose recommendations should be followed.

9.2 Pre-enrollment procedures

Screening may occur over the course of several contacts/visits, up to and including before vaccination on day 0. All inclusion and exclusion criteria must be assessed within 56 days before enrollment, unless otherwise specified in the eligibility criteria (or below in this section).

After the appropriate informed consent has been obtained and before enrollment, the following procedures are performed:

- Medical history, documented in the case history record;
- Assessment of whether the volunteer is at low risk for HIV infection;
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
    - For volunteers who consent to mucosal (semen, rectal, or cervicovaginal) sample collection, screen for:
        - Any rectal condition that represents a contraindication to rectal secretion sampling, such as an active infection or inflammation of the colorectal area (such as an HSV-2 outbreak or inflamed hemorrhoids or colitis/diarrhea); and
        - Any active genital tract infection such as genital lesions or ulcers, or vaginal or penile discharge
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots (record the complete generic name for all medications);
- 12-lead ECG with interpretation (Section 9.4.1.3);
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
    - Screening HIV test,
    - HBsAg,
    - Anti-HCV Abs,
    - Syphilis test,
    - Complete blood count (CBC) with differential and platelets,
    - Chemistry panel (ALT, AST, alkaline phosphatase, and creatinine),

- Cardiac troponin,
- Urine dipstick (urinalysis if indicated, see Section 9.9),
- Urine or serum pregnancy test (volunteers who were born female); Persons who are not of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing;
- Pap smear (for volunteers who were born female and are age 21 or older). Pap smear is not required:
  - if volunteer disagrees to provide cervicovaginal secretion samples
  - if volunteer has reported having had a Pap smear within previous 5 years with most recent result normal or ASCUS [atypical squamous cells of undetermined significance] with no evidence of high-risk HPV), or
  - if high-risk HPV testing was not conducted, and volunteer has documented Pap smear within the 3 years prior to enrollment, with the latest result reported as normal or ASCUS.

- Administration of behavioral risk assessment questionnaire;

- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html);

- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.7; and

- Discussion of pregnancy prevention. A pregnant or breastfeeding person may not be enrolled in this trial. Specific criteria and assessment of contraception and pregnancy status are described in study inclusion criteria. Discussion of pregnancy prevention includes advising a participant who was born female and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

9.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The HVTN CRS registers the participant by scheduling the day 0 visit (enrollment) via the Web-based randomization system, and requests the randomization assignment. Circumstances may require a participant's enrollment visit to be changed. This may exceed the 4-day randomization time limit.

At all vaccination visits, the following procedures are performed before vaccination:

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of baseline reactogenicity parameters;
- Assessment of concomitant medications (as described in Section 9.2);
- Cardiac symptom assessment;
- Assessment of any new or unresolved AEs/intercurrent illnesses; and
- Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.
- Optional mucosal specimen collection
- Clinical laboratory tests for study participants who agree to provide (semen, rectal, or cervicovaginal) mucosal secretion samples, including:
  - Syphilis
  - Gonorrhea and chlamydia
  - Trichomonas vaginalis (for participants providing cervicovaginal samples),
  - Bacterial vaginosis (for participants providing cervicovaginal samples),
  - Yeast (for participants providing cervicovaginal samples, if clinically indicated).

Following completion of all procedures in the preceding list, and if results indicate that vaccination may proceed, vaccination is prepared and administered (Sections 8.3 and 8.4).

Administration of all injections during a vaccination visit must be accomplished within 1 calendar day.

Immediately following vaccination, the participant remains in the clinic for observation. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the participant is given the postvaccination symptom log and is instructed on how to complete it. The site will make arrangements to obtain daily reports of reactogenicity events from the participant during the reactogenicity period (as described in Section 9.10).

The following procedures will be performed at all vaccination visits. These procedures may be performed prior to or following vaccination:

- Risk reduction counseling (as described in Section 9.7);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.8); and

- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation); and

Additional procedures will be performed at scheduled visits as specified in Appendix G:

- Behavioral risk assessment;
- Social impact assessment questionnaire;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate; and
- Specimen collection (should be completed prior to vaccination)

9.4 Follow-up visits

The following procedures are performed at all scheduled follow-up visits:

- Risk reduction counseling (as described in Section 9.7);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.8); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation);
- Assessment of new or continuing concomitant medications (as described in Section 9.2);
- Cardiac symptom assessment; and
- Assessment of new or unresolved AEs/intercurrent illnesses.

Additional procedures will be performed at scheduled follow-up visits as specified in Appendix G:

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study;
- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;

- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;

- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;

- Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing;

- Optional mucosal specimen collection

- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (Section 9.2),
  - ECG and Cardiac troponin (if clinically indicated). and
  - Urine dipstick (urinalysis if appropriate; see Section 9.9);

- Clinical laboratory tests for study participants who agree to provide (semen, rectal, or cervicovaginal) mucosal secretion samples, including:
  - Syphilis
  - Gonorrhea and chlamydia
  - Trichomonas vaginalis (for participants providing cervicovaginal samples),
  - Bacterial vaginosis (for participants providing cervicovaginal samples),
  - Yeast (for participants providing cervicovaginal samples, if clinically indicated),

- Specimen collection.

9.4.1 Cardiac safety monitoring

Myo/pericarditis has been observed in recipients of vaccinia vaccinations used to protect against smallpox. It has been an *uncommon* occurrence in vaccinia recipients. Myo/pericarditis has not been documented to occur with MVA. Within HVTN protocols approximately 400 volunteers have received MVA vaccinations and there have been no MVA associated cases of myo/pericarditis. However, the safety of trial participants is the major priority for the HVTN and the Protocol Team, and thus enhanced cardiac safety monitoring has been added to this protocol to detect potential cardiac effects of MVA vaccine.

ECGs are performed at screening to establish a baseline and to exclude participants with pre-existing ECG findings that may interfere with post vaccination cardiac assessments. They are subsequently performed as clinically indicated. ECG interpretation will be centralized, and provided by a contract ECG laboratory staffed by a cardiologist who has experience with interpreting ECGs from several other vaccinia and MVA trials [36]. This

should provide consistency in the application of ECG screening criteria for enrollment, in the quality of the ECG data, and in assessing ECG changes as in other MVA studies [37].

9.4.1.1 Cardiac symptoms assessment

At all MVA/placebo vaccination visits and at subsequent visits as indicated in Appendix G, participants will be questioned specifically about symptoms and signs suggestive of myo/pericarditis or other cardiovascular complications as listed below:

- Shortness of breath,
- Chest pain/discomfort,
- Palpitations,
- Unexplained fatigue, and
- Fever, chills, myalgia/arthralgias.

Upper respiratory tract infections (URIs) alone are not suggestive of myopericarditis, and do not require a workup for such. In addition, no work up is required for transient symptoms that do not appear to be clinically significant.

If cardiopulmonary symptoms are reported, the study staff will perform a cardiopulmonary evaluation (vital signs, auscultation of the heart and lungs, and symptom-directed physical exam). Any report of these or any other signs and symptoms suggestive of any new cardiovascular condition will prompt an appropriate diagnostic evaluation as medically indicated.

9.4.1.2 Cardiac troponin

Testing for cTnI or cTnT, biomarkers for cardiac tissue injury, is performed for specific symptoms after enrollment as specified in Section 9.4.1.4. cTnI or cTnT tests will be performed at local laboratories. Sites should consult their local laboratories for information on specific handling requirements.

Any cTnT or cTnI result above the institutional upper limit of normal should be reported by phone or email to the HVTN Clinical safety specialist within 24 hours (telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site, https://members.hvtn.org/protocols/hvtn114), and repeated as soon as possible along with an additional ECG.

9.4.1.3 ECG testing

A 12-lead ECG is required at screening, and as clinically indicated. ECG equipment will be provided by the HVTN or accessed locally by the sites. ECG interpretation will be provided by a contract ECG laboratory.

9.4.1.4 Evaluation of suspected myo/pericarditis

The classic presentation of myo/pericarditis may not always be apparent with very early involvement. Since apparently benign symptoms may be suggestions of or mimic myo/pericarditis, there should be a low threshold for additional investigation of chest

sensation or symptoms referable to the chest. As with evaluation for all potentially serious health problems in study participants, the protocol team recommends that the clinic physician be involved in the evaluation and clinical decision making associated with cardiac symptoms.

Any participant who develops symptoms suggestive of possible myo/pericarditis (such as chest pain, dyspnea, palpitations, congestive heart failure) following MVA/placebo vaccination will be evaluated with an ECG and cTnT or cTnI by study staff as long as performing these tests in the research setting does not interfere with prompt medical care of the participant. Symptoms or findings that lead to a cardiac evaluation or referral for suspected myo/pericarditis should be reported by phone or email to the Clinical safety specialist within 24 hours (telephone numbers and email addresses are found on the protocol home page, https://members.hvtn.org/protocols/hvtn114).

The participant with symptoms and cardiac enzyme findings and/or ECG findings consistent with suspected or probable myo/pericarditis related to vaccine according to the CDC case definition [38], attached as Appendix G to this protocol, will be referred to a cardiologist for consultation and care. The site will communicate a request to the cardiologist that the initial evaluation include any of the following tests that have not been done previously for evaluation of that specific cardiac event: an ECG, cTnT or cTnI, and echocardiography. The site will request permission from the participant for access to medical records related to the evaluation. An AE of myo/pericarditis with a suspected causal relationship to vaccine would be followed by study staff until resolution and the participant will be contacted 1 year after the event to complete follow-up of the AE.

Any episode of myo/pericarditis at any grade must be reported to clinical safety specialist immediately and reported as an SAE, as described in Section 11.2.3. Study staff will follow any AE of myo/pericarditis until resolution.

9.5 Mucosal secretion sampling

Mucosal secretion samples will be collected at baseline (prior to the first vaccination) and at other timepoints indicated in Appendix F and Appendix G. Participants who have consented to any or all mucosal sampling procedures (Appendix A), and who meet the screening criteria for these procedures, will provide samples of rectal fluids, semen (participants born male only), and/or cervicovaginal secretions (participants born female only).

- Participants who consent to provide optional cervicovaginal, rectal, or semen secretions will be tested for the following infections at the mucosal sampling visits: gonorrhea, chlamydia, and syphilis. Participants who were born female who consent to provide cervicovaginal samples will be tested for trichomoniasis and bacterial vaginosis and may be tested for hyphae/budding yeast (if clinically indicated). Test results will be provided to participants and all participants who test positive for 1 or more of these infections will receive counseling as well as treatment or referral for treatment as appropriate. Sample collection will not be performed or may be deferred to a later date within the visit window if a contraindication to sampling (eg, active genital tract infection [GTI]) is present (as indicated below).

- Rectal fluid sampling (optional, both sexes). For participants born female, a pregnancy test must be performed and be negative prior to any rectal mucosal

sampling. Participants should abstain from receptive anal sex, insertion of any foreign object or substance into the anus (including but not limited to cleaning products [creams, gels, lotions, pads, etc.], lubricant, enemas, and douching even with water), and using perianal or intra-anal steroid or other anti-inflammatory cream in or around the anus for 48 hours prior to sample collection. Rectal secretion sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, within the visit window. In addition, rectal sampling will not be performed (or may be deferred to a later date within the visit window) if there is a contraindication to rectal secretion sampling, such as an active infection or inflammation of the colorectal area (such as an HSV-2 outbreak or inflamed hemorrhoids or colitis/diarrhea) or if the participant has any active GTI.

- Cervicovaginal secretion sampling (optional, only for participants who were born female). Participants who are age 21 or older must report having had a Pap smear within the 5 years prior to enrollment, with the latest result reported as normal or ASCUS (atypical squamous cells of undetermined significance) with no evidence of high-risk HPV; if high-risk HPV testing was not conducted, the participant must report having had a Pap smear within the 3 years prior to enrollment, with the latest result reported as normal or ASCUS. For participants capable of becoming pregnant, a pregnancy test must be performed and must be negative prior to any cervicovaginal mucosal sampling. Cervicovaginal mucosal sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, within the visit window. In addition, cervicovaginal sampling will not be performed (or may be deferred to a later date within the visit window) if a participant has an active ulcerative genital lesion or is known to have an active GTI at the scheduled timepoint. Participants providing cervico+vaginal secretion samples should be advised as follows:
  - Do not use anything with spermicide, lubricants, or topical/intravaginal medications (eg, topical yeast infection treatments) for 48 hours before the samples are collected;
  - Do not douche for 48 hours before the samples are collected;
  - Do not have vaginal sex or insert any foreign object or substance into the vagina for 48 hours before the samples are collected;
- Semen sampling (optional, only for participants who were born male). Participants providing semen samples are asked to refrain from ejaculation, using anything with lubricants, putting saliva on the penis, or having oral sex for at least 48 hours prior to specimen collection. In addition, semen sampling will not be performed (or may be deferred to a later date within the visit window) if a participant is known to have an active GTI at the scheduled timepoint.

9.6 Annual health contact

Participants will be contacted at 2 years following initial study injection (Appendix H). At this contact, CRS staff will collect the information listed below. Clinic visits will only be required if HIV confirmatory testing is necessary (Section 9.7.1); however, a clinic visit may be arranged for other reasons.

- Confirmation of vital status; if deceased, attempt to learn cause and date of death;

- If participant is alive, record the participant's responses to questions regarding any occurrence of the following events since the last HVTN study contact:
  - Life-threatening adverse experiences;
  - Persistent or significant disability/incapacity;
  - Hospitalizations and reasons;
  - Other important medical events that may jeopardize the participant or may require intervention to prevent 1 of the other outcomes listed above;
  - New chronic conditions requiring more than 30 days of medical intervention or medication;
  - New diagnosis of HIV infection; and
  - Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded, and adverse events will be assessed for relationship to study product(s).

9.6.1 Interim contacts

CRSs may report safety information obtained at a contact other than the annual contact. These contacts are reported as interim visits.

9.7 HIV counseling and testing

HIV counseling will be performed in compliance with the CDC's guidelines or other local guidelines for HIV counseling and referral. HIV testing will be performed in accordance with the current HVTN HIV testing algorithm following enrollment.

Participants will be counseled routinely during the trial on the avoidance of HIV infection and on the potential negative social impacts of testing Ab positive due to the vaccine. They will also be counseled on the risks of HIV Ab testing outside of the HVTN CRSs and will be discouraged from doing so during study participation and/or during any period of vaccine-induced positive serology.

Study staff will take particular care to inform study participants of the likelihood of routine HIV testing being offered or performed outside the study CRS at emergency rooms, clinics, and medical offices. Such testing has become more likely due to the CDC's revised guidelines for HIV counseling and testing, as well as policy changes in many countries to make HIV testing more frequent and routine. CRS staff should inform participants of their right to opt out of HIV testing outside the study site. CRS staff should inform study participants if local and/or state policies and regulations permit medical providers to perform HIV testing without first informing patients. If this is the case, then CRS staff should advise study participants that they may decline testing preemptively. CRS staff should also inform participants if positive results must be reported to local public health authorities. CRS staff should also inform participants of the need to maintain study blinding by getting HIV testing only at the study CRS. CRS staff should provide participants with CRS contact information and should encourage participants to ask medical providers to contact the CRS. The CRS can verify that the participant is in an HIV vaccine clinical trial and should only be tested at the study CRS.

Potential participants identified as being HIV infected during screening are not enrolled. All participants who become HIV infected during the study will be terminated from this study. Potential and enrolled participants identified as HIV infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.7.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

The study product may elicit an Ab response to HIV proteins. Therefore, vaccine-induced positive serology may occur in this study. Several precautionary measures will be taken to distinguish intercurrent HIV infection from vaccine-induced positive serology. These precautionary measures include:

- Participants will have physical examinations at visits specified in Appendix G. Signs or symptoms of an acute HIV infection syndrome, an intercurrent illness consistent with HIV-1 infection, or probable HIV exposure would prompt a diagnostic workup per the HVTN algorithm for Recent Exposure/Acute Infection Testing to determine HIV infection.

- HIV testing will be performed at multiple timepoints throughout the study (Appendix G). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN Laboratory Manual of Operations), which is able to distinguish vaccine-induced Ab responses from actual HIV infections.

- All participants can receive HIV-1 diagnostic testing from the site following their last scheduled visit until they are told that they did not receive an HIV vaccine or that they do not have vaccine-induced seropositivity.

- All participants who received vaccine product and who have vaccine-induced positive or indeterminate HIV-1 serology (as measured by the standard anti-HIV Ab screening tests) at or after the study is unblinded will be offered poststudy HIV-1 diagnostic testing (per the HVTN poststudy HIV-1 testing algorithm) periodically and free of charge as medically/socially indicated (approximately every 6 months) unless or until HIV Ab testing is no longer the standard test in clinical settings.

9.7.2 VISP registry

Experimental HIV vaccines may induce Ab production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called “vaccine-induced seropositivity” (VISP) (Section 9.7.1). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

9.8 Contraception status

Contraception status is assessed and documented at every scheduled clinic visit for a participant who was born female and who is sexually active in a way that could cause that participant to become pregnant. Prior to enrollment and throughout the study, staff will ask participants to verbally confirm their use of adequate contraceptive methods. A participant who was born female and is sexually active in a way that could cause that participant to become pregnant should be reminded at all scheduled clinic visits of the importance of using contraception and should be referred to specific counseling, information, and advice as needed. (Specific contraception requirements are listed in Section 7.1). This reminder should be documented in the participant's study record.

Self-reported infertility—including having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation—must be documented in the participant's study record.

9.9 Urinalysis

Dipstick testing may be performed in the clinic or the lab, as long as the required elements (glucose, protein, and hemoglobin) are tested. The examination is performed on urine obtained by clean catch.

If the screening dipstick is transiently abnormal due to menses or infection, document this issue in the participant's source documentation. For infection, provide appropriate treatment and/or referral. Following resolution, repeat the dipstick and, if within the eligibility limits specified in the protocol, the participant may be enrolled.

Follow-up urinalysis should be deferred if a participant is menstruating, but should be performed as soon as possible. If a follow-up dipstick is abnormal due to a participant's menstrual period, document in the comment section of the case report form (CRF) and repeat the dipstick once the participant is no longer menstruating. A micro-urinalysis is not required.

9.10 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0 [November 2014], except as noted in Section 11.2.2).

The reactogenicity assessment period is 3 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms using a postvaccination symptom log and to contact the site daily during the assessment period. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 3 to resolution. Participants are instructed to contact the clinic for events that arise during the period between vaccination and the next scheduled visit. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions, and lymph nodes. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 3 full days after), or those meeting SAE/adverse events requiring expedited reporting to DAIDS criteria, are recorded on an adverse experience log form.

Table 9-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	HVTN CRS staff
	Early: 25-60 minutes after vaccination	HVTN CRS staff
	Between early assessment and 11:59pm day 0	HVTN CRS staff or participant
1	Between 12:00am and 11:59pm day 1	HVTN CRS staff or participant
2	Between 12:00am and 11:59pm day 2	HVTN CRS staff or participant
3[b]	Between 12:00am and 11:59pm day 3	HVTN CRS staff or participant

[a] Day of vaccination

[b] New or unresolved reactogenicity symptoms present on day 3 are followed until resolution

9.10.1 Assessment of systemic and local symptoms

Systemic symptoms include increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, nausea, and vomiting. Local symptoms include pain and/or tenderness proximal to the injection site. The daily maximum severity reached for each symptom during the assessment period is reported.

Body temperature is measured by oral or infrared thermometry and reported in degrees Celsius. If temperature is measured in Fahrenheit, the conversion to Celsius should be documented in the participant's chart note. A measurement is taken once daily during the assessment period and should be repeated if participant is feeling feverish.

9.10.2 Assessment of injection site

Typical injection site reactions are erythema/induration/swelling. The maximum horizontal and maximum vertical measurements for all injection site reactions are recorded.

All injection site reactions are monitored until resolution. Areas of erythema/induration/swelling 25 cm^2 or more are followed daily; otherwise, the frequency of follow-up is based on clinician judgment.

9.10.3 Assessment of lymph nodes

This assessment is required only when reactogenicity assessments are performed by HVTN CRS staff, not by the participant.

Only the proximally draining lymph nodes are assessed (eg, axillary nodes on the same side of the body for injections given in the deltoid). Lymph nodes are first evaluated for enlargement and tenderness. If they are found to be enlarged, measurements are taken to determine the size (widest diameter) of the enlarged node(s).

9.11 Visit windows and missed visits

Visit windows are defined in HVTN 114 Study Specific Procedures. For a visit not performed within the window period, a Missed Visit form is completed. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

Procedures performed at an interim visit are usually toxicity/safety assessments (including local safety labs) and HIV testing. With the exception of HIV testing, these procedures are performed only if they were required at the missed visit or if clinically indicated. HIV testing may be performed as deemed appropriate by the study staff. Blood samples for immunogenicity assays are not typically collected at interim visits.

If a missed visit required vaccination, please see Section 7.3.2 and Section 7.3.3 for resolution.

9.12 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, platelet count, and chemistry panel), pregnancy testing, social impact assessment, and HIV test.

9.13 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. In case of required termination, enrollment in an observational study should be offered to the participant. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome.

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN Site Lab Reference Manual provides further guidelines for operational issues concerning the clinical and processing laboratories. The manual includes guidelines for general specimen collection, special considerations for phlebotomy, specimen labeling, whole blood processing, HIV screening/diagnostic testing, and general screening and safety testing.

Tube types for blood collection are specified in Appendix F. For tests performed locally, the local lab may assign appropriate tube types.

In specific situations, the blood collection tubes may be redirected to another laboratory or may require study-specific processing techniques. In these cases, laboratory special instructions will be posted on the protocol-specific section of the HVTN website.

10.2 Total blood volume

Required blood volumes per visit are shown in Appendix F. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-week) period.

10.3 Primary immunogenicity timepoints

The primary immunogenicity timepoints in this study are at visits 4 (day 14) (ie, 2 weeks after the first vaccination visit) and 7 (day 126) (ie, 2 weeks after the second vaccination visit). Endpoint assays for humoral and cellular responses are performed on participants at the primary immunogenicity timepoints and will also be performed at baseline. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on participants at other timepoints; the schedule is shown in Appendix F.

10.4 Endpoint assays: cellular

10.4.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific CD4+ and CD8+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct. ICS parameters will include cytokines such as IFN-γ, IL-2, and TNF-α, and may include other cytokines to identify T cells of specific functionality (such as Th2 and Th17). Markers of cytotoxic potential (Granzyme B, perforin and CD57) may also be included. Data will be reported as percentages of CD4+

or CD8+ T cells responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

Flow cytometry will also be used to phenotypically characterize peripheral follicular helper T cells (pTfh). Identification of pTfh will be based on expression of CXCR5 and PD-1 on CD4+ T cells, and may include additional markers.

10.5 Endpoint assays: humoral

10.5.1 HIV-1 multiplex Ab assay

Total binding IgG (IgG1, IgG2, IgG3, IgG4) and IgA Ab to clade B and AE isolates will be assessed on serum samples from study participants taken at the primary immunogenicity timepoints and baseline. This includes Env-specific Ab responses for gp120, gp41, V1/V2, V3, and the IDR of gp41. Specimens from other timepoints as well as other HIV antigens may also be assayed based on the results of the initial assay.

10.5.2 Neutralizing Ab assay

HIV-1–specific neutralizing Ab assays will be performed on serum samples from all study participants taken at baseline and at the primary immunogenicity timepoints. Specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoints. Assays will be performed with the vaccine strain and heterologous strains of HIV-1 that exhibit tier 1 (highly sensitive) and tier 2 (moderately sensitive) neutralization phenotypes [39].

10.6 Lab assay algorithm

The Lab Assay Algorithm lists assays to characterize cellular, humoral, and innate immune responses as well as host genetics that may be conducted to determine endpoints in HVTN vaccine trials. The type of assay(s) employed will be dependent on the response obtained by the primary immunogenicity assays at relevant timepoints. Please note that the Lab Assay Algorithm will be updated periodically to include new assays.

10.7 Mucosal studies

As an exploratory analysis, cervicovaginal secretions, rectal secretions, and semen samples will be collected at timepoints indicated in Appendix F, then processed and assessed for HIV-specific IgA and IgG. Additional exploratory humoral and/or cellular assays may be performed on these samples.

10.8 Exploratory studies

As an exploratory analysis, blood samples will be collected at timepoints indicated in Appendix F, then processed and assessed for ADCC responses and antibody avidity of

Env-specific Ab for V2, V3, and IDR of gp41. Additional humoral assays may be performed on these samples.

Samples may be used for other testing and research related to furthering the understanding of HIV immunology or vaccines. In addition, cryopreserved samples may be used to perform additional assays to support standardization and validation of existing or newly developed methods.

10.9 Other use of stored specimens

The HVTN stores specimens from all study participants indefinitely, unless a participant requests that specimens be destroyed or if required by IRB/EC, or RE.

Other use of specimens is defined as studies not described in the protocol.

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include limited genetic testing and, potentially, genome-wide studies. This research is done only to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will occur only after review and approval by the HVTN, the IRB/EC of the researcher requesting the specimens, and the CRS's IRBs/ECs if required.

The protocol sample informed consent form is written so that the participant either explicitly allows or does not allow their samples to be used in other research when they sign the form. Participants who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will remain in this study and their samples will only be used for the studies described in this protocol. If a participant decides against allowing other research using his or her samples, or at any time rescinds prior approval for such other use, the study site investigator or designee must notify HVTN Regulatory Affairs in writing. In either case, HVTN Regulatory Affairs directs the HVTN Lab Program not to use samples from these participants for such other uses.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on other use of specimens.

10.10 Biohazard containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC and the NIH or other applicable agencies.

All dangerous goods materials, including Biological Substances, Category A or Category B, must be transported according to instructions detailed in the International Air Transport Association Dangerous Goods Regulations.

11 Safety monitoring and safety review

11.1 Safety monitoring and oversight

11.1.1 HVTN 114 PSRT

The HVTN 114 PSRT is composed of the following members:

- DAIDS medical officer representative,
- Protocol chair and cochair,
- Protocol Team leader,
- Core medical monitor, and
- Clinical safety specialist.

The clinician members of HVTN 114 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, project manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 114 PSRT meetings.

11.1.2 HVTN SMB

The SMB is a multidisciplinary group consisting of biostatisticians, clinicians, and experts in HIV vaccine research that, collectively, has experience in the conduct and monitoring of vaccine trials. Members of the SMB are not directly affiliated with the protocols under review.

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months during the main study, as defined in Section 3 (for safety reviews during the annual health contact period, please see Section 11.4.3). The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. To increase the sensitivity for detecting potential safety problems, the SMB will review safety data aggregated across multiple protocols that use the same or similar vaccine candidates. The SMB conducts additional special reviews at the request of the HVTN 114 PSRT.

Study sites will receive SMB summary minutes and are responsible for forwarding them to their IRB/EC and any applicable RE.

11.1.3 SDMC roles and responsibilities in safety monitoring

The roles and responsibilities of the SDMC in relation to safety monitoring include:

- Maintaining a central database management system for HVTN clinical data;

- Providing reports of clinical data to appropriate groups such as the HVTN 114 PSRT and HVTN SMB (Section 11.1.2);

11.1.4 HVTN Core roles and responsibilities in safety monitoring

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 114 PSRT AE review criteria (Section 11.3);

- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (Section 11.3);

- Querying HVTN CRSs for additional information regarding reported clinical data; and

- Providing support to the HVTN 114 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

Sites must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results, concomitant medications) before the end of the next business day after receiving the information. The forms should not be held in anticipation of additional information at a later date. If additional information is received at a later date, the forms should be updated and resubmitted before the end of the next business day after receiving the new information.

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0 [November 2014], available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx, except:

- Weight loss is required to be reported as an adverse event only if it is considered to be deleterious to the participant's health (HVTN 114 SSP)

- Sinus arrhythmia will not be reported as an AE;

- PR interval $\leq$ 0.219 sec will not be reported as an AE. The definition of a Grade 1 mild prolonged PR interval (Adult > 16 years) that will be used is 0.22 sec - 0.25 sec;

- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:

    - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;

    - Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area;

    - Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;

    - Grade 4 is: Potentially life-threatening consequences (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue).

- The grading of Insomnia events will consider the criteria within the Insomnia parameter as well as the general AE functional table such that:

    - Grade 1 Insomnia is defined as: Mild difficulty falling asleep, staying asleep, or waking up early causing no or minimal interference with usual social and functional activities with intervention not indicated;

    - Grade 2 Insomnia is defined as: Moderate difficulty falling asleep, staying asleep, or waking up early, causing greater than minimal interference with usual social and functional activities with intervention indicated;

    - Grade 3 Insomnia is defined as: Severe difficulty falling asleep, staying asleep, or waking up early, causing inability to perform usual social and functional activities with intervention or hospitalization indicated.

    If a definition of insomnia falls between 2 grades, the final grading will be selected based on the degree of interference with usual social and functional activities caused by the symptoms.

All AEs are reported to the SDMC on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting to DAIDS (Section 11.2.3) and (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.3).

Sites are expected to notify the Clinical safety specialist of any serious safety concern requiring their attention (Table 11-1). Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn114). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

In the case of email notification, the Clinical safety specialist will reply during working hours (US Pacific Time) to confirm that the email has been received and reviewed. If email service is not available, the HVTN CRS should notify the Clinical safety specialist of the event by telephone, then submit CRFs.

In addition, site investigators are required to submit AE information in accordance with IRB/EC and any applicable RE requirements.

11.2.3 Expedited reporting of adverse events to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 (January 2010) of the *Manual for Expedited Reporting of Adverse Events* to DAIDS (DAIDS EAE Manual), which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/. The SAE Reporting Category will be used for this study.

The internet-based DAIDS Adverse Experience Reporting System (DAERS) must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AE reports may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact CRMSsupport@niaid.nih.gov or from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AE reports by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about expedited AE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

The study products for which expedited reporting are required are:

- MVA/HIV62B and control for MVA/HIV62B
- AIDSVAX B/E and control for AIDSVAX B/E

In addition to the expedited Reporting Category identified above, other AEs that must be reported in an expedited manner are: all myopericarditis events.

While the participant is in the main study reporting period (Section 3), the SAE Reporting Category will be used.

If the participant has completed the main study and is in the “annual health contact reporting period” (Section 3) the SUSAR Reporting Category will be used. In addition, per Section 9.5, all adverse events that are serious are collected on the LTFU Event Log and reported to the SDMC.

After the participant has completed the annual health contact period and is off study, sites must report SUSARS if the study site staff becomes aware of the events on a passive basis (eg, from publicly available information).

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports). However, because safety is a primary study endpoint, the Sponsor Medical Officer will not be unblinded to study treatment assignment when there is an assessment of relatedness of the SAE with the study product(s); and the safety report will be sent to the FDA based on the blinded attribution assessment.

If the PSRT believes unblinding of the site principal investigator to treatment assignment will assist with the clinical management of the SAE, the PSRT will consult the independent HVTN SMB for a recommendation. In the event the HVTN SMB determines that unblinding is indicated, the SMB will inform the site physician of the participant's treatment assignment in such a manner as to maintain the study blind of the PSRT and study team. For additional impact and management of SAEs on the study, refer to Section 11.3.

11.2.4 Expedited reporting of AEs to pertinent national and local regulatory authorities

Site IoRs/designees outside of the United States will file expedited reports to appropriate national regulatory authorities in accordance with their requirements and EAE information and any other relevant safety information to their ECs/IRBs in accordance with EC/IRB requirements.

11.3 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccinations will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 114 PSRT AE review are summarized in Table 11-1. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the HVTN 114 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

Table 11-1 AE notification and safety pause/AE review rules

Event and relationship to study products	Severity	HVTN CRS action[a]	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate HVTN 114 PSRT notification
SAE, related	Grade 3	Email and submit forms immediately	Prompt HVTN 114 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Prompt HVTN 114 PSRT AE review to consider pause

[a] Phone numbers and email addresses are found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn114).

[b] Does not include subjective reactogenicity symptoms (injection site pain, tenderness, fatigue/malaise, myalgia, arthralgia, chills, headache, nausea).

For all safety pauses, HVTN Core notifies the HVTN 114 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs. When an immediate safety pause is triggered, HVTN Core notifies the SMB.

Once a trial is paused, the HVTN 114 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. HVTN Core notifies the participating HVTN CRSs, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of study vaccinations. Based on the HVTN 114 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 114 PSRT notification or prompt HVTN 114 PSRT AE review is triggered, HVTN Core notifies the HVTN 114 PSRT as soon as possible during working hours (US Pacific Time)—or, if the information was received during off hours, by the morning of the next work day. If a prompt HVTN 114 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN requires that each CRS submit to its IRB/EC protocol-related safety information (such as IND safety reports, notification of vaccine holds due to the pause rules, and notification of other unplanned safety pauses). CRSs must also follow all applicable RE reporting requirements.

In addition, all other AEs are reviewed routinely by the HVTN 114 PSRT (Section 11.4.2).

11.4 Review of cumulative safety data

Routine safety review occurs at the start of enrollment and then throughout the study.

Reviews proceed from a standardized set of protocol-specific safety data reports. These reports are produced by the SDMC and include queries to the HVTN CRSs. Events are tracked by internal reports until resolution.

11.4.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 114 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 114 PSRT reviews clinical safety reports on a weekly basis and conducts calls to review the data as appropriate. After the injections and the final 2-week safety visits are completed, less frequent reporting and safety reviews may be conducted at the discretion of the HVTN 114 PSRT. HVTN Core reviews reports of clinical and laboratory AEs. Events identified during the review that are considered questionable, inconsistent, or unexplained are referred to the HVTN CRS clinic coordinator for verification.

11.4.3 Annual Health Contacts quarterly review

After the main study period, a monitoring team reviews safety reports quarterly during the annual health contacts period. This monitoring team comprises a DAIDS Medical Officer, Core medical monitor, and a Clinical safety specialist.

11.5 Study termination

This study may be terminated early by the determination of the HVTN 114 PSRT, a pertinent national regulatory authority, NIH, Office for Human Research Protections, or vaccine developer(s). In addition, the conduct of this study at an individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

12 Protocol conduct

This protocol and all actions and activities connected with it will be conducted in compliance with the principles of GCP (ICHe6), and according to DAIDS and HVTN policies and procedures as specified in the *HVTN Manual of Operations,* DAIDS Clinical Research Policies and Standard Procedures Documents including procedures for the following:

- Protocol registration, activation, and implementation;
- Informed consent, screening, and enrollment;
- Study participant reimbursement;
- Clinical and safety assessments;
- Safety monitoring and reporting;
- Data collection, documentation, transfer, and storage;
- Participant confidentiality;
- Study follow-up and close-out;
- Unblinding of staff and participants;
- Quality control;
- Protocol monitoring and compliance;
- Advocacy and assistance to participants regarding negative social impacts associated with the vaccine trial;
- Risk reduction counseling;
- Specimen collection, processing, and analysis;
- Ancillary studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 114 *Study Specific Procedures.*

12.1 Social impacts

Participants in this study risk experiencing discrimination or other personal problems, resulting from the study participation itself or from the development of VISP. The HVTN CRS is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a social impact, a designated NIAID or HVTN Core representative can be contacted.

Social harms are tabulated by the SDMC and are subjected to descriptive analysis. The goal is to reduce their incidence and enhance the ability of study staff to mitigate them when possible.

Summary tables of social impact events will be generated weekly, and made available for review by the protocol chairs, protocol team leader, and the designated NIAID representative.

12.2 Compliance with NIH guidelines for research involving products containing recombinant DNA

Because this study is evaluating products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.* Information about the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled at the site. Investigators at each site are responsible for obtaining IBC approval per NIH guideline *section IV-B07-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of initial protocol registration for this trial. If this protocol is amended, investigators should follow the requirements of their IBC.

12.3 Emergency communication with study participants

As in all clinical research, this study may generate a need to reach participants quickly to avoid imminent harm, or to report study findings that may otherwise concern their health or welfare.

When such communication is needed, the CRS will request that its IRB/EC and any applicable RE expedite review of the message. If this review cannot be completed in a timeframe consistent with the urgency of the required communication, the site should contact the participant first, and then notify the IRB/EC and any applicable RE of the matter as soon as possible.

13 Version history

The Protocol Team may modify the original version of the protocol. Modifications are made to HVTN protocols via clarification memos, letters of amendment, or full protocol amendments.

The version history of, and modifications to, Protocol HVTN 114 are described below.

Protocol history and modifications

Date: June 20, 2016

Protocol version: 1.0
Protocol modification:
Original protocol

14 Document references (other than literature citations)

Other documents referred to in this protocol, and containing information relevant to the conduct of this study, include:

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf.
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/
- Division of AIDS Protocol Registration Manual. Available at http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/prmanual.pdf
- U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Version 2.0 [November 2014]. Available at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/safetyandpharmacovigilance/manualforexpeditedreporting.aspx
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 114 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 114 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN Laboratory Manual of Operations. Accessible through the HVTN website.
- HVTN Manual of Operations. Accessible through the HVTN website.
- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/ps/publications/dgr/Pages/index.aspx
- HVTN Laboratory Assay Algorithm

- HVTN algorithm for diagnosis of HIV infections. Part of the HVTN Laboratory Manual of Operations.

- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf

- Participants' Bill of Rights and Responsibilities. Accessible through the HVTN website.

- NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Available at http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

- NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research. Available at http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html.

- Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks, July 2008.

- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://phacs.nichdclinicalstudies.org/publicDocs/DAIDS_SourceDocPolicy.pdf

- Title 21, Code of Federal Regulations, Part 50. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=21:1.0.1.1.19&idno=21

- Title 45, Code of Federal Regulations, Part 46. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=45:1.0.1.1.25&idno=45

See Section 16 for literature cited in the background and statistics sections of this protocol.

15 Acronyms and abbreviations

Ab	antibody
ADCC	antibody-dependent cellular cytotoxicity
AE	adverse event
ALT	alanine aminotransferase
ART	antiretroviral therapy
AST	aspartate aminotransferase
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence intervals
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
FDA	US Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IDR	immunodominant region
IFN-γ	interferon gamma
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	intrauterine device
LTFU	loss to follow-up
MAR	missing at random
MCAR	missing completely at random

MMR	measles, mumps, and rubella
MSM	men who have sex with men
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NICD	National Institute for Communicable Diseases (Johannesburg, South Africa)
NIH	US National Institutes of Health
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PSRT	Protocol Safety Review Team
RAB	DAIDS Regulatory Affairs Branch
RE	regulatory entity
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPF CEF	specific pathogen free chick embryo fibroblasts
SPT	DAIDS Safety and Pharmacovigilance Team
TB	tuberculosis
UW-VSL	University of Washington Virology Specialty Laboratory
VISP	Vaccine induced seropositivity
VLP	virus-like particle

* CRSs were formerly referred to as HIV Vaccine Trial Units (HVTUs). Conversion to use of the term CRS is in process, and some HVTN documents may still refer to HVTUs.

16 Literature cited

1. UNAIDS. Ethical considerations in biomedical HIV prevention trials. **2007**.

2. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research. **1979**.

3. Council for International Organizations of Medical Sciences (CIOMS). International ethical guidelines for biomedical research involving human subjects. Bull Med Ethics **2002**;17-23.

4. Centers for Disease Control and Prevention. HIV in the United States: At a Glance. http://www.cdc.gov/hiv/statistics/basics/ataglance.html. 11-25-2014. Last accessed: 3-4-2015

5. Porco TC, Martin JN, Page-Shafer KA, Cheng A, Charlebois E, Grant RM, Osmond DH. Decline in HIV infectivity following the introduction of highly active antiretroviral therapy. AIDS **2004**;18:81-8.

6. Hall HI, Frazier EL, Rhodes P, Holtgrave DR, Furlow-Parmley C, Tang T, Gray KM, Cohen SM, Mermin J, Skarbinski J. Differences in human immunodeficiency virus care and treatment among subpopulations in the United States. JAMA Intern Med **2013**;173:1337-44.

7. Rosenberg E, Sullivan P, Kelley C, Sanchez T, Luisi N, del Rio C, Salazar L, Frew P, Peterson J. Race and Age Disparities in HIV Incidence and Prevalence Among MSM in Atlanta, GA (Abstract 38) . Conference on Retroviruses and Opportunistic Infections, Boston, Massachusetts, USA, March 3-6, **2014**

8. Johnson AS, Hall HI, Hu X, Lansky A, Holtgrave DR, Mermin J. Trends in diagnoses of HIV infection in the United States, 2002-2011. JAMA **2014**;312:432-4.

9. UNAIDS. World AIDS Day Report. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/JC2434_WorldAIDSday_results_en.pdf. 2012. Last accessed: 6-24-2013

10. Mendoza Y, Martinez AA, Castillo MJ, Gonzalez C, Garcia-Morales C, Avila-Rios S, Reyes-Teran G, Armien B, Pascale JM, Bello G. Human immunodeficiency virus type 1 (HIV-1) subtype B epidemic in Panama is mainly driven by dissemination of country-specific clades. PLoS ONE **2014**;9:e95360.

11. Bautista-Arredondo S, Colchero MA, Romero M, Conde-Glez CJ, Sosa-Rubi SG. Is the HIV epidemic stable among MSM in Mexico? HIV prevalence and risk behavior results from a nationally representative survey among men who have sex with men. PLoS ONE **2013**;8:e72616.

12. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P,

Robb ML, Michael NL, Kunasol P, Kim JH. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med **2009**;361:2209-20.

13. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med **2012**;366:1275-86.

14. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, Dugast AS, Schoen MK, Rolland M, Suscovich TJ, Mahan AE, Liao L, Streeck H, Andrews C, Rerks-Ngarm S, Nitayaphan S, de Souza MS, Kaewkungwal J, Pitisuttithum P, Francis D, Michael NL, Kim JH, Bailey-Kellogg C, Ackerman ME, Alter G. Polyfunctional Fc-Effector Profiles Mediated by IgG Subclass Selection Distinguish RV144 and VAX003 Vaccines. Sci Transl Med **2014**;6:228ra38.

15. Yates NL, Liao HX, Fong Y, DeCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang ZY, Seaton KE, Berman PW, Alpert MD, Evans DT, O'Connell RJ, Francis D, Sinangil F, Lee C, Nitayaphan S, Rerks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Pinter A, Zolla-Pazner S, Gilbert PB, Nabel GJ, Michael NL, Kim JH, Montefiori DC, Haynes BF, Tomaras GD. Vaccine-Induced Env V1-V2 IgG3 Correlates with Lower HIV-1 Infection Risk and Declines Soon After Vaccination. Sci Transl Med **2014**;6:228ra39.

16. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, Bonsignori M, Moody MA, Fong Y, Chen X, Poling B, Nicholson CO, Zhang R, Lu X, Parks R, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Gilbert PB, Kim JH, Michael NL, Montefiori DC, Haynes BF. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proc Natl Acad Sci U S A **2013**;110:9019-24.

17. Goepfert PA, Elizaga ML, Seaton K, Tomaras GD, Montefiori DC, Sato A, Hural J, Derosa SC, Kalams SA, McElrath MJ, Keefer MC, Baden LR, Lama JR, Sanchez J, Mulligan MJ, Buchbinder SP, Hammer SM, Koblin BA, Pensiero M, Butler C, Moss B, Robinson HL. Specificity and 6-month durability of immune responses induced by DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 virus-like particles. J Infect Dis **2014**;210:99-110.

18. Goepfert PA, Elizaga ML, Sato A, Qin L, Cardinali M, Hay CM, Hural J, Derosa SC, Defawe OD, Tomaras GD, Montefiori DC, Xu Y, Lai L, Kalams SA, Baden LR, Frey SE, Blattner WA, Wyatt LS, Moss B, Robinson HL. Phase 1 Safety and Immunogenicity Testing of DNA and Recombinant Modified Vaccinia Ankara Vaccines Expressing HIV-1 Virus-like Particles. J Infect Dis **2011**;203:610-9.

19. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, Koblin BA, Buchbinder SP, Keefer MC, Tomaras GD, Frahm N, Hural J, Anude C, Graham BS, Enama ME, Adams E, DeJesus E, Novak RM, Frank I, Bentley C, Ramirez S, Fu R, Koup RA, Mascola JR, Nabel GJ, Montefiori DC, Kublin J, McElrath MJ, Corey L,

Gilbert PB. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med **2013**;369:2083-92.

20. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, Hu D, Tappero JW, Choopanya K. Randomized, Double-Blind, Placebo-Controlled Efficacy Trial of a Bivalent Recombinant Glycoprotein 120 HIV-1 Vaccine among Injection Drug Users in Bangkok, Thailand. J Infect Dis **2006**;194:1661-71.

21. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmor M, Del Rio C, McElrath MJ, Casimiro DR, Gottesdiener KM, Chodakewitz JA, Corey L, Robertson MN. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet **2008**;372:1881-93.

22. Moog C, Dereuddre-Bosquet N, Teillaud JL, Biedma ME, Holl V, Van HG, Heyndrickx L, Van DA, Katinger D, Vcelar B, Zolla-Pazner S, Mangeot I, Kelly C, Shattock RJ, Le GR. Protective effect of vaginal application of neutralizing and nonneutralizing inhibitory antibodies against vaginal SHIV challenge in macaques. Mucosal Immunol **2014**;7:46-56.

23. Liu P, Williams LD, Shen X, Bonsignori M, Vandergrift NA, Overman RG, Moody MA, Liao HX, Stieh DJ, McCotter KL, French AL, Hope TJ, Shattock R, Haynes BF, Tomaras GD. Capacity for infectious HIV-1 virion capture differs by envelope antibody specificity. J Virol **2014**;88:5165-70.

24. Karasavvas, N, Karnasuta, C, Nguay, V, Vasan, S, Trichavaroj, R, de Souza, MS, Savadsuk, H, Inthawong, D, Rittiroongrad, S, Madnote, S, Nitayaphan, S, Pitisuttithum, P, Rerks-Ngarm, S, O'Connell, RJ, Michael, NL, and Kim, JH. Investigation of Antibody Responses Induced in RV305 a Late Boost Vaccination of HIV-1 Uninfected Volunteers that Participated in RV144, a Thai Trial, AIDS Vaccine, 7-10 October 2013, Barcelona, Spain (P03.68 LB). http://epostersonline.com/aidsvax2013/?q=node/1472&page=1. 2013

25. Nilsson C, Godoy-Ramirez K, Hejdeman B, Brave A, Gudmundsdotter L, Hallengard D, Currier JR, Wieczorek L, Hasselrot K, Earl PL, Polonis VR, Marovich MA, Robb ML, Sandstrom E, Wahren B, Biberfeld G. Broad and potent cellular and humoral immune responses after a second late HIV-modified vaccinia virus ankara vaccination in HIV-DNA-primed and HIV-modified vaccinia virus Ankara-boosted Swedish vaccinees. AIDS Res Hum Retroviruses **2014**;30:299-311.

26. Moorthy VS, Imoukhuede EB, Keating S, Pinder M, Webster D, Skinner MA, Gilbert SC, Walraven G, Hill AV. Phase 1 evaluation of 3 highly immunogenic prime-boost regimens, including a 12-month reboosting vaccination, for malaria vaccination in Gambian men. J Infect Dis **2004**;189:2213-9.

27. Pitisuttithum P, Berman PW, Phonrat B, Suntharasamai P, Raktham S, Srisuwanvilai LO, Hirunras K, Kitayaporn D, Kaewkangwal J, Migasena S, Sheppard HW, Li E, Chernow M, Peterson ML, Shibata R, Heyward WL, Francis DP. Phase I/II study of a candidate vaccine designed against the B and E subtypes of HIV-1. J Acquir Immune Defic Syndr **2004**;37:1160-5.

28. Nitayaphan S, Pitisuttithum P, Karnasuta C, Eamsila C, de Souza M, Morgan P, Polonis V, Benenson M, VanCott T, Ratto-Kim S, Kim J, Thapinta D, Garner R, Bussaratid V,

Singharaj P, El Habib R, Gurunathan S, Heyward W, Birx D, McNeil J, Brown AE. Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. J Infect Dis **2004**;190:702-6.

29. Spearman P, Tomaras G, Montefiori.D, Huang Y, Ahmed H, Elizaga M, Hural J, McElrath J, Ouedraogo L, Pensiero M, Butler C, Kalams S, Overton E, Barnett S, and the NIH/NIAID HIV Vaccine Trials Network. Rapid Development of Cross-Clade Neutralizing Antibody Responses in Human Volunteers after Priming with Clade B gp120/gp140 Protein and Boosting with Clade C gp140 Protein. Poster AIDS Vaccine **2012**, 9-12 September, Boston, Massachusetts, USA.

30. Lai L, Kwa SF, Kozlowski PA, Montefiori DC, Nolen TL, Hudgens MG, Johnson WE, Ferrari G, Hirsch VM, Felber BK, Pavlakis GN, Earl PL, Moss B, Amara RR, Robinson HL. SIVmac239 MVA vaccine with and without a DNA prime, similar prevention of infection by a repeated dose SIVsmE660 challenge despite different immune responses. Vaccine **2012**;30:1737-45.

31. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat **1998**;52:119-26.

32. Zar JH. Biostatistical Analysis, 4th Ed. Prentice Hall, **1998**.

33. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics **1999**;55:625-9.

34. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med **1997**;16:81-102.

35. Huang Y, Gilbert P, Montefiori D, Self S. Simultaneous evaluation of the magnitude and breadth of a left- and right-censored multivariate response, with application to HIV vaccine development. Statistics in Biopharmaceutical Research **2009**;1:81-91.

36. Elizaga ML, Vasan S, Marovich MA, Sato AH, Lawrence DN, Chaitman BR, Frey SE, Keefer MC. Prospective surveillance for cardiac adverse events in healthy adults receiving modified vaccinia Ankara vaccines: a systematic review. PLoS ONE **2013**;8:e54407.

37. Sano J, Chaitman BR, Swindle J, Frey SE. Electrocardiography screening for cardiotoxicity after modified Vaccinia Ankara vaccination. Am J Med **2009**;122:79-84.

38. Update: cardiac-related events during the civilian smallpox vaccination program--United States, 2003. MMWR Morb Mortal Wkly Rep **2003**;52:492-6.

39. Seaman M, Janes H, Hawkins N, randpre L, Devoy C, Giri A, Coffey R, Harris L, Wood B, Daniels M, Bhattacharya T, Lapedes A, Polonis V, McCutchan F, Gilbert P, Self S, Korber B, Montefiori D, Mascola J. Tiered Categorization of a Diverse Panel of HIV-1 Env Pseudoviruses for Assessment of Neutralizing Antibodies. J Virol **2010**;84:1439-52.

Appendix A Sample informed consent form

Title: A phase 1 clinical trial to evaluate the immunogenicity of AIDSVAX B/E bivalent gp120 vaccine and MVA/HIV62B in healthy, HIV-1–uninfected adult participants who previously received MVA/HIV62B in DNA/MVA or MVA/MVA regimens in HVTN 205

HVTN protocol number: HVTN 114

Site: [Insert site name]

Thank you for participating in HVTN 205 and your interest in this follow-up research study. We contacted you because you got the study vaccines, not the placebo, in HVTN 205. Please read this consent form or ask someone to read it to you. If you decide to join the study, we will ask you to sign or make your mark on this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test two HIV vaccines. HIV is the virus that causes AIDS.

Up to 100 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

1. **We are doing this study to answer several questions.**

   - Are these study vaccines safe to give to people who received the HIV vaccines in HVTN 205?
   - Are these people able to take the study vaccines without becoming too uncomfortable?
   - How do these people's immune systems respond to the study vaccines? (Your immune system protects you from disease.)
   - Are the immune responses different from what we saw in HVTN 205?

2. **The study vaccines cannot give you HIV.**

   The study vaccines are not made from actual HIV. It is impossible for the study vaccines to give you HIV. Also, they cannot cause you to give HIV to someone else.

3. We do not know if the study vaccines will decrease, increase, or not change your risk of becoming infected with HIV if you are exposed to the virus.

Sites: Any change to the language in this section requires approval from HVTN Regulatory Affairs.

Several studies have tested whether HIV vaccines can reduce the risk of getting HIV from another person. In some studies, people who got the vaccine seemed to have the *same* risk of getting HIV as people who did not get the vaccine. In one study, people who got the vaccine seemed to have a *lower* risk of getting HIV than people who did not get the vaccine. In studies with a different vaccine, some people who got the vaccine had a *higher* risk of getting HIV than people who did not get the vaccine.

This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. The study staff can tell you about the differences.

We do not know whether the vaccines in this study will affect your risk of getting HIV from another person. The risk could be higher, lower, or unchanged. It's very important to avoid exposure to HIV during and after the study. We will tell you how to avoid HIV.

4. These study vaccines are experimental.

The study vaccines are called MVA/HIV62B and AIDSVAX B/E. From here on, we will call them MVA and AIDSVAX or the study vaccines. They are experimental HIV vaccines. That means we do not know whether the vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These vaccines are used only in research studies.

The vaccines are being provided by GeoVax, Inc. and by Global Solutions for Infectious Diseases (GSID).

You received the MVA vaccine when you were in HVTN 205. The MVA vaccine is made from a virus called Modified Vaccinia Ankara (MVA) virus, which was developed to protect against smallpox. The virus in the vaccine has been changed so that it will not spread in your body. It also will not be contagious to people around you. The MVA vaccine will instruct the body to make a few of the proteins that are found in HIV. These proteins may cause the body to have an immune response. Immune responses are part of the body's defense against disease.

The MVA vaccine was given to about 223 participants in HVTN 205 and to 123 people in two other HVTN studies. In those studies, the vaccine did not cause serious health problems.

The AIDSVAX vaccine is made of man-made proteins that are similar to proteins from the outer surface of the HIV virus. Your body's immune system may respond to this study vaccine by making antibodies that recognize and fight against HIV proteins. Antibodies are special proteins made by the body that can recognize and prevent infections. The AIDSVAX vaccine has been given to over 10,000 participants in different studies.

The MVA vaccine and the AIDSVAX vaccine have not been given together in people before.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, vomiting, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching at the injection site. Most people can still do their planned activities after getting a vaccine. Rarely, people experience side effects that limit their normal activities or make them go to the doctor.

Rarely, a vaccine can cause an allergic reaction, including a rash, hives, or difficulty breathing. Allergic reactions can be life-threatening. You should tell us if you have ever had a bad reaction to any injection or vaccine.

Very rarely, a vaccine causes an autoimmune disease in a person, or makes an autoimmune disease worse. An autoimmune disease happens when your immune system attacks your own body, instead of attacking an infection.

Risks of the study vaccines:

In addition to general risks of vaccines, the MVA and AIDSVAX vaccines have some other possible risks. This section lists the side effects we know about. There may be others that we don't yet know about, even serious ones. We will tell you if we learn about any new side effects.

Risks of the MVA vaccine

The MVA vaccine is similar to the smallpox vaccine that has been used worldwide to protect against smallpox. The smallpox vaccine may cause certain heart problems in some people. This can happen in about 1 in every 175 people who receive the smallpox vaccine. This result has not been seen in any study using an MVA vaccine in healthy people. The MVA vaccine in this study is much weaker than smallpox vaccine, and is designed to avoid these problems. In other earlier studies, this MVA vaccine has been given to more than 300 healthy people and has not caused any heart problems or serious side effects. It is very unlikely, but is possible that MVA vaccines, including this one, could be associated with heart problems. If you join the study, we will ask you about symptoms related to heart problems. Let us know right away if you are having any problems like extreme tiredness, chest pain, or difficulty breathing.

If we suspect a heart problem during the study, we may do additional testing to check your heart. We may refer you to a heart doctor for diagnosis and treatment. We will keep in close touch with you until the problem is over. We will ask you to sign a form to allow us to review your medical records for the heart problem.

In earlier studies of the MVA vaccine, some people have had lymph node (gland) swelling and some had pain or tenderness in the neck or armpit area near the injection site. Two people had severe allergic reactions that were treated and got better the same day. One person reported feeling shaky and short of breath. Another person had nasal congestion. Some people had temporary lab changes such as a low white blood cell count. These symptoms were mild or moderate and usually improved or went away within a few days.

The MVA vaccine used in this study was made by a different process than the MVA vaccine used in HVTN 205. This is because GeoVax is preparing for future studies where

larger amounts of vaccine will be needed. Although it is the same vaccine, the different process may cause your body to react differently than it did in HVTN 205.

Risks of the AIDSVAX vaccine:

This AIDSVAX vaccine has been given to thousands of people. The most common symptoms at the injection site were pain and tenderness. In some cases, this resulted in some limited arm movement which went away on its own within a few days. Less often, some people had injection site hardness. The most common reactions in the body were swollen glands, and muscle or joint aches.

A few people had changes in blood and urine test results following injections. The changes did not cause health problems. We do not know if these changes were caused by the study vaccines.

One person experienced a serious allergic reaction immediately after a vaccination with AIDSVAX. The person was treated and recovered completely in a few hours.

We do not know if participants in this study will have similar side effects to those seen in earlier studies.

Joining the study

5. It is completely up to you whether or not to join the study.

Take your time in deciding. If it helps, talk to people you trust, such as your doctor, friends or family. If you decide not to join this study, or if you leave it after you have joined, your other care at this clinic and the benefits or rights you would normally have will not be affected.

If you join this study, you may not be allowed to join other HIV vaccine or HIV prevention studies now or in the future. You cannot be in this study while you are in another study where you receive a study product. Also during the study, you should not donate blood or tissue.

If you choose not to join this study, you may be able to join another study.

Site: Remove item 6 if you use a separate screening consent that covers these procedures.

6. If you decide to join the study, we will screen you to see if you are eligible.

Screening involves a physical exam, HIV test and health history. A physical exam may include, but is not limited to:

- Checking your weight, temperature and blood pressure
- Looking in your mouth and throat
- Listening to your heart and lungs
- Feeling your abdomen (stomach and liver)

- Doing a genital exam (which includes touching that area)

We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for: syphilis, Hepatitis B, and Hepatitis C. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were born female, we will test you for pregnancy.

To see if you can join the study, we will do a heart test called an electrocardiogram (ECG, also known as EKG). For the ECG, we will place leads (suction cups, attached with gel or stickers) on your chest, arms and legs and you will need to lie still for several seconds. This test will also let us see if there are any changes in your heart health later in the study.

We will review the screening results with you. The screening results may show you are not eligible to join the study, even if you want to. You cannot be in another research study where you receive a study product and be enrolled in this study.

If you are allergic to eggs or egg products, you cannot be in this study.

We will ask you about your history of drug use. Using some drugs may mean that you cannot join this study.

Sites: adapt the following section so it is applicable to the care available at your site.

7. **If we find that you have a health problem during screening or during the study:**

   - We will tell you about the care that we can give here for free.

   - For the care that we cannot give, we will explain how we will help you get care elsewhere.

   For health problems that are unrelated to the study, we will not pay for medical costs.

8. **If you were born female and could become pregnant, you must agree to use birth control to join this study.**

   Site: List approved birth control methods here if you do not want to hand out the separate Approved Birth Control Methods sheet.

   You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 3 weeks before your first injection until 6 months after your last study injection (the time of your last scheduled clinic visit). We will talk to you about effective birth control methods. They are listed on a handout that we will give to you. *Site: Delete the preceding sentence if you include the birth control sheet in this consent form.* If you join the study, we will test you for pregnancy at some visits, including before each study injection.

Being in the study

If you meet the study requirements and want to join, here is what will happen:

9. You will come to the clinic for scheduled visits about [#] times over 10 months.

Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

Visits can last from [#] to [#] hours.

You may have to come for more visits if you have a lab or health issue.

We may contact you after the main study ends (for example, to tell you about the study results).

10. We will give you [Site: Insert compensation] for each study visit you complete.

This amount is to cover the costs of [Site: Insert text]

Site: Insert any costs to participants (eg, birth control costs for female participants who could become pregnant).

US sites only:

Payments you receive for being in the study may be taxable. The clinic staff may need to ask you for your Social Security number for tax reasons.

You do not have to pay anything to be in this study.

11. We will give you 1 or both of the study vaccines.

Not everyone in this study will get the same study vaccines. Some people will only get the MVA vaccine, some will only get the AIDSVAX vaccine, and some will get both vaccines. If you get only one of the vaccines, you will also get a placebo injection. A placebo is a substance that does not contain vaccine. In this study, the placebo is sterile salt water. Everyone gets two injections at both injection visits.

If you got only MVA vaccinations in HVTN 205, you have a:

- 1-in-2 chance of getting only the MVA vaccine and placebo in this study
- 1-in-2 chance of getting both the MVA and AIDSVAX vaccines in this study

If you got both DNA and MVA vaccinations in HVTN 205, you have a:

- 1-in-3 chance of getting only the MVA vaccine and placebo in this study
- 1-in-3 chance of getting only the AIDSVAX vaccine and placebo in this study
- 1-in-3 chance of getting both the MVA and AIDSVAX vaccines in this study

The clinic staff have no say in which of the study vaccines you will get. They will not know which one you are getting, and neither will you. Only the pharmacist at your site will have this information while the study is going on.

You will have to wait until everyone completes their final study visits to find out which study vaccines you got. This could be up to 2 years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

12. We will give you the study products on a schedule.

You will be in 1 of 5 groups. As shown below, which group you are in depends partly on which vaccine(s) you got in HVTN 205. The group that you are assigned to in this study is completely random, like flipping a coin. Whichever group you are in, you will get 1 injection into both of your upper arms at both injection visits. If you cannot get an injection in both arms, then one arm may be used. There are 2 injection visits, 4 months apart.

Site: A picture version of the injection schedule has been provided in Appendix B. You may insert it below in place of (or in addition to) the text version or give it as a separate document to volunteers if you believe it will be helpful to them. You are not required to do either.

Injection Schedule

Group	Vaccine(s) received in HVTN 205	First injection visit	4 months later
1	MVA only	MVA + Placebo	MVA + Placebo
2	MVA only	MVA + AIDSVAX	MVA + AIDSVAX
3	DNA and MVA	MVA + Placebo	MVA + Placebo
4	DNA and MVA	MVA + AIDSVAX	MVA + AIDSVAX
5	DNA and MVA	AIDSVAX + Placebo	AIDSVAX + Placebo

You will have to wait in the clinic for about a half hour after each set of injections to see if there are any problems. Then for that night and for 3 more days, you will need to write down how you are feeling and if you have any symptoms. Contact the clinic staff if you have any issues or concerns after receiving an injection. If you have a problem, we will continue to check on you until it goes away.

13. In addition to giving you the study products, we will:

- Do regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;
- Perform physical exams;
- Do pregnancy tests if you were born female;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study;

- Take urine and blood samples.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 17 mL and 200 mL (a little more than 1 tablespoon to a little less than 1 cup). Your body will make new blood to replace the blood we take out.

Site: You may want to add a sentence to the end of the previous paragraph contextualizing the blood volumes described (eg, "To compare, people who donate blood in the US can give a total of about 500 mL in an 8-week period."). Modify the example for cultural relevance and alter blood volumes as necessary.

Site: Insert Appendix E, Table of procedures (for informed consent form) in this section or distribute it as a separate sheet if it is helpful to your study participants. You are not required to do either.

We will be looking for side effects. We will review the results of these procedures and tests with you at your next visit, or sooner if necessary. If any of the results are important to your health, we will tell you.

14. If you agree, we will also collect rectal fluid and cervical fluid or semen.

We want to see how the vaccines affect the parts of the body where people may be exposed to HIV: their rectum, vagina, and penis. At the end of this form we will ask if you allow us to collect rectal fluid, and cervical fluid (if you were born female) or semen (if you were born male). We would like to collect these samples at 5 clinic visits. You can decide not to give any of these samples and still be in the study. If you do not allow us to collect these samples before the first injection, we will not collect any other samples.

If you agree to provide these samples, we will test you for the sexually transmitted infections gonorrhea, chlamydia, and syphilis at each sampling visit. If you were born female, we will also test you for Trichomonas vaginalis and bacterial vaginosis at these visits. We may also take vaginal swabs to test for yeast infection.

We will give you the results of these tests. If you need care, we will tell you about the care we can give you at this clinic. We will also tell you about care we can help you get elsewhere.

For each type of sample, we will ask you to avoid certain activities for a period of time before we collect them. This will help make sure your samples give accurate lab readings.

Rectal fluid collection

We will collect rectal fluid by placing a small absorbent sponge or swab in the rectum using a plastic tube about 2 cm wide (a little less than an inch). The tube will go in about 2 ½ inches. This will take about 5 minutes.

For the 2 days before we collect your rectal fluid, we will ask you to follow these instructions:

- Do not have receptive anal intercourse

- Do not put anything into your anus, including cleaning products (creams, gels, lotions, pads, etc.), lubricant, enemas or douches (even with water)

- Do not use any anti-inflammatory creams in or around your anus.

We will not collect rectal fluid if we think you may have an anal or rectal infection. You should tell us if your rectal area is sore.

We will not collect rectal fluid if you are pregnant or menstruating. We will try to schedule another visit to collect these samples if you are menstruating.

Cervical Fluid

In order to provide cervical fluid samples, you must have had a Pap smear within the last 3 to 5 years with the most recent result being normal. (If you haven't had a Pap smear within the last 3 to 5 years and would like to get one, we will tell you where you can get one.)

To collect cervical fluid, we will insert a speculum (a device that opens the vagina) into your vagina. Then we will place a small sponge in the opening of the cervix for about 2 minutes to absorb the fluid.

For the 2 days before we collect your cervical fluid, we will ask you these things:

- Do not use any spermicide, lubricants, douche (even with water), or medication in or around your vagina;

- Do not have vaginal intercourse or insert anything into your vagina.

We will not collect cervical fluid if you are pregnant or if we think you may have a cervical or vaginal infection. We will not collect cervical fluid if you are menstruating but we will try to schedule another visit to collect these samples.

Semen

You may provide the semen at home or here at the clinic. We will ask you to ejaculate into a plastic cup, which we will give to you. The clinic must receive the semen sample within 2 hours or less after it is collected.

We will ask you not to ejaculate, use anything with lubricants, put saliva on the penis, or have oral sex for at least 2 days before providing the semen.

15. We will counsel you on avoiding HIV infection.

We will ask you personal questions about your HIV risk factors such as sexual behavior and drug use. We will talk with you about ways to keep your risk of getting HIV low. Some topics we may discuss include:

- What you think may cause risky behavior for you.

- Methods to avoid getting HIV.

These may include not having sex, using condoms, or behavior changes, such as cutting down on alcohol. We will talk with you about which methods of HIV prevention may be right for you.

16. We will test your samples for this study.

We will send your samples (without your name) to a lab to see how your immune system responds to the study products.

The researchers may also:

- Take cells from your samples and grow more of them. We may grow more of your cells over time, so that they can continue to contribute to this study.

- Do limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. Limited genetic testing involves only some of your genes, not all of your genes (your genome). The researchers will not look at all of your genes, only the genes related to the immune system and diseases.

These tests are for research purposes only. The lab will not give the results to you or this clinic, and the results will not become part of your study record.

These tests will be done in labs in the United States.

Site: Delete next section if using separate consent for use of samples and information in other studies

17. When we take samples from you for this study, we take extra samples in case we have to repeat tests. When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies by HVTN or other researchers. We will call these "extra samples."

This section gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

Do I have to agree? No. You are free to say yes or no, or to change your mind after you sign this form. At your request, we will destroy all extra samples that we have. Your decision will not affect your being in this study or have any negative consequences here.

Where are the samples stored? Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* Your samples will be stored in the HVTN's central repository in the United States.

PDC: Adjust previous sentence if samples will be stored only in the United States. Also, add text above informing participants of any international shipping of their samples.

How long will the samples be stored? There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

Will I be paid for the use of my samples? No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

Will I benefit from allowing my samples to be used in other studies? Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not needed for your medical care. They are not part of your medical record. The studies are only being done for research purposes.

Will the HVTN sell my samples and information? No, but the HVTN may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

How do other researchers get my samples and information? When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN keeps track of your decision about how your samples and information can be used.

What information is shared with other researchers? The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

What kind of studies might be done with my extra samples and information? The studies will be related to HIV, vaccines, the immune system and other diseases. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.

- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

Who will have access to my information in studies using my extra samples?

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

18. We will do our best to protect your private information.

US sites: Check HIPAA authorization for conflicts with this section.

Your study records and samples will be kept in a secure location. We will label all of your samples and most of your records with a code number, not your name or other personal information. However, it is possible to identify you, if necessary. We will not share your name with the lab that does the tests on your samples, or with anyone else who does not need to know your name.

Sites: Any change to the following highlighted text requires approval from HVTN Regulatory Affairs.

We do need to share your name with the HVTN in case you need proof in the future that you participated in an HIV vaccine study. The HVTN will keep your name in a secure file with these items:

- The name of your study
- Your age or date of birth
- Your study ID number
- What study product(s) you received

There are no HIV test results kept in this file. The HVTN will not share any information that could identify you without your agreement. The HVTN will remove your name from the file if you do not want it there.

Clinic staff will have access to your study records. Your records may also be reviewed by groups who watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. These groups include:

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,

- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- GeoVax, Inc., GSID, and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. At this clinic, we have to report the following information:

Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.).

- [Item 1]
- [Item 2]
- [Item 3]

US sites: Include the following boxed text. You can remove the box.

We have a Certificate of Confidentiality from the US government, to help protect your privacy. With the certificate, we do not have to release information about you to someone who is not connected to the study, such as the courts or police. Sometimes we can't use the certificate. Since the US government funds this research, we cannot withhold information from it. Also, you can still release information about yourself and your study participation to others.

The results of this study may be published. No publication will use your name or identify you personally.

When the study is done, we may share the information from the study with others so they can see it and use it. We will not share any information that will let someone identify you.

19. We may stop your injections or take you out of the study at any time. We may do this even if you want to stay in the study and even if you were scheduled for additional injections.

This may happen if:

- you do not follow instructions,
- the researcher thinks that staying in the study might harm you,
- you get HIV,
- you enroll in a different research study where you receive another study product, or
- the study is stopped for any reason.

If we stop your injections, we may ask you to stay in the study to complete other study procedures.

20. If you become pregnant during the study, we will continue with some procedures but not injections.

We will do this for as long as it is safe for you and your developing baby.

If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

21. If you get infected with HIV during the study, we will help you get care and support.

You will not be able to stay in this study. We will counsel you about your HIV infection and about telling your partner(s). We will tell you where you can get support and medical care, and about other studies you may want to join. *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

Other Risks

22. There are other risks to being in this study.

This section describes the other risks and restrictions we know about. There may also be unknown risks, even serious ones. We will tell you if we learn anything new that may affect your willingness to stay in the study.

Risks of routine medical procedures:

In this study, we will do some routine medical procedures. These are taking blood and giving injections. These procedures can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore, bleeding, and (rarely) muscle damage or infection at the injection site. Taking blood can cause a low blood cell count (anemia), making you feel tired.

ECG testing:

The gel or stickers used to keep the leads in place during the ECG may irritate your skin and cause redness. It is possible that you may feel stress or anxiety about having the ECG test done, or about the test results.

Risks of sampling rectal and genital fluids:

For semen collection, you may be asked not to ejaculate for a few days before providing the sample. You may find this inconvenient.

Collection of cervical fluids may cause some discomfort. This discomfort is similar to what happens during a pap smear at a routine physical exam. It does not usually last very long.

Collection of rectal secretions may involve use of an anoscope. This may cause temporary discomfort.

During any of these procedures, you may feel anxious or embarrassed. If you feel uncomfortable in any way, please tell us and we will try to help you.

Personal problems/discrimination/testing HIV antibody positive:

About 10 to 20% of people who join HVTN studies report personal problems or discrimination because of joining an HIV vaccine study. Family or friends may worry, get upset or angry, or assume that you are infected with HIV or at high risk and treat you unfairly as a result. Rarely, a person has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

The body makes antibodies to fight or prevent infection. Most vaccines cause the body to make antibodies as a way of preventing infection. Your body may make antibodies to HIV because you received HIV study vaccines. The study vaccines are likely to cause you to test positive on some types of HIV antibody tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccines, a routine HIV test done outside this clinic is likely to say you have HIV, even if you don't. If you have VISP from participating in HVTN 205, these study vaccines may make it last longer. If you had VISP that has already gone away, these study vaccines may make it come back. We do not know how long it will last.

Because you may have VISP, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccines. We can provide you with free HIV testing for as long as you need it. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

It is unlikely, but you could test negative at the end of the study and positive some time later, even though you don't have HIV. This could happen if different HIV tests come into use. We will give you a phone number to call for more information.

Site: Modify the following paragraph if applicable. If someone believes you are infected with HIV even if you are not, you could face discrimination and other problems. For example, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you do have a positive HIV antibody test caused by the study vaccines, you will not be able to donate blood or organs. Your family and friends may treat you differently. We will give you a brochure that tells you more about testing HIV positive because of an HIV vaccine, and how you can avoid some of these problems.

If you become pregnant during or after the study and have VISP, we don't know if the antibodies could be passed to your baby. We know that this happens with other vaccines, like tetanus vaccine. These antibodies from the mother are not a danger to the baby, and

they go away over time. If the baby continues to have VISP, we can do this testing for free for as long as it is needed. For most babies antibodies from the mother last for about six months.

You should always tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive and the delivery staff believes you have an HIV infection, your baby may be started on antiretroviral treatment when it is not needed. If this happens, we can arrange for you and the baby to have a test that can tell the difference between true HIV infection and a VISP result.

Embarrassment/anxiety:

You may feel embarrassed when we ask about your HIV risks, such as having sex and using drugs. Also, waiting for your HIV test results or other health test results could make you feel anxious. You could feel worried if your test results show that you are infected with HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of disclosure of your personal information:

We will take several steps to protect your personal information. Although the risk is very low, it is possible that your personal information could be given to someone who should not have it. If that happened, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information if you would like it.

Risks of genetic testing:

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

U.S. Sites, include the following paragraph. In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work. GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

Unknown risks:

We do not know if the study vaccines will increase, decrease, or not change your risk of becoming infected with HIV if exposed. If you get infected with HIV, we do not know how the study vaccines might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. It could be that a future HIV vaccine may not work as well for you because you got the study vaccines. Currently, no HIV vaccine has been approved for use.

We do not know how the study vaccines will affect a pregnant participant or a developing baby.

Benefits

23. The study may not benefit you.

We do not know whether getting the study vaccines might benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams that you get while in this study might detect health problems you don't yet know about.

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccines later become approved and sold, there are no plans to share any money with you.

Your rights and responsibilities

24. If you join the study, you have rights and responsibilities.

You have many rights that we will respect. You also have responsibilities. We list these in the Participant's Bill of Rights and Responsibilities. We will give you a copy of it.

Leaving the study

25. Tell us if you decide to leave the study.

You are free to leave the study at any time and for any reason. Your care at this clinic and your legal rights will not be affected, but it is important for you to let us know.

We will ask you to come back to the clinic one last time for a physical exam, and we may ask to take some blood samples. We will also ask about any personal problems or benefits you have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

Injuries

Sites: Do not make changes to the following section without obtaining approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org.

26. If you get sick or injured during the study, contact us immediately.

Your health is important to us. *(Sites: adjust the following 2 sentences if applicable to the care available at your site)* We will tell you about the care that we can give here. For the care that we cannot provide, we will explain how we will help you get care elsewhere.

If you become sick or injured in this study, there is a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met. The funds may come from different groups, as described below.

The developers have agreed to pay medical costs for study-related injuries that are determined to be caused by their study products.

For study-related injuries that cannot be funded as described above, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale-appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

Some injuries are not physical. For example, you might be harmed emotionally by being in an HIV vaccine study. Or you might lose wages because you cannot go to work. However, there are no funds to pay for these kinds of injuries, even if they are study related.

You may disagree with the decision about whether your injury is study related. If you wish, the HVTN will ask independent experts to review the decision. You always have the right to use the court system if you are not satisfied.

Annual health contact

27. After your clinic visits end, we will contact you once more, 2 years after your first injection.

We will contact you by phone or email *[Site: Modify mode of contact as appropriate]* to ask questions about your health. If you prefer to answer these questions in person, you can come to the clinic to do this.

If we have any concerns about your health, we may need to have more contact with you. You are also welcome to contact us at any time if you have concerns about your health related to being in the study.

If we ask you to come to the clinic, we will give you [Site: Insert compensation amount] for each visit. This amount is to cover the costs of [Site: Insert text].

If someone outside this study clinic told you that you are infected with HIV, we will ask you to come back to the clinic for another HIV test. We will draw about 15 mL (1 tablespoon) of blood. We may ask you to come back more than once for this testing.

Because we will want to contact you one more time, please tell us if your address or phone number changes, if you are moving away, or if you do not want us to contact you anymore.

You can tell us at any time that you don’t want this health contact. If you do so, you will not lose any benefits or rights you would normally have.

All other information that is discussed earlier in this consent also applies to the annual health contact.

Questions

28. If you have questions or problems at any time during your participation in this study, use the following important contacts.

If you have questions about this study, contact
[name and telephone number of the investigator or other study staff].

If you have any symptoms that you think may be related to this study, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name/title/phone of person on IRB or other appropriate organization].

If you want to leave this study, contact
[name and telephone number of the investigator or other study staff].

Your permissions and signature

29. In section 14 of this form, we told you about optional collection of rectal fluid samples, cervical fluid or semen samples. Please write your initials or make your mark in the boxes next to the options you choose.

☐ I agree to provide rectal fluid.

☐ I do not agree to provide rectal fluid.

☐ I agree to provide semen or cervical fluid.

☐ I do not agree to provide semen or cervical fluid.

Site: Delete the following section if using a separate consent for use of samples and information in other studies.

30. In Section 17 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please choose only one of the

options below and write your initials or make your mark in the box next to it. The HVTN keeps track of your decision about how your samples and information can be used.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include limited genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my cells, or genome wide studies.

31. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

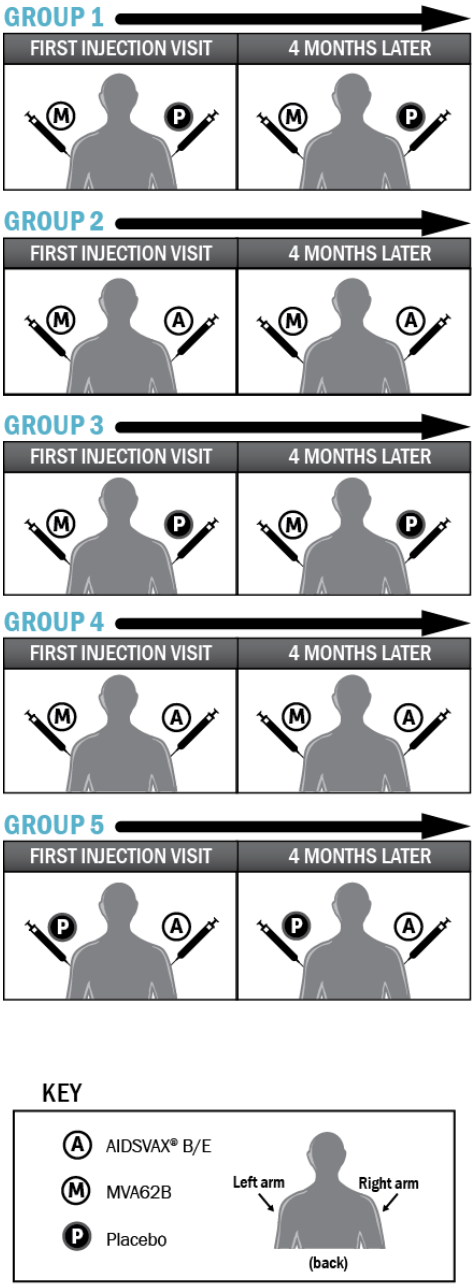
Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix B Injection schedule for sample informed consent form

This table shows the injections you will get while you are in the study. This table does not show all of your study visits.

HVTN 114 injection schedule:



Appendix C Approved birth control methods (for sample informed consent form)

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby.

If you were born female and are sexually active in a way that could lead you to get pregnant, you must agree to use effective birth control, from 3 weeks before your first injection until 6 months after your last study injection.

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your “tubes tied”) or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a female partner or partners;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, you need to use male or female condoms to protect yourself from HIV infection.

Appendix D Sample consent form for use of samples and information in other studies

Title: A phase I clinical trial to evaluate the immunogenicity of AIDSVAX B/E bivalent gp120 vaccine and MVA/HIV62B in healthy, HIV-1 uninfected adult participants who previously received MVA/HIV62B in DNA/MVA or MVA/MVA regimens in HVTN 205

HVTN protocol number: HVTN 114

Site: [Insert site name]

When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies. We will call these "extra samples."

This form gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

1. Do I have to agree?

No. You are free to say yes or no, or to change your mind after you sign this form. At your request, we will destroy all extra samples that we have. Your decision will not affect your being in this study or have any negative consequences here.

2. Where are the samples stored?

Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* Your samples will be stored in the HVTN's central repository in the United States.

3. How long will the samples be stored?

There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

4. Will I be paid for the use of my samples?

No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

5. Will I benefit from allowing my samples to be used in other studies?

Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not needed for your medical care. They are not part of your medical record. The studies are only being done for research purposes.

6. Will the HVTN sell my samples and information?

No, but the HVTN may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

7. How do other researchers get my samples and information?

When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN keeps track of your decision about how your samples and information can be used.

8. What information is shared with other researchers?

The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

9. What kind of studies might be done with my extra samples and information?

The studies will be related to HIV, vaccines, the immune system and other diseases. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.

- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

10. What are the risks of genetic testing?

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

U.S. Sites, include the following paragraph

In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work. GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

11. Who will have access to my information in studies using my extra samples?

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

Questions

12. If you have questions or problems about allowing your samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact
[name and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact [name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact
[name/title/phone of person on IRB or other appropriate organization].

13. Please choose only one of the options below and write your initials or make your mark in the box next to it. The HVTN keeps track of your decision about how your samples and information can be used.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include limited genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above and also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my cells, or genome wide studies.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix E Table of procedures (for sample informed consent form)

	Time after 1st injection visit								
Procedure	Screening visit(s)	First injection visit	1 week	2 weeks	4 months	4 months & 1 week	4½ months	7 months	10 months
Injections		√			√				
Medical history	√								
Complete physical	√								√
Brief physical		√	√	√	√	√	√	√	
Urine test	√			√			√		
ECG (EKG)♦	√								
Blood drawn	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female) *	√	√		√ †	√		√ †	√	√ †
HIV testing and pretest counseling	√				√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√
Rectal fluid/Cervical fluid/Semen collection (optional) ‡		√		√	√		√		√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

♦ An ECG may be repeated at later visits in some cases.

* Persons who had a complete hysterectomy (removal of the uterus) or had their ovaries removed (verified by medical records), are not required to have a pregnancy test.

† Only for participants born female who agree to provide optional rectal and cervical fluid samples.

‡ Includes urine/swab testing for gonorrhea and chlamydia, and blood testing for syphilis each time samples are collected. For persons providing cervical samples, testing will include swabs for trichomoniasis, bacterial vaginosis, and (possibly) yeast infection.

Appendix F Laboratory procedures

					Tube volume (mL)									
				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
				Day:		D0	D7	D14	D112	D119	D126	D196	D303	
				Month:	Screening visit[3]	M0	M0.25	M0.5	M4	4.25	M4.5	M7	M10	
						VAC1			**VAC2**					
Procedure	**Ship to[1, 2]**	**Assay Location[2]**	**Tube[4]**	**Tube size (vol. capacity)[4]**										**Total**
BLOOD COLLECTION														
Screening or diagnostic assays														
HBsAg/anti-HCV	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	5
Syphilis	Local Lab	Local Lab	SST	5mL	5	5[11]	—	5[11]	5[11]	—	5[11]	—	5[11]	30
HIV diagnostics[9]	UW-VSL	UW-VSL	EDTA	10mL	10	—	—	—	10	—	—	10	20	50
Safety labs														
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	—	5	—	—	5	5	—	20
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	5	—	—	5	5	—	20
Cardiac Troponin[6]	Local lab	Local lab	Na Hep	5mL	5	—	—	5	—	—	5	—	—	15
Immunogenicity assays[7]														
Humoral assays														
HIV-1 binding Ab	CSR	Duke	SST	8.5mL	—	8.5	—	8.5	8.5	—	8.5	8.5	8.5	51
Neutralizing Ab	CSR	Duke	SST	8.5mL	—	8.5	—	8.5	8.5	—	8.5	8.5	8.5	51
Ab avidity	CSR	Duke	SST	8.5mL	—	y	—	y	y	—	y	y	y	0
ADCC	CSR	Duke	SST	8.5mL	—	y	—	y	y	—	y	y	y	0
Cellular assays														
HIV-specific ICS	CSR	FHCRC	ACD	8.5mL	—	42.5	—	42.5	—	—	42.5	—	42.5	170
Phenotyping (pTfh)	CSR	FHCRC	ACD	8.5mL	—	—	17	—	—	17	—	—	—	34
Specimen storage														
PBMC	CSR		ACD	8.5mL	—	42.5	—	68	—	—	68	—	68	246.5
Serum	CSR		SST	8.5mL	—	51	—	34	34	—	34	34	34	221
Visit total					**35**	**158**	**17**	**181.5**	**66**	**17**	**181.5**	**71**	**186.5**	**913.5**
56-Day total					**35**	**193**	**210**	**391.5**	**66**	**83**	**264.5**	**71**	**186.5**	
URINE COLLECTION														
Urinalysis	Local lab	Local lab			X	—	—	X	—	—	X	—	—	
Pregnancy test[8]	Local lab	Local lab			X	X	—	X[10]	X	—	X[10]	X	X[10]	
Chlamydia/Gonorrhea[11]	Local lab	Local lab			—	X	—	—	—	—	X	—	—	
CERVICAL/VAGINAL SWAB COLLECTION[12]														
Trichomonas vaginalis	Local lab	Local lab			—	X	—	X	X	—	X	—	X	
Bacterial vaginosis	Local lab	Local lab			—	X	—	X	X	—	X	—	X	
Yeast	Local lab	Local lab			—	X	—	X	X	—	X	—	X	
MUCOSAL SAMPLE COLLECTION (OPTIONAL)[11]														
Semen	CSR	Duke			—	X	—	X	X	—	X	—	X	
Cervical secretion	CSR	Duke			—	X	—	X	X	—	X	—	X	
Rectal secretion	CSR	Duke			—	X	—	X	X	—	X	—	X	

[1] CSR = central specimen repository

[2] HVTN Laboratory Program includes laboratories at UW-VSL, FHCRC, Duke-DHVI, Duke-NAB, and Duke-ADCC. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke = Duke University Medical Center (Durham, North Carolina, USA).

[3] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[4] Local labs may assign appropriate alternative tube types for locally performed tests.

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.3 (postenrollment).

[6] Blood will be drawn for cardiac troponin test if clinically indicated.

[7] Immunogenicity assays will be performed at M0 (for binding Ab assay), M0.5, and M6.5. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other timepoints.

[8] Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[9] At an early termination visit for a withdrawn or terminated participant (Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 9 above.

[10] Pregnancy testing at the indicated visit is only required of participants who are born female and are providing a cervical and/or rectal secretion sample.

[11] Syphilis testing by serology and Chlamydia and gonorrhea testing by urine or swab will only be performed if the participant agrees to provide a mucosal sample.

[12] Cervical/vaginal swabs will only be collected from participants who agree to provide a cervical secretion sample; testing is for Trichomonas vaginalis, bacterial vaginosis, and (if clinically indicated) yeast.

[13] Optional mucosal specimens may be collected once the participant has been found to have met mucosal specimen collection criteria specified in the SSP.

y = 17mL of SST blood collected for binding and neutralizing Ab assays will also cover specimen needs for Ab avidity and ADCC assays; no separate blood draw is needed.

Appendix G Procedures at HVTN CRS (cont. on following page)

Visit:	01[a]	02	03	04	05	06	07	08	09	Post
Day:		D0	D7	D14	D112	D119	126	D196	D303	
Month:		M0	M0.25	M0.5	M4	M4.25	M4.5	M7	M10	
Procedure	Scr.	VAC1			VAC2					
Study procedures[b]										
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	X	—
ECG[c]	X	—	—	—	—	—	—	—	—	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	—	—
Cardiac symptom assessment	—	X	X	X	X	X	X	X	X	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	—
Pregnancy prevention assessment[d]	X	X	X	X	X	X	X	X	X	—
Behavioral risk assessment	X	—	—	—	X	—	—	X	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	X	—	—	X	X	—
Outside testing questionnaire	—	—	—	—	—	—	X	—	X	—
Concomitant medications	X	X	X	X	X	X	X	X	X	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	—
HIV infection assessment[e]	X	—	—	—	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	X	—	—	X	X
Mucosal specimen collection (optional)	—	X	—	X	X	—	X	—	X	—
Local lab assessment										
Urine dipstick	X	—	—	X	—	—	X	—	—	—

[a] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[b] For specimen collection requirements, see Appendix F.

[c] ECG is required at screening and may also be performed at additional timepoints as clinically indicated (Section 9.4.1.3).

[d] Pregnancy prevention assessment is required only for participants who were born female and are capable of becoming pregnant.

[e] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

Visit:	01[f]	02	03	04	05	06	07	08	09	Post
Day:		D0	D7	D14	D112	D119	126	D196	D303	
Month:		M0	M0.25	M0.5	M4	M4.25	M4.5	M7	M10	
Procedure	Scr.	VAC1			VAC2					
Pregnancy (urine or serum HCG)[g]	X	X	—	X[h]	X	—	X[h]	X	X[h]	—
CBC, differential, platelet	X	—	—	X	—	—	X	X	—	—
Chemistry panel (Section 9.2)	X	—	—	X	—	—	X	X	—	—
Cardiac troponin[i]	X	—	—	—	—	—	—	—	—	—
Syphilis	X	X[j]	—	X[j]	X[j]	—	X[j]	—	X[j]	—
Hepatitis B/Hepatitis C	X	—	—	—	—	—	—	—	—	—
Chlamydia/gonorrhea (urine)[j]	—	X	—	X	X	—	X	—	X	—
Trichomonas vaginalis (cervical/vaginal swab)[k]	—	X	—	X	X	—	X	—	X	—
Bacterial vaginosis (cervical/vaginal swab)[k]	—	X	—	X	X	—	X	—	X	—
Yeast, if clinically indicated (cervical/vaginal swab)[k]	—	X	—	X	X	—	X	—	X	—
Pap smear[l]	X	—	—	—	—	—	—	—	—	—
Vaccination procedures										
Vaccination[m]	—	X	—	—	X	—	—	—	—	—
Reactogenicity assessments[n]	—	X	—	—	X	—	—	—	—	—
Poststudy										
Unblind participant	—	—	—	—	—	—	—	—	—	X

[f] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[g] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine initial eligibility may be performed at screening, but must also be done on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[h] Only for participants who were born female and who agree to provide rectal and/or cervical secretion samples.

[i] Blood draw for cardiac troponin is required at screening and may also be performed at additional timepoints as clinically indicated (Section 9.4.1.2).

[j] On-study syphilis testing by serology and Chlamydia and gonorrhea testing by urine or swab will only be performed if participant agrees to provide a mucosal sample.

[k] Cervical/vaginal swabs will only be collected from participants who agree to provide a cervical secretion sample; testing is for Trichomonas vaginalis, bacterial vaginosis, and (if clinically indicated) yeast.

[l] Only for volunteers born female who consent to provide cervical secretion samples. Also not required if volunteer has documented Pap smear within previous 5 years with most recent result normal or ASCUS with no evidence of high-risk HPV, or if high-risk HPV testing was not conducted, Pap smear within the 3 years prior to enrollment, with the latest result reported as normal or ASCUS.

[m] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration, except for results of a serum pregnancy test, if indicated. Lab tests may be drawn within the 3 days prior to vaccination.

[n] Reactogenicity assessments performed daily for at least 3 days postvaccination (Section 9.10).

Appendix H Procedures at CRS for annual health contacts

Contact[a] Day	728
Month	24
Procedures	
Vital status and health events[b]	X

[a] Clinic visits are not required, except that any participant reporting a diagnosis of HIV infection will be asked to come to the clinic so that HIV status can be confirmed.
[b] See Section 9.6.

Appendix I Case definition of myo/pericarditis for use in adverse events monitoring

Myo/pericarditis

Myo/pericarditis is defined as a spectrum of disease caused by inflammation of the myocardium and/or pericardium. Patients might have symptoms and signs consistent with myocarditis, pericarditis, or both. For the purpose of surveillance reporting, patients with myocarditis or pericarditis will be reported as having myo/pericarditis. These categories are intended for surveillance purposes and not for use in individual diagnosis or treatment decisions.

Case Definition for Acute Myocarditis

A suspected case of acute myocarditis is defined by the following criteria and the absence of evidence of any other likely cause of symptoms or findings below:

- Presence of dyspnea, palpitations, or chest pain of probable cardiac origin in a patient with either one of the following:
  - Electrocardiogram (ECG) abnormalities beyond normal variants, not documented previously, including
    - ST-segment or T-wave abnormalities,
    - Paroxysmal or sustained atrial or ventricular arrhythmias,
    - AV nodal conduction delays or intraventricular conduction defects, or
    - Continuous ambulatory electrocardiographic monitoring that detects frequent atrial or ventricular ectopy

    or
  - Evidence of focal or diffuse depressed left-ventricular (LV) function of indeterminate age identified by an imaging study (e.g., echocardiography or radionuclide ventriculography).

A probable case of acute myocarditis, in addition to the above symptoms and in the absence of evidence of any other likely cause of symptoms, has one of the following:

- Elevated cardiac enzymes, specifically, abnormal levels of cardiac troponin I, troponin T, or creatine kinase myocardial band (a troponin test is preferred);
- Evidence of focal or diffuse depressed LV function identified by an imaging study (e.g., echocardiography or radionuclide ventriculography) that is documented to be of new onset or of increased degree of severity (in the absence of a previous study, findings of depressed LV function are considered of new onset if, on follow-up studies, these findings resolve, improve, or worsen); or
- Abnormal result of cardiac radionuclide imaging (e.g., cardiac MRI with gadolinium or gallium-67 imaging) indicating myocardial inflammation.

A case of acute myocarditis is confirmed if histopathologic evidence of myocardial inflammation is found at endomyocardial biopsy or autopsy.

Case Definition for Acute Pericarditis

A suspected case of acute pericarditis is defined by the presence of

- Typical chest pain (i.e., pain made worse by lying down and relieved by sitting up and/or leaning forward) and
- No evidence of any other likely cause of such chest pain.

A probable case of acute pericarditis is a suspected case of pericarditis, or a case in a person with pleuritic or other chest pain not characteristic of any other disease, that, in addition, has one or more of the following:

- Pericardial rub, an auscultatory sign with one to three components per beat,
- ECG with diffuse ST-segment elevations or PR depressions without reciprocal ST depressions that are not previously documented, or
- Echocardiogram indicating the presence of an abnormal collection of pericardial fluid (e.g., anterior and posterior pericardial effusion or a large posterior pericardial effusion alone).

A case of acute pericarditis is confirmed if histopathologic evidence of pericardial inflammation is evident from pericardial tissue obtained at surgery or autopsy.