Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Robb ML, Eller LA, Kibuuka H, et al. Prospective study of acute HIV-1 infection in adults in East Africa and Thailand. N Engl J Med 2016;374:2120-30. DOI: 10.1056/NEJMoa1508952

RV217 protocol documents

**Further details on statistical methods are provided in the manuscript.*

PROTOCOL RV 217

HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand

Short Title: Early Capture HIV Cohort (ECHO)

Study Conducted By
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Study Funded By
Division of AIDS (DAIDS)
U.S. Military HIV Research Program

RV 217/WRAIR #1373 Main Protocol
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Summary

This is a multi-center, non-randomized clinical observational study to be conducted in two parts, a pilot study to assess feasibility (Part A) and the full study (Part B). The purpose of the study is to characterize recruitment, retention, human immunodeficiency virus (HIV) prevalence, HIV incidence and biological characteristics of acute HIV infection in high-risk volunteers in Africa and Southeast Asia. The first part of the trial, Part A, is a small pilot study to establish and optimize the recruiting, retention and study compliance at each participating site. Based upon this pilot, the study will expand to full enrollment as planned (Part B) with operational procedures and study event schedules adjusted as needed with a corresponding amendment prior to initiating Part B. The study itself, as conducted in both Parts A and B, incorporates two phases. The main study activity, or phase I, is the observational cohort or surveillance activity. In contrast to standard cohort studies which are designed with regular visits at monthly to six monthly intervals, this study proposes to collect very small blood samples via finger stick collections similar to diabetic monitoring of blood glucose. These "small blood volume" (SBV) visits will occur twice weekly (until the participant completes visit G, and then will occur once per month) and afford the opportunity to diagnose HIV infection prior to the advent of detectable antibody by the most sensitive techniques available. The acceptability and feasibility of twice weekly monitoring is unknown and for this reason, a pilot part of the study is planned to optimize methods and engage target communities effectively (Part A). Participants with incident HIV infections observed during phase I of the study will be asked to enter long-term follow-up in phase II of the study. Phase II of the study observes HIV infected individuals for an extended period to evaluate early events, chronic viral burden and early disease progression. The protocol is organized into a base protocol, which describes both Parts A and B as well as both phases I and II and the scientific rationale and analysis plan. The site-specific addenda describe in greater detail implementation plans for the study at each site. Protocol team members for each of the four enrollment sites are listed in the relevant site-specific addenda.

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TABLE OF CONTENTS

SCHEMA

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HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand

Objectives

PRIMARY OBJECTIVES

1) Define the risk behavior, prevalence and incidence of HIV infection and retention of a high risk cohort of adults in Thailand, Uganda, Kenya and Tanzania.
2) Obtain 150 acute HIV infections (AHI) with at least 30% captured within Fiebig stages I and II to support the full characterization of host responses and viral dynamics.
3) Observe an increased incidence with at least 30% of the incident cases identified prior to the advent of detectable antibody.

SECONDARY/EXPLORATORY OBJECTIVES

1) Assess and optimize HIV diagnostic strategies in HIV primary infection across multiple subtypes and risk groups.
2) Define the genetic diversity and evolution of HIV-1 in the prevalent and incident HIV cases with particular emphasis on characterization of acute, primary HIV infection.
3) Characterize immune activation, innate and adaptive cellular immunity in the early acute HIV-1 infection.
4) Characterize B cell responses in peripheral and mucosal compartments arising in early acute HIV-1 infection.
5) Characterize genetic polymorphisms in genes controlling host restriction, innate and adaptive Immunity, and their influence on HIV acquisition and early control of HIV infections.
6) Characterize clinical events including endemic infection as risk factors for HIV acquisition.

Study Design

Phase I: Non-randomized, cohort, prospective, 24-month observational study to be conducted in two parts. Part A is a pilot study to establish and optimize operations and study design features to meet study objectives prior to opening the study to full enrollment at all sites in Part B. The study will conduct a screening visit and initial

follow-up in all enrolled volunteers. This will include both HIV negative and HIV prevalent cases. Prevalence is estimated to be high in these populations and as many as 1000-1500 volunteers will be enrolled and evaluated in the first two visits with an enrollment target of 500 HIV negative, high-risk volunteers per site. Subsequently, only HIV negative volunteers will be followed for a period of approximately 24 months, except for a small number of HIV positive individuals for masking to minimize risk of stigmatization. After the initial two visits, volunteers will be seen at the research clinic approximately every 3 months. In addition, at locations that are convenient to the volunteers, small blood volume samples will be collected using microvettes twice weekly. These samples will be analyzed and results returned to the site within 48 hours to permit new HIV positive participants to be identified while they remain antibody negative, HIV nucleic acid test positive. All newly infected participants will be referred to phase II of the study.

Phase II: study of HIV incident cases arising within phase I. HIV incident volunteers identified at any follow-up visit will be asked to consent to participate in phase II to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of at least 50 months from the time of entering the AHI phase. Very frequent visits using a more intensive collection of samples initially will occur to characterize acute HIV pathogenesis and then at regular three month intervals for the duration of the study.

Participants

The study population will include men and women, aged 18-50 years old, who may be members of the following high risk groups: female and male sex workers (SWs), barworkers (BWs), sexually transmitted infection clinic attendees (STIAs), motorcycle passenger transporters (Boda-Boda), transgenders (TG), and men who have sex with men (MSM). Although these populations are known to be among "Most at-Risk Populations" or MARPs, not all members of these occupational groups engage in equivalent risk behavior. Enrollment will therefore engage members of these populations whose behavior places them at higher risk.

Part A (pilot study), will enroll primarily SWs and BWs in Africa and SWs, TGs and MSM in Thailand. Each site will enroll up to 200 volunteers. Part B (full study) will enroll all of the MARPs to attain enrollment of 300 HIV negative, MARPs volunteers at each site in the following groups. Some shifting of enrollment numbers per site might occur in order to complete the study at the same time at all sites.

Kenya: SW; STIA

Tanzania: BW

Uganda: SW; STIA, BW and Boda-Boda

Thailand: SW, MSM and TG

Study Duration

Duration of enrollment will vary by site according to HIV prevalence and volunteer interest. Approximately nine months will be required for enrollment into Part A at all sites. Part B will commence when a site achieves 3 Fiebig 1/ 2 AHI and documents an increased incidence. Part B will require an additional 12-18 months to complete enrollment and all HIV negative, high-risk volunteers participate in phase I for 24 months. Thus complete enrollment and follow-up for phase I, the surveillance component of the study will require 57 months (21 months for Part A and 36 months for Part B). HIV infections will be followed for a minimum of 50 months and total duration from first enrollment, follow-up and data analysis will require at least 10 years.

Study Funded By

Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)

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LIST OF ABBREVIATIONS AND DEFINITIONS

TERM	DEFINITION
ACASI	Audio Computer Administered Self-Interview
ACD	acid citrate dextrose
ADCC	antibody dependent cellular cytotoxicity
AFRIMS	Armed Forces Research Institute of Medical Sciences
Ag/Ab	antigen/antibody
AHI	acute HIV infection
AI	associate investigator
AIDS	Acquired Immunodeficiency Syndrome
ALT	alanine transaminase
APOBEC	apolipoprotein B-editing catalytic polypeptide-like subunit
Boda-Boda	motor cycle passenger transporters
BW	Barworkers
CAB	Community advisory board
CAP	College of American Pathologists
CBC	complete blood count
CCR5+	chemokine receptor 5
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence interval
CLSI	Clinical Laboratory Standards Institute
CNV	copy number variation
CRF	case report form
CRR	continuing review report
CTL	cytotoxic T lymphocyte
CVL	cervicovaginal lavage
DAIDS	Division of AIDS
DC	dendritic cells
DCAC	Data Coordinating and Analysis Center
DHSP	Division of Human Subjects Protection
DLDM	Department of Laboratory Diagnostics and Monitoring
DMO	data management officer
DMS	data management supervisor
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate

DoD	Department of Defense
DSMB	Data Safety Monitoring Board
EDTA	ethylenediaminetetraacetic acid
EI	exposed infected
EIA	enzyme immune assay
ELISA	enzyme-linked immunosorbent assay
EM	expectation maximization
env	envelope
ERC	ethical review committee
EU	exposed uninfected
FL	full-length
FSW	female sex worker
GALT	gut associated lymphoid tissue
GCP	Good Clinical Practice
GEIS	Global Emerging Infections Surveillance
GLP	Good Laboratory Practices
GWAS	Genome-wide association studies
HAART	Highly Active Anti-Retroviral Therapy
HEPS	highly exposed persistently seronegative
HISIS	HIV Superinfection Study
HIV	human immunodeficiency virus
HIVNET	HIV Network
HJF	Henry M. Jackson Foundation for Advancement of Military Medicine
HLA	human leukocyte antigen
HPTN	HIV Prevention Trials Network
HSR	hypersensitivity reactions
HSV-2	Herpes Simplex Virus Type 2
HTA	high transmission area
HW	Hardy-Weinberg
ICF	informed consent form
IFNγ	interferon gamma
Ig	immunoglobin
IgG	immunoglobin G
IgM	immunoglobin M
IP	internet protocol
IRB	institutional review board
KEMRI	Kenya Medical Research Institute
KIR	killer immunoglobulin receptor
LBV	large blood volume
LD	linkage disequilibrium
LFT	liver function tests
LFU	lost to follow-up
LLV	lower level viremia

LMU	Ludwig-Maximilians-University of Munich
LPS	lipopolysacharide
m	monomeric
MARPs	most at-risk populations
MEPS	Military Entrance Processing Stations
MHA	multi-region hybridization assay
MMRP	Mbeya Medical Research Programme
MO	Medical Officer
MOP	Manual of Operations
MoPH	Ministry of Public Health
MSM	men who have sex with men
MSW	male sex worker
MUWRP	Makerere University Walter Reed Project
MVA	modified vaccinia virus Ankara
NAAT	nucleic acid amplification technologies
Nab	neutralizing antibodies
NAD	nicotinamide adenine dinucleotide
NCCLS	National Community for Clinical Laboratory Standards
NCR	natural cytoxicity receptors
NGO	non-governmental organization
NHS	normal human serum
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	natural killer
OE	Onto-Express
OHRP	Office for Human Research Protections
p	polymeric
PAMP	pathogen-associated molecular patterns
PAM-R	prediction analysis of microarrays in the R workbench
PAVE	Partnership for AIDS Vaccine Evaluation
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEP	post-exposure prophylaxis
PEPFAR	President's Emergency Plan for AIDS Relief
PHSC	Public Health Service Center
PI	principal investigator
PrEP	pre-exposure prophylaxis
RCC	Regulatory Compliance Center
RDS	respondent driven sampling
RLU	relative luminescence units
RNA	ribonucleic acid
RPR	rapid plasma reagin
RR	rate ratios

RSS	Research Support System
RTA	Royal Thai Army
RT-PCR	reverse transcription polymerase chain reaction
s	secretory
SAM	significance analysis of microarrays
SBV	small blood volume
SC	seroconverter
SE	southeast
SGA	single genome amplification
SIV	simian immunodeficiency virus
SN	seronegative
SNP	single nucleotide polymorphisms
SOE	schedule of events
SRV	steadily rising viremia
ST	Smart Maximal Antibody Response Tube, SMARTube
STI	sexually transmitted infections
STIA	sexually transmitted infection clinic attendees
SW	sex worker
TG	Transgender
TLR	toll-like receptors
TOU	Test of Understanding
TRIM5	TRIpartite Motif-5
UNAIDS	The Joint United Nations Programme on HIV/AIDS
US	United States
USA	United States of America
USAMC	United States Army Medical Component
USAMRMC	United States Army Medical Research and Materiel Command
USAMRU-K	United States Army Medical Research Unit-Kenya
USFDA	United States Food and Drug Administration
USG	United States Government
MHRP	United States Military HIV Research Program
VCT	voluntary counseling and testing
VRC	Vaccine Research Center
VTN	Vaccine Trials Network
WB	Western Blot
WBC	white blood cells
WDC	withdrawal of consent
WGA	whole genome amplification

WRAIR	Walter Reed Army Institute of Research
WRP	Walter Reed Project

1.0 INTRODUCTION

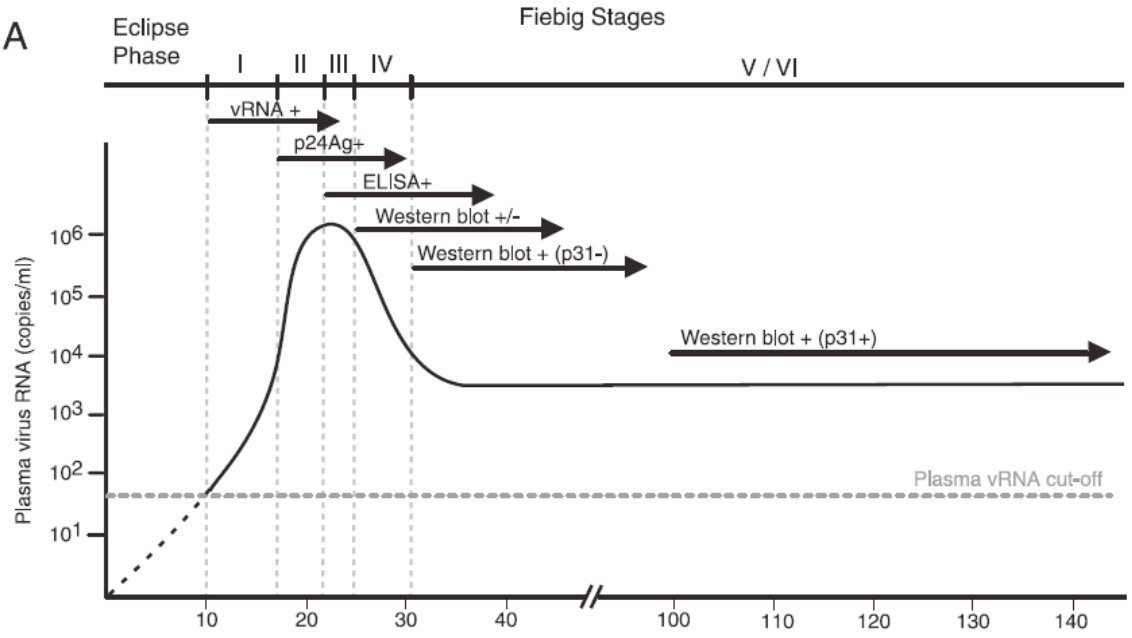
The HIV vaccine field has been roiled by the results of STEP, the Merck/NIH-VTN phase IIB "proof of concept" efficacy trial (Science 2007). The failure of this candidate vaccine to prevent infection or afford discernable control of viremia early after infection has called into question the merits of any vaccine strategy focused entirely upon induction of T cell mediated immune responses. Further, the apparent increase in transmission rate in a subset of participants not only alters the risk to benefit analysis of prophylactic vaccine trials but underscores our limited knowledge of transmission events and factors that modulate transmission efficiency. A consensus has formed that a more fundamental understanding of the basic pathogenesis of HIV infection and disease is required (Science 2008, Walker 2008). Further, the design of future "proof of concept" efficacy trials will need to carefully consider designs which limit experimental vaccine exposure to the minimum number of volunteers required.

HIV disease progression rates vary substantially and correlate with early viremic set-point (Mellors 1997; Lyles 2000). Individuals who exert poor control over viral replication in this early phase of infection are destined to progress rapidly to immune deficiency and death and conversely those who are able to substantially control viral replication enjoy relatively prolonged survival. Rarely, individuals are able to suppress viral replication below levels of detection for extended periods and appear to remain healthy for an indefinite period (Deeks 2007). Recent work has shown that the gut associated lymphoid tissue (GALT), the predominant reservoir of CD4+ T cells, suffers massive depletion of memory CD4+ T-cells during the first weeks following SIV infection of macaques (Veazey 1998; Mattapallil 2005) or HIV infection in humans (Guadalupe 2003; Brenchley 2004). Further, the primary target for HIV in the acute infection are memory T cells with specificity for HIV antigens (Douek 2004; Lichterfield 2007; Strapans 2004). The extent to which the GALT is preserved correlates with survival in non-human primate models receiving candidate preventive SIV vaccines (Mattapallil 2006). In addition to the critical role of early viral replication and the viral set-point, it has been shown that T cell activation levels independently are associated with CD4+ T cell decline and that this parameter is also established as an early set point as humans emerge from the acute phase of infection (Deeks 2004). A recent report finds a strong correlation between viral load in the acute phase of illness and viral load set point (Kelley 2007). This study also showed a higher acute viral burden was associated with more symptomatic primary HIV illness. Individuals with symptomatic primary infection have higher viral load set-points and higher mortality rates (Henrad 2000; Lavreys 2006).

These observations suggest that the long-term course of HIV disease progression is established in the first weeks of infection. It is critical to understand the early events associated with GALT T-cell depletion and the mechanisms responsible for the variable control of viral replication. This work in humans has been limited to individuals who have passed the critical time points where the dynamics of host-viral interaction dictate the level of acute viral replication and influence the ultimate steady state of chronic viremia. It is hoped that understanding what events distinguish the elite controllers from those with a typical course of HIV infection or from those who progress rapidly will afford insights leading to novel prevention and treatment strategies. It is apparent that the events that define these differences occur very early after infection.

The current classification of acute infection relies upon the hierarchical humoral immune response and the dynamic characteristics of HIV detection as defined by Fiebig et. al., (Fiebig 2003), and outlined below in Figure 1(Salazar-Gonzalez 2008).

Figure 1 Fiebig Stages



The earliest phase of infection, the eclipse phase, precedes HIV ribonucleic acid (RNA) detection and is estimated to last from a few days to 11 days (Kahn 1998). Fiebig stage I follows with detection of viral RNA only. Fiebig stage II is characterized by an increased RNA signal and detection of p24 antigen. Fiebig stage III is similar to stage II but includes a positive sensitive enzyme immuno-assay (EIA) identifying HIV specific antibodies. Fiebig stage IV, V and VI ensue with an indeterminate Western blot, followed by a positive Western blot absent a p31 signal and finally a fully positive Western blot, respectively.

The duration of each Fiebig stage for non-B clade HIV infections is not known. The relationship of the eclipse phase and subsequent Fiebig stages, in terms of duration or amplitude of viral load, to the viral load at set-point and the long term clinical course is not known. It is plausible that innate immunity and genetically determined host defense mechanisms are critical determinants to preservation of the GALT and the long-term effectiveness of the host adaptive response to HIV. This study seeks to identify acute infection in stages I and II prior to establishing peak HIV viremia and potentially to characterize the eclipse phase to better define the early pathogenic events, characterize host-viral interactions and explore their relation to long term outcomes in terms of viral set-point and immune system integrity.

1.1 State of MHRP Vaccine Research

The primary mission of the United States Military HIV Research Program (MHRP) is to develop strategies to prevent HIV infection and the major objective of this effort is establishing a vaccine for prevention of HIV infection across the globe. There are two obstacles that arise towards the achievement of this objective: 1) the determinants of protective immunity to HIV are unknown; and, 2) the role of genetic diversity in limiting protective immunity is unknown. The MHRP is advancing a vaccine strategy for efficacy testing in Thailand to address the first obstacle in an environment with a single subtype of HIV in circulation (subtype E). Clinical testing of vaccines in East Africa permits an evaluation of the role of genetic diversity as subtypes A, C and D are contributing in varying proportions to the HIV epidemic in the region.

Together with partner organizations, MHRP has identified at least one site in each of the East African countries of Uganda, Tanzania, and Kenya, and another in Thailand that could serve as vaccine efficacy trial sites. The study proposed here is part of a series of studies aimed at developing Phase IIb-III clinical trial capability through identification and preparation of cohorts for possible phase III HIV vaccine efficacy trials. Efforts in each country are independent collaborations between the MHRP, local partners, academic centers and/or host country medical research institutes. However, an attempt it made to integrate activities at all sites. By combining capabilities, training and experience from each site, MHRP seeks to achieve a sufficiently cohesive organization to conduct a single trial at all the sites in each of the countries or to contribute collaboratively as a network to larger multi-national clinical trials.

As noted above, there is a need for further basic research defining early events in HIV infection, which might guide vaccine development. To this goal, the proposal addresses both basic cohort definition and a series of exploratory objectives aimed at discovery of correlates of protection from HIV infection or disease.

The MHRP has three vaccine products in clinical development. These are the ALVAC/gp120 prime/boost clade B and CRF01 AE vaccines currently in efficacy testing in Thailand, the Vaccine Research Center (VRC) deoxyribonucleic acid (DNA) prime/recombinant Adenovirus type 5 boost which have been evaluated at MHRP in East African sites and will be undergoing efficacy testing on a small scale in 2009 and finally a DNA prime, modified vaccinia virus Ankara MVA boost vaccine under development within MHRP.

The results of the Phase III HIV vaccine clinical trial involving more than 16,000 adult volunteers in Thailand demonstrated that an investigational HIV vaccine regimen was safe and modestly effective in preventing HIV infection in the modified intent-to-treat analysis. According to final results released by the trial sponsor, the U.S. Army Surgeon General, the prime boost combination of ALVAC® HIV and AIDSVAX® B/E lowered the rate of HIV infection by 31.2% compared with placebo.

In the final analysis, 74 placebo recipients became infected with HIV compared to 51 in the vaccine regimen arm. The efficacy result is statistically significant as the lower bound of the 95% confidence interval is above zero. The vaccine regimen had no effect on the amount of virus in the blood of volunteers who became HIV-infected during the study.

This study, RV144, tested a prime-boost vaccine strategy that combined two vaccines based on strains (subtypes) of HIV that circulate in Thailand. The prime canary pox vector called ALVAC-HIV vCP1521, was developed by Sanofi Pasteur; the booster vaccine, AIDSVAX B/E, that was previously tested in two efficacy studies and developed by VaxGen but now licensed to Global Solutions for Infectious Diseases. The results of RV 144 demonstrate that an efficacious HIV vaccine for the prevention of HIV vaccine is now possible, and we plan to utilize the cohorts developed through this proposal for further efficacy testing.

The VRC candidate vaccine may also be tested in its current formulation in these cohorts should the small efficacy trial commencing in 2009 show interim safety. It is expected that the MHRP DNA and MVA product will be ready for efficacy testing in about five to six years. Clearly, identifying and characterizing high-risk cohorts is needed for anticipated efficacy trial needs eventuating over the next three to six years.

The feasibility and acceptability of working in these high-risk populations with frequent visits to ascertain very early HIV infection will be established through conduct of RV 217 parts A and B. If successful, it is clear that this population would be suitable for a number of intervention studies aimed at reduction of secondary transmission, alteration of long term prognosis and potentially, radical cure of HIV. These studies may include behavioral, anti-retroviral and vaccine interventions alone or in combination. The investigators are very interested in these studies and applying lessons learned from the proposed study to the optimal design and execution of acute HIV infection intervention research.

1.2 Cohort Development Activities

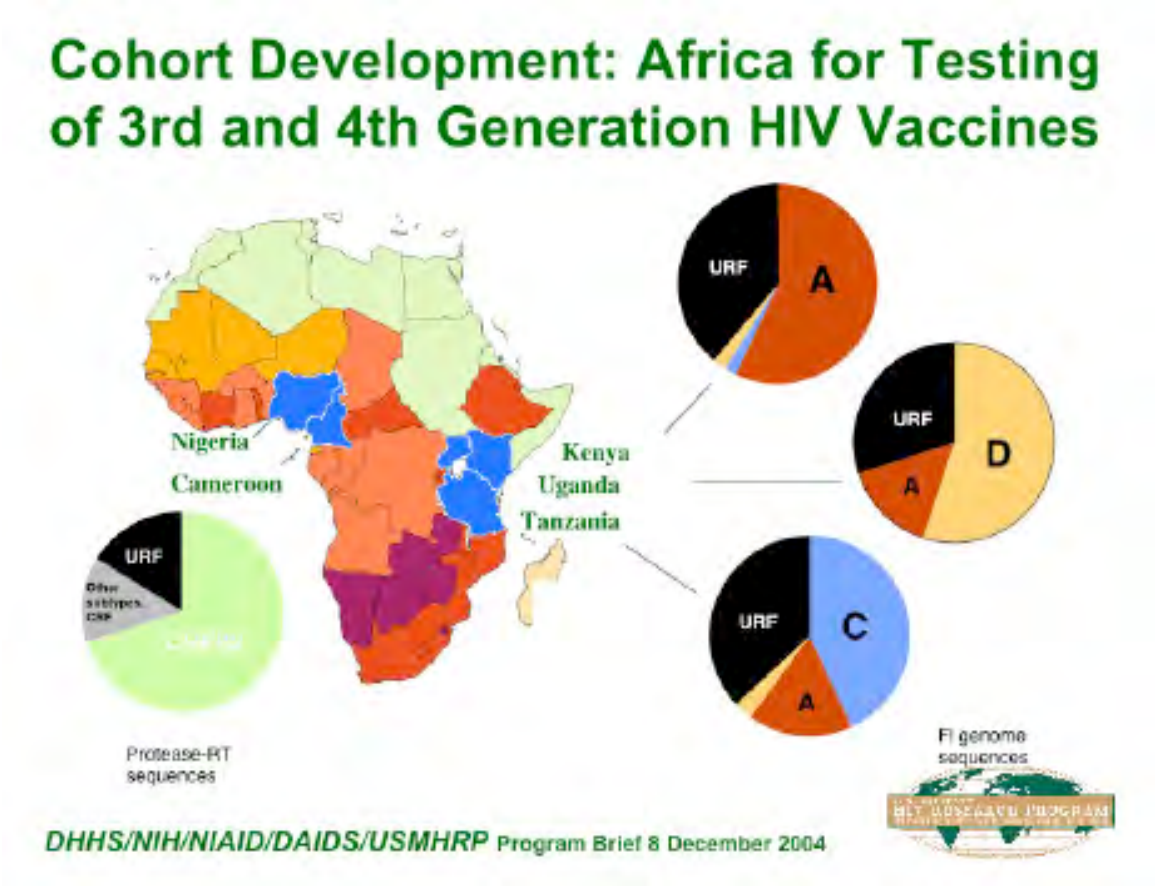
The MHRP has sought long-term partnerships to conduct HIV vaccine research in keeping with the goals, processes and considerations discussed above. Initial cohort development activities in Thailand were conducted to support evaluation of the ALVAC/gp 120 prime-boost vaccine strategy. These efforts included seven studies at various sites within Thailand. The highly commendable AIDS control program in Thailand has limited potential populations for the conduct of further efficacy trials to high-risk populations, such as sex workers, MSM, and transgendered individuals. To further achieve strategic requirements, populations with high incidence of multiple HIV subtypes must be identified. A review of the prevalence rates and distribution of genetically defined subtypes indicates that Africa provides a setting with severe epidemics of multiple subtypes. In view of the need for political stability and will to address HIV/AIDS, the presence of an infrastructure and cadre of trained scientific collaborators as well as other considerations, the MHRP has expanded its cohort development activities to support future efficacy trials in three African countries: Uganda, Tanzania, and Kenya. As shown below, these sites provide the opportunity to evaluate vaccines within communities with four of the prevalent sub-types of HIV (A, C, D and CRF02_AG (IbNG), along with the two sub-types prevalent in Thailand (B and CRF01_AE).

The epidemic in all three East African participating countries is substantial and in most respects similar among the three (UNAIDS 2007). Within each of these three countries the MHRP is currently conducting vaccine cohort development activities that have thus far identified

populations with prevalence and incidence sufficient to contribute to HIV vaccine trials with reasonable efficiency (12-month incidence greater than 1.3% - 1.8%).

In addition, the sites offer an important difference in respect to the molecular epidemiology of the epidemic at each site based upon full-length sequence data. The distribution of subtypes is shown below in Figure 2. More detailed analysis identifies the major subtype in each region contributing disproportionately to the recombinant forms found in that region. For example, in Kenya, pure A subtype is most common and most recombinants include substantial subtype A genetic contributions.

Figure 2: Cohort Development: Africa for Testing of 3rd and 4th Generation HIV Vaccines



Of critical importance, the incidence and follow-up characteristics have been evaluated in the context of closed cohorts. To some extent, experiences at the three African sites have varied. At the Tanzania site, relatively high follow-up rates were identified in all three of the site's cohorts and in the general population cohort no decline in incidence was observed over time. By contrast, the tea plantation cohort in Kericho has observed steadily declining incidence over time and had low follow-up rates early but have shown considerable improvement over time with adjustments to compensation, follow-up schedules, and research study staff participation and study "ownership."

RV 217 seeks to not only define the cohort characteristics of Most at-Risk Populations (MARPs), but also seeks to identify very early HIV infections. It is possible to model closed cohort characteristics in an open cohort design. The retention of these MARPs is not known and replacement enrollments for loss to follow-up and non-compliance may be needed to acquire the number of acute infections specified. Details of the procedures and circumstances for additional enrollment are noted in the protocol Manual of Operations (MOP).

1.3 Most At-risk Populations (MARPs)

Current Phase IIb/III HIV vaccine efficacy study protocols are focusing upon populations with annual incidence of at least 2% and preferably 3% with yearly loss to follow-up of 10% or less. Although current MHRP sites may reach these metrics with respect to a 2% incidence and 90% annual follow-up, when age constraints are imposed to identify the higher risk elements of these cohorts, it is considered necessary to explore MARPs as potential participants along with our established cohorts. The purpose of this study is to develop data for key parameters used to assess the suitability of these additional populations.

In addition, this cohort activity represents an opportunity to determine if such MARPs are interested in participating in HIV vaccine research and provides a vehicle for positive community engagement as well as HIV education, prevention, and treatment referrals where applicable in MARPs often in need of such services.

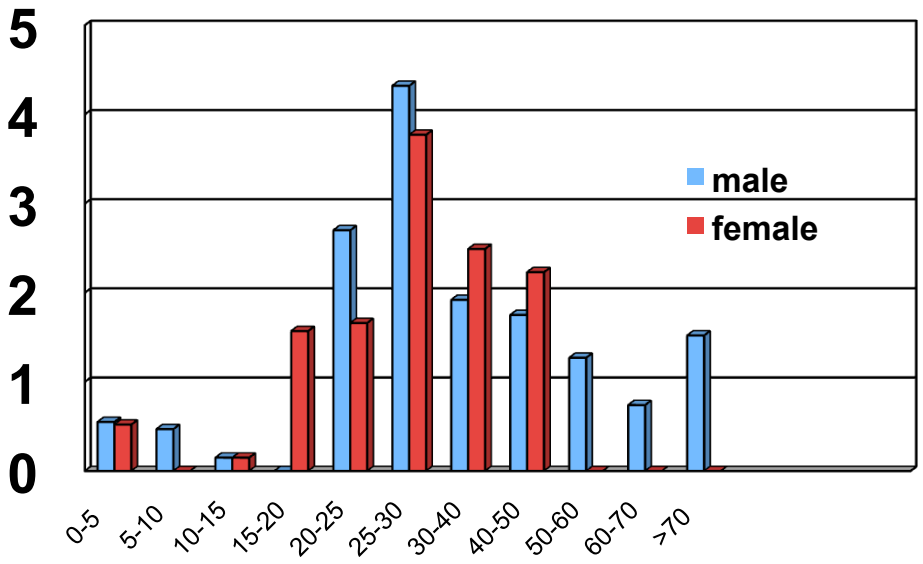
The populations to be included in this study have been the subjects of considerable research and their increased risk for HIV infection is well established (Estebanez 1993). Each site has assessed likely incidence of study sub-populations based upon direct experience, published information or personal communication from those performing studies in these groups. Incidence rates are shown below:

sex workers (SW)/barworkers (BW): Kenya 3%,
SW only: Uganda 4%
BW, motor cycle passenger transporters (Boda-Boda): Uganda 3%, Tanzania 7%
sexually transmitted infection clinic attendees (STIAs): Kenya and Uganda 3%
SW, men who have sex with men (MSM), transgender (TG): Thailand--similar cohorts in Bangkok suggest an incidence of 4% (3.5 – 5%);

Despite the high incidence in these MARPs, concern has been raised about the potential to include these groups in intervention studies due to high mobility and unacceptable rates of loss to follow-up. Recent work from the Tanzania group has successfully demonstrated an operational approach to one of these high risk groups, bar workers, which maintained an aggregate follow-up of 75% over three years (Hoffmann 2004). At that site, a cohort of 600 female BWs was established with the aim of studying their HIV-1 status, subtypes of HIV-1 and immunological and virological correlates of infection. Data collection based upon 3-month follow-ups included laboratory data for HIV and selected sexually transmitted infection (STI), clinical and behavioral data.

The HIV-incidence over a 3-year follow-up was 7 per 100 person-years (Hoffmann 2004). Newer unpublished data, obtained through the ongoing EMINI surveillance, a population based, observational cohort of 20,000 individuals, could confirm high prevalence and incidence rates for HIV in "high-transmission-areas". In this study nine distinct geographical areas with a total population of 220,000 individuals were registered and a subset of 10% was randomly selected for the surveillance. Five of the nine sites are close to the transafrican highway and called "high-transmission-area" for HIV. We found HIV incidence rates of up to 4% in this study group. As this surveillance is done in the general population and does not focus on high-risk behavior or occupations, we expect a higher incidence in high-risk groups, similar to the findings of the HIV Superinfection Study (HISIS) described above.

Figure 3 HIV Incidence in 5 "high-transmission-areas" in the Mbeya Region from 2006/2007 to 2007/2008



The overall HIV-prevalence was 68.0% in the 600 participants initially enrolled into the cohort. The yearly HIV-incidence was 15.2, 6.2 and 2.4 per 100 person-years in year 1, 2 and 3 respectively (Riedner 2006). The most difficult task concerning a cohort involving a MARP would be to take account of the mobility of the study participants. In the cohort described above the baseline survey determined that only 12% of the eligible population worked more than three years in the same workplace. The remarkable retention of 75% over three years (Hoffmann 2004) can be attributed to the trust built within the target group, their employers, and local authorities before the study participants were enrolled and that this trust was maintained throughout the study.

Other data support a high incidence in the populations targeted for enrolment in RV 217. Several studies have documented high incidence rates in female sex workers (FSWs) (3.2-9.8%), bar-workers (4.27-5%), and truck drivers (3-4%) in the African setting (Kaul 2004; Laga 1993; Van Loggerenberg 2008; Rakwar 1999; Jackson 1997; Riedner 2006; Watson-Jones 2008; Baeten

2000). In two studies, the temporal trend of infections identified a preponderance of incident cases arising in the first 6-12 months of study (Riedner 2006; Baeten 2000).

1.4 Clinical Events Including Endemic Infection As Risk Factors For HIV Acquisition

Results of the recent STEP study of the Merck Ad5 vaccine showed a correlation between pre-existing Ad5 antibodies and increased susceptibility to infection to an Ad5 based vaccine (Science 2007). One possible explanation for increased susceptibility is that the Ad5 vaccine vector boosted an existing primed-Ad5 adaptive memory immune response and the resultant secondary immune response predisposed volunteers to an increased susceptibility to HIV infection, perhaps by a mechanism as simple as stimulating the production of more chemokine receptor 5 (CCR5+) CD4+ T-cells, the primary target of early HIV infection. If a vaccine indirectly increased susceptibility to HIV infection by stimulating an immune response then it is reasonable to hypothesize that other non-vaccine stimulants of adaptive immunity would also increase HIV susceptibility. Since many diseases are known to induce robust humoral and cellular immune responses, we hypothesize that individuals with co-incident malaria, helminthes, or sexually transmitted infections will have an increased risk of HIV transmission. An increase in HIV replication has been documented in association with malaria infection *in vitro* (Xiao 1998; Frobel 2004) and *in vivo* (Hoffmann 1999; Kublin 2005), and recently, that acute HIV infection (AHI) is highly prevalent in patients suspected of malaria (Bebell 2008). To explore this issue, we propose to estimate the association of HIV infections with recent malaria infection, sexually transmitted infections, other infectious diseases as discerned by interim medical history, physical examination and laboratory markers of parasitic infestation, gut endothelial integrity and systemic immune activation.

1.5 Scientific Background

1.5.1 HIV Diagnostics

Primary HIV infection causes diverse non-specific clinical symptoms that typically do not lead to HIV diagnosis (Daar 2001), and the usual serological methods for laboratory diagnosis of established infection do not turn positive for weeks to several months (Horsburgh 1989, Ciesielski 1997). The seroconversion window duration has narrowed due to emergence of 3rd generation EIAs which detect Immunoglobulin M (IgM) as well as Immunoglobulin G (IgG), and 4th generation assays that detect both antibody and p24 antigen (Ly 2001). While these advances have incrementally narrowed the seroconversion window, little is known about relative performance of these assays using non-subtype B seroconversion panels. The majority of studies that allow for direct comparison of assay sensitivity during early seroconversion have utilized samples from patients in developed nations with subtype B infection, leaving questions about the generalizability of such data, particularly since most available assays are based on subtype B strains (Peeters 2003). Studies to date have assessed subtype detection using viral culture material or chronically infected individuals, whereas seroconversion panels tested have been from patients infected with subtype B (Ly 2001). Implementation of combination tests in many

locations is constrained by cost and laboratory factors that would be at least partially addressed if antigen/antibody (Ag/Ab) rapid tests were available.

In less cost-constrained environments, early diagnostic strategies have focused on detection of either viral antigen (Fiscus JCM 2007) or nucleic acid (Busch AIDS 2005) for diagnosis of primary infection. Here again, studies comparing methodologies for detection of acute infections have been conducted using subtype B seroconversion panels (Busch Transfusion 2005). A study to evaluate methods of detecting clients with acute HIV infection in Malawi compared the Roche Amplicor Monitor RNA assay v1.5 to p24 antigen assays and several antibody tests (Fiscus JID 2007), but the methodology employed did not include serial specimen collection that would allow for assay-assay comparison.

A stereotyped pattern of sequential appearance of diagnostic markers has been defined for subjects with primary subtype B infection consisting of sequential appearance of RNA, p24 antigen, and anti-HIV antibodies (Fiebig 2003). While proper characterization of early infection stage affords the ability to describe viral and immune dynamics among infected individuals, the sequence and duration of laboratory stages are not known for patients from developing nations with non-B subtype infections.

Most HIV surveillance has relied on seroprevalence estimates using samples collected from sentinel populations. However, incident infection surveillance provides a more timely and proximate estimation of recent transmission, yielding much greater utility to prevention programs and site selection for interventions such as microbicides and vaccines. Major efforts have been underway to develop methods of incidence estimation using a single blood sample. All such approaches rely on the well-documented crescendo anti-HIV humoral immune response that occurs during the first several months of infection. The Centers for Disease Control and Prevention (CDC) has been the leader in the field, and currently considers three approaches worthy of further study. First, incident cases may be reactive using the sensitive EIA but non-reactive using a less sensitive assay (Gouws 2002; Janssen 1998). Secondly, CDC has been developing a strategy to estimate the proportion of anti-HIV-1 IgG in total IgG following seroconversion (Hargrove 2008; Parekh 2002). Finally, incidence may be estimated using HIV antibody avidity (Suligoi 2008; Loschen2008; Chawla 2007, Martro 2005; Suligoi 2003). Development of these strategies has been limited by lack of appropriate samples to allow for statistical analysis, which requires sequentially collected samples from the same individual including an HIV-negative test and positive tests <1 year after the last negative test, as well as three or more positive tests spaced at ≥ 3 month intervals (personal communication, Bernard Branson, CDC 02MAY08).

Smart Maximal Antibody Response Tube, SMARTube, for *in vitro* stimulation of anti-HIV antibody from *in vivo* primed B lymphocytes

The Smart Maximal Antibody Response Tube, SMARTube HIV & HCV (ST), is a tissue culture tube containing 2 ml of culture medium with a proprietary cocktail of stimuli which promote proliferation and differentiation of HIV or HCV antigen primed B lymphocytes from fresh whole blood specimens. Two published reports outlined below and discussions with the product developer suggest that this pretreatment device accelerates production of anti-HIV antibody to levels detectable by commercially available serological diagnostic assays and thus enables detection and diagnosis of window period seronegative acute/primary HIV infected individuals

weeks to months prior to seroconversion (Novikov I 2009; Mumo J 2008). In corporation of this assay into ECHO screening may provide a powerful means to resolve the infection status of ECHO participants who periodically test Aptima reactive, but seemingly do not progress; thus, providing a means to discriminate between Aptima false reactive ("spurious blips") and the actual detection of RNA signal and resolution of HIV infection status.

Novikov and Jehuda-Cohen report the development of this "Stimmulogy" assay and its use to identify seronegative HIV infected individuals among two Ethiopian émigré populations immigrating to Israel in 1992 and 1998. Parallel serological testing of paired de-identified pre and ST stimulated herapinized samples was conducted. Pre ST plasma samples were tested directly by standard EIA. For ST samples, 1 ml of heparinized whole blood was added to the culture tube and incubated in a 5% CO_2 humidified incubator at 37^0C for five days. Post incubation ST plasma was tested by conventional HIV enzyme immunoassays (EIA) following adjustment of EIA diluents to compensate for dilution into the smart media. Studies with Smart media and post incubation stimulated ST plasma demonstrated that neither ST plasma, nor Smart media impacted the signal to cut-off for EIAs in conventional HIV EIAs. Of 285 samples from the 1992 population, 7/285 were HIV infected by both standard and ST EIA. An additional 8/285 (2.7%) were identified as HIV EIA reactive, HIV Western Blot positive post ST stimulation. Of these, 5/8 pre stimulation and 2/8 post stimulation samples were PCR positive. Of the 1998 population samples, 26/537 (4.84%) pre stimulated samples were HIV positive. Post ST stimulation an additional 2/537 window period seronegative HIV infected individuals were identified (Novikov I 2009). Mumo et al 2009 using the same approach examining adult and student Kenyan blood donors demonstrated similar findings.

In addition to the aforementioned studies, Mumo et al, 2009 demonstrated that this assay identified HIV infected individuals weeks to months prior to seroconversion and detection by standard serological screening assays. Of 20 pregnant women visiting an antenatal clinic and tested for HIV antibody, 7/20 were HIV infected by both standard and ST EIA. Among the 13 seronegative women, 5/20 were HIV antibody reactive post ST stimulation. Serconversion estimates based on taking the midpoint between the time of last seronegative and first seroreactive sample by standard EIA of serial samples indicated that ST stimulation detected seronegative individuals 3-10 months earlier than standard EIA (Mumo J 2008). This phenomena, earlier detection weeks to months prior to seroconversion, has also reportedly been demonstrated in current clinical trials underway with this device (personal communication, Dr. Jehuda-Cohen).

Finally, application of this technology to recent infection detection has been proposed. While not yet fully mature as a concept, a Stimulation Index for estimating incidence based on a semi-quantitative comparison of antibody levels in plasma to ST stimulated plasma has been proposed and is under consideration and development.

These provocative findings strongly suggest, albeit they have not been independently corroborated, that ST stimulation can accelerate the production of anti-HIV antibody from HIV primed lymphocyte populations leading to early detection and diagnosis in seronegative HIV infected individuals. If true, these findings also suggest that the immune system may be capable of regulating viral exposure and subsequent progress of infection for prolonged periods in some individuals; that the eclipse phase extends well beyond current consideration.

1.5.2 Viral Evolution

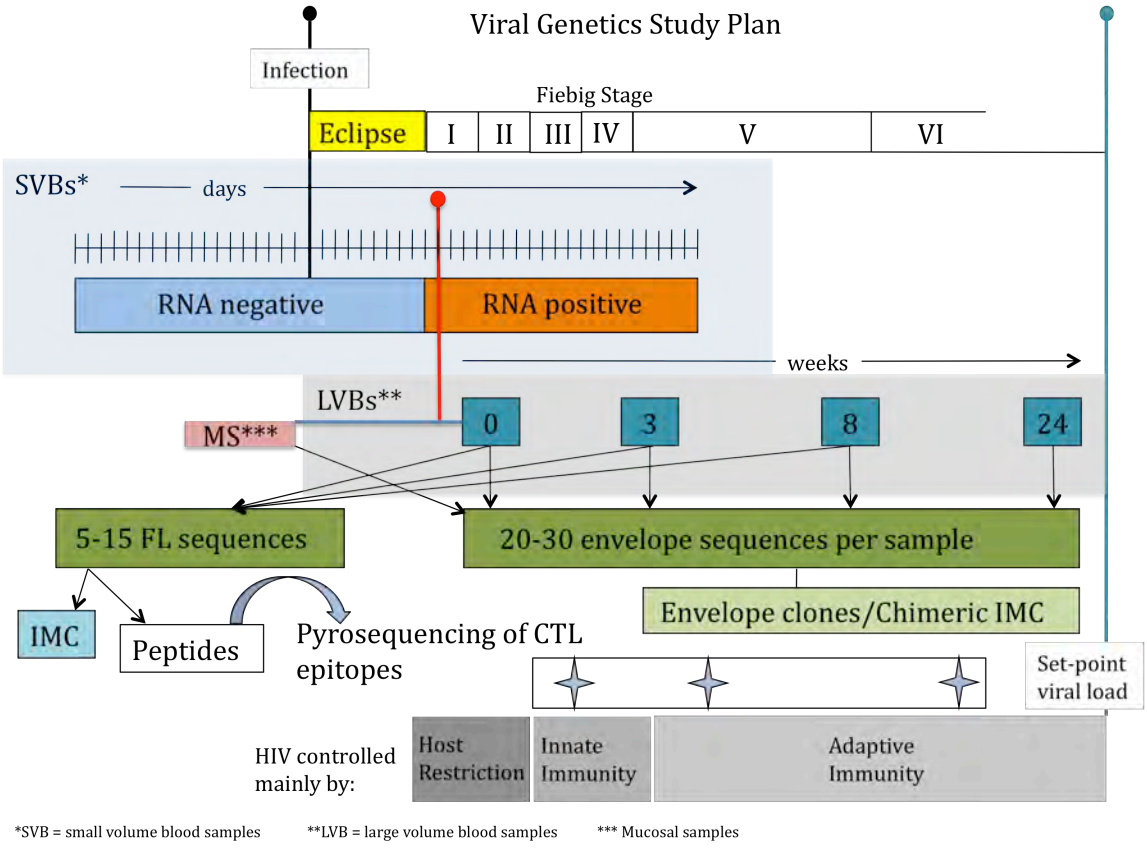
From the moment of infection, multiple lines of host defense impinge on HIV-1 (Goff 2004; Hilleman 2004). First, host restriction factors, such as co-receptor polymorphisms (Carrington 1999a; O'Brien 2000), Apolipoprotein B-editing catalytic polypeptide-like subunits (APOBECs) (Harris 2004; Malim 2006; Holmes 2007) and TRIM5-alpha (Kaumanns 2006; Speelmon 2006; Li 2007), control the establishment of a productive infection, influence the magnitude of the initial viremia, and have a continuing impact on the subsequent development of effective viral control. Next, innate immune mechanisms, already in place before viral infection occurs, take control of virus spread in the infected individual, using both cellular (NK) and humoral (antibody dependent cellular cytotoxicity (ADCC)) effector mechanisms to identify and lyse infected cells and provide for efficient viral recognition and clearance (Little 1999; Jacobs 2005; Boyton 2007). Concomitantly, the adaptive immune response is triggered, and its effectiveness is dependent on the outcome of the earlier interactions (da Silva 2003; Douek 2006; Davenport 2007; Nabel 2007). If host restriction and innate immunity are relatively ineffective, the adaptive responses may be mounted in an environment of massive immune system damage, dis-coordination, and unfavorable cytokine milieu, and the adaptive immune system is faced with a viral quasispecies that results from unbridled replication and a corresponding exponential increase in quasispecies diversity (Coffin 1996; Ferbas 1996; Liu 2002; Troyer 2005; Lemey 2007; Tebit 2007). In a positive feedback loop, poorly constituted adaptive responses lead to further uncontrolled viral replication, and diminish the long-term benefits that would ordinarily accrue from effective adaptive cellular and humoral responses in the years to come. The commonly used outcome measure for host-virus interaction, viral load set-point, is the aggregate result of all of these host defenses, is further modulated by the host genetic (Gottlieb 2008) background and the infecting viral strains, and is not established until an equilibrium has been reached between the virus and host, typically around 6 months post-infection. During the first six months of infection, significant changes occur in the diversity and neutralization susceptibility of HIV-1 envelope (Wei 2003; Derdeyn 2004; Gottlieb 2008; Liu 2008) and some cytotoxic T lymphocyte (CTL) responses have already generated escape variants that come to dominate the population (Karlsson 2007; Liu 2007; Loh 2008); investigation of the earliest responses to adaptive immune responses is a critical element of HIV vaccine development.

A significant gap in knowledge exists regarding the specifics, timing, range, and relative effectiveness of early control mechanisms for HIV-1. Barriers to knowledge have been both logistical, driven by the relatively short interval between introduction of HIV-1 into the human host and the onset of effective viral control (Busch 1997; Kahn 1998; Little 1999), and technical, related to the inherent difficulty of access to and efficient sampling a diverse viral quasispecies during the earliest time period. Here we propose both traditional and novel approaches to define the initial viral quasispecies, in order to gather direct evidence for selection by host restriction factors, innate immune mechanisms, and from the earliest manifestations through the complete development of adaptive cellular and humoral immunity. Novel features of this proposal include: 1) an exploratory look-back study for the presence and characteristics of proviral DNA in the clinical eclipse phase (Fiebig 2003), after infection but prior to the first detectable plasma RNA (Fiebig 2005); 2) serial, in-depth early sampling of the envelope quasispecies before, during, and after the ontogeny of high affinity antiviral antibodies; 3) application of pyrosequencing, capable of sampling at least 1000 templates from the initial viral quasispecies, to investigate the contribution of CTL escape variants, present before the adaptive cellular

response is mounted, to early escape from CTL; and 4) use of the yeast-based recombination/cloning system to efficiently generate infectious molecular clones of HIV-1 representing the earliest accessible viral strain(s) and some of the selected variants arising early in infection.

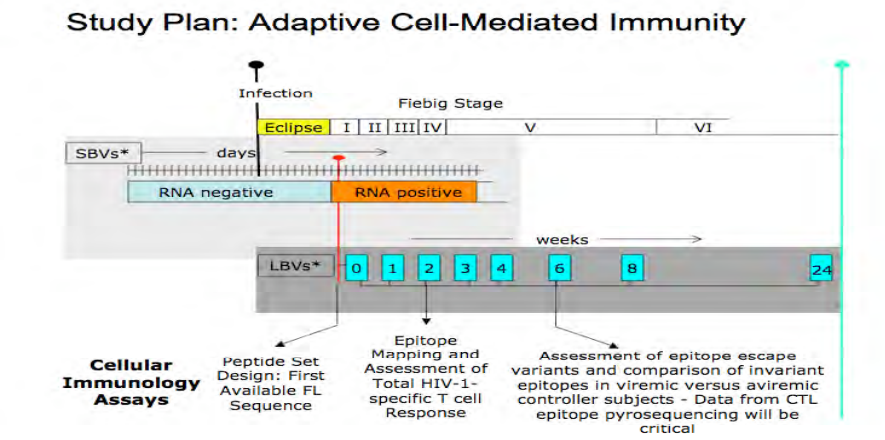
The viral genetics of the approximately 150 acute seroconverters will be studied within a strong contextual framework of accurate and consistent Fiebig staging (Fiebig 2003), early intensive clinical follow-up of seroconverters, maintenance of large, relevant, exposed uninfected cohorts, host genetic analysis, evaluation of innate and adaptive immune responses and the early impact of HIV-1 infection on gene expression profiles in key target cells, evaluation concurrent infection with selected endemic pathogens and of generalized immune activation, and a nested sub-study of mucosal damage and mucosal responses to HIV-1. Finally, the study will include four of six globally prevalent HIV-1 subtypes/ circulating recombinant forms (McCutchan 2006; Taylor, 2008) and two genetically distinctive human populations (Cao 2004) and (Kikak, G, unpublished data).

Figure 4 Viral Genetics Study Plan



1.5.3 Cellular Immunology

Figure 5 Adaptive Cell-Mediated Immunity



Adaptive T Cell Immune Responses to HIV-1

Defining the precise correlates of the cellular immune response to HIV-1 that are associated with control of HIV-1 replication has been the focus of intense study over the past two decades. The identification of such correlates has been pursued as part of a rational strategy to produce an HIV-1 vaccine capable of best mimicking the cellular immune response associated with control of HIV-1 viremia. Once defined, such correlates will also help establish the necessary benchmarks for candidate vaccine down selection and efficacy testing. While indisputable proof that $CD8^+$ T cell activity is the causative selective pressure that controls viral load in either acute or chronic infection is not available (Letvin 2006; Letvin 2007; Walker 2008), an overwhelming abundance of circumstantial evidence supports this notion: the association of $CD8^+$ T cell activity with virus clearance during acute infection (Borrow 1994; Koup 1994; Brander 2002); the clear association of certain human leukocyte antigen (HLA) class I alleles with delayed progression to Acquired Immunodeficiency Syndrome (AIDS) (Gao 2001; O'Brien 2001; Carrington 2003; Gao 2005; Altfeld 2006; Martin 2007) and the molecular imprinting of HLA-allele associated mutations in the viral proteome both at the population level and within infected individuals (Kiepiela 2004; Bhattacharya 2007; Brumme 2007; Rousseau 2008); the positive selection of virus escape mutations within, and proximal to epitopes targeted by $CD8^+$ T cells (Wilson 1999; Kelleher 2001; Draenert 2004; Leslie 2006), the transmission of these escape mutants to a new host (Goulder 2001; Leslie 2004; Leslie 2005; Goepfert 2008), and the subsequent reversion of these escape mutants in hosts that cannot make an immune response against them (Allen 2004; Feeney 2004; Leslie 2004; Allen 2005; Goepfert 2008). Furthermore, the quality of the HIV-specific $CD8^+$ T cells in controlled versus uncontrolled infection differs with respect to both their functional capacity and their surface phenotype. Described functional abnormalities of $CD8^+$ T cells in uncontrolled HIV-infection include a reduced capacity to proliferate which is linked to a reduction in both autocrine IL-2 production and surface CD28

expression (Trimble et al., 2000; Migueles 2002; Topp 2003; Lichterfeld 2004; Rethi 2005), reduced production of Interferon gamma (IFNγ), and a general decrease in the number of cells with a multifunctional phenotype (Betts 2004; Betts 2005; Betts 2006; Streeck 2008). The differentiation status of HIV-specific $CD8^+$ T cells is also compromised in HIV-infection, resulting from the disregulation of expression of a variety of surface molecules including, but not limited to, Immunoglobulin (Ig)-superfamily molecules, TNFRSF receptors and ligands and chemokine receptors (Day 2006; Trautmann 2006; Wang 2007; Elrefaei 2008; Holm 2008). Hence, it has become apparent that measuring any given parameter in a univariate approach does not provide a meaningful assessment of the quality of the adaptive cellular immune response against HIV-1. A systematic multi-factorial approach to studying the cellular immune response as early as possible after infection with HIV-1 would identify critical facets of the adaptive immune response associated with control or delay of initial peak viremia, and help establish important immune parameters that should be measured during vaccine trial assessment.

Analysis of HIV Antigen Specific Immune Responses in Uninfected Volunteers

Transient elevation of type I interferons and other cytokines and chemokines occurs prior to peak viremia in acute HIV infection, followed by TNF-α and IFN-γ, suggesting that systemic activation of plasmacytoid dendritic cells leads to a cascade of to activation and recruitment of other immune cells including NK cells and eventually T cells. Recently, Tiemessen, CT. *et al* demonstrated that HIV-1 specific responses in NK cells could be detected in the $CD3^-/CD8^+$ compartment only in the presence of plasma from fresh heparinized whole blood in infants born to HIV^+ mothers who remained uninfected (Tiemessen 2009). The ability to reproduce and build upon this finding in other cohorts, especially those with other types of repetitive high risk HIV exposure, is critical to understanding innate HIV specific immune protection.

T-cell Immune Activation

HIV-1 infection is associated with immune activation that involves T-cell lymphocytes and is characterized by an increased expression of cell surface markers some of which include CD25, CD38, CD69, HLA DR, β-2-microglobulin and neopterin (Fauci 1993). The most common measure of activation is CD38, an ectoenzyme important to the metabolism of T lymphocytes, and HLA-DR, which measures a class II major histocompatibility complex antigen. The degree of immune activation for any HIV infected individual is widely variable but during chronic infection is thought to be associated with level of viremia, CD4 T cell counts, and disease progression (Giorgi 2002; Barry 2003; Hazenberg 2003; Eggena 2005), and have prognostic value for individuals on antiretroviral therapy (Tilling 2002; Ondoa 2005). Moreover, it is thought that baseline activation is higher in HIV seronegative Africans due to other infections as compared to Europe and North America (Kassu 2001). Additionally, it would be useful to describe the effects of infecting HIV-1 subtype on the immune system as measured through activation and regulatory T cell surface markers. Hence, it would be informative to assess T cell activation pre-infection, and at successive time-points post-infection to follow the evolution of this phenomenon. This approach may also reveal pre-existing immune activation correlates that increase risk of infection or the rapidity with which the virus replicates early in infection.

Regulatory T cells

Regulatory T-cells have been proposed to down regulate the immune reponse within the CD4 and CD8 T-cell compartments and loss of their function due to HIV-1 infection may be associated with hyperimmune activation and subsequent CD4 depletion observed in natural

infection resulting in disease progression (Eggena, 2005). Some of the commonly distinguishable markers of regulatory T cells include CD25 (IL-2 receptor) bright expression, CD62L (L-selectin) high, and Foxp3 (forkhead box protein 3 transcription factor). Most studies have addressed immune activation and the role of regulatory T-cells in the context of cross sectional studies or longitudinal analysis of HIV positive volunteers on antiretroviral therapy, therefore not much is characterized about early HIV infection. Additionally, it would be useful to describe the effects of infecting HIV-1 subtype on the immune system as measured through activation and regulatory cell surface markers.

Natural Killer Cells

The specific role of innate immunity during HIV-1 infection is poorly defined, but may offer some insight into control, clearance, and moderation of infection and disease progression. NK cells are a subset of bone marrow derived large granular lymphocytes that are responsible for anti-tumor or anti-viral target recognition, lysis, and cytokine and chemokine production during initial infection until the adaptive arm can fully respond (Yokoyama 2004). It is known that T cells are to some extent linked with controlling HIV-1 infection and certain functional profiles may be associated with slow HIV-1 disease progression in infected individuals (Betts et al., 2006), indicating the importance of a vigorous adaptive immune response. Despite the association of adaptive T cells with partial control of HIV-1, decline of peak viremia during acute infection is poorly understood. During the early events in acute HIV-1 infection before the adaptive arm can fully respond the innate arm of the immune response may play a critical role in initial viral control, driving the adaptive arm, and may influence disease progression. Rapid expansion of certain subsets of NK cells, with a preferential increase in cytolytic NK cells, occurs in acute HIV-1 infection and is reduced after the emergence of HIV-1 specific CD8 T cells (Alter 2007b). Additionally, NK cell cytolytic response to HIV-1 is as potent as CD8 T cells *in vitro* (Kottilil 2003) and thus could be a strong containment force during this initial phase of infection. NK cells are regulated by a vast array of stimulatory and inhibitory receptors designed for a non-antigen specific recognition of infection and divided into 4 classes: killer immunoglobulin-like receptors (KIR), C-type lectin receptors, the natural cytotoxicity receptors (NCR), and the toll-like receptors (TLR) (Biassoni 2001). Chronic HIV-1 infection has been associated with a switch from inhibitory to activating C-type lectin receptor expression (Mela 2005) and a down regulation of NCRs (De Maria 2003). More recently focus has been placed on certain KIRs that interact with HLA molecules on potential target cells. The activating receptor KIR3DS1 interacts with certain HLA-B alleles and is associated with delayed progression to AIDS (Martin 2002), decreased NK cell activation and increased function (Long 2008). Furthermore, interactions between KIR and HLA have been shown to directly influence the functional ability of NK cells to control HIV-1 replication (Alter 2007a). KIR2DL3 is associated with NK cell activation, loss of function and inversely correlates to CD4 percentage in perinatally HIV-1 infected children (Ballan 2007). There is also evidence that certain KIR and HLA interactions may affect the risk of HIV transmission (Jennes 2006).

Further studies are needed to understand how subsets of NK cells contribute to protection and pathogenesis of HIV-1 infection. Here we propose to study the frequency, function, and phenotype of NK cells in acute HIV-1 infection. Analysis of phenotypic changes will focus on subset distribution defined by CD56 and CD16 expression, and expression of inhibitory and activating receptors. Functional assessment of NK cells will be made based on degranulation, cytokine, and chemokine production after stimulation with media, MHC^{null} K562 cell line, or PMA/ionomycin. The data on NK cells will be analyzed in relation to the immune responses

detected, and potential associations with viral subtype, host genetic factors and disease progression will be determined.

Functional Genomics and HIV Surrogate Marker Exploration

Functional genomics is a powerful tool to investigate host responses to infection and therapy. The success of gene chips is due, in part, to their ability to interrogate the entire genome, offering the broadest and most comprehensive coverage of the transcriptome. Many genetic polymorphisms affect disease trajectory, drug response or propensity to develop drug toxicities. Known host determinants of HIV-1 disease progression include genes for chemokine receptors (eg, CCR5 delta 32) and HLA molecules (eg, B57 and B27), natural killer cell killer immunoglobulin-like receptor (KIR3DS1), and APOBEC3F and APOBEC3G proteins. There are likely more unknown host genetic determinants yet to be discovered. While the search for elusive correlates of protection from HIV disease progression continues, we propose to use global gene expression profiling because it offers the most comprehensive coverage of the human genome - especially those genes involved in immunity or response to viral infection. Global gene expression profiling of the earliest emergent adaptive HIV-specific T cells in acute infection provides the most likely approach to discovery of novel correlate(s) of protection. In combination with other novel and exploration assay systems it would from a central component of a systematic multi-factorial approach to identify critical facets of the adaptive immune response associated with control or delay of initial peak viremia.

The Proteome Analysis of Activated CD4+ T-Cells Infected with Different Clades of HIV-1

MHC class I molecules are expressed on nucleated cells whereas MHC class II molecules are predominantly expressed on professional antigen presenting cells. For the clearance of intracellular pathogens, epitopes bound to either MHC class I or MHC class II molecules are presented at the cell surface for interaction with $CD8_+$ T-cells and $CD4_+$ T-cells, respectively (Cresswell 2005; Ramachandra 2009). Antigen processing and presentation is a complicated process and there are significant differences between MHC class I and class II pathways, although antigens that enter the MHC class II pathway are capable of being presented by MHC class I molecules and vice versa (Giodini 2010).

MHC class I processing involves many proteins such as ubiquitination proteins, chaperone proteins, loading and transporter proteins, and proteases. These proteins need to work in a defined order for the antigens in the cytosol to be proteolytically cleaved and presented on the cell surface as an 8-10 amino acid epitope bound to an MHC class I molecule (Rock 2004). In MHC class II processing, antigens are taken up by the cell through endocytosis / phagocytosis. The antigens are processed using a different series of proteases and chaperone molecules and presented on the cell surface as a 12-15 amino acid epitope bound to an MHC class II molecule (Ramachandra 2009). Disruption of any protein(s) involved in the MHC class I or class II pathways can either negate the presentation of antigenic epitopes on these molecules or lead to an altered epitope repertoire.

Intracellular pathogens have devised numerous methods for evading the host's immune response. One principal mechanism is by interfering with antigen processing and presentation thus preventing the appearance of foreign peptides on the cell surface (Loureiro 2006). The predominant protease is the proteasome complex that is required for the creation of a vast majority of MHC class I precursor epitopes. The proteasome consists of two forms, the

constitutive proteasome found in all cell types and the immunoproteasome found in cells activated with interferongamma. The proteasome is a barrel shaped complex of 4 rings consisting of 28 subunits. The alpha subunits (a1-a7) make up the outer rings and the beta subunits (b1-b7) make up the inner rings. The three active enzymatic subunits of the constitutive proteasome, b1, b2, and b5 are replaced with inducible subunits (b1i, b2i, and b5i) for the formation of the immunoproteasomes, leading to changes in the enzymatic activity of the proteasome complex and an altered epitope repertoire (Klotzel 2004; Steers 2008).

HIV-1 interferes with antigen processing and presentation by disrupting the composition of the immunoproteasome and by down regulating the MHC class I molecule. HIV-1 Tat, an early stage antigen prevents the b1i subunit incorporation into the immunoproteasome (Gavioli 2004) and HIV-1 Gag-p24, a late stage antigen alters the immunoproteasome composition by interfering with the PA28b and b2i subunits (Steers 2009). The alteration of the immunoproteasome composition at different stages of the viral replication cycle could potentially alter the repertoire of MHC class I precursor epitopes. HIV-1 Nef protein decreases the expression of MHC class I molecules at the cell surface (Stove 2006) thus preventing the HIV-1-peptide-MHC complex from interacting with cytotoxic CD8+ T-cell lymphocytes. Presently, the impact of HIV-1 infection on other proteins intricately involved in MHC class I processing and presentation is unknown.

1.5.4 Humoral Immunology

Historically, vaccines that induce antibodies have been the most effective strategy to combat viral diseases such as polio, hepatitis, measles, and influenza. While antibodies are known to play an important role in protection in these diseases, the importance of antibodies in human immunodeficiency virus type 1 (HIV-1) protection and pathogenesis remains to be further defined (Zolla-Pazner 2004; Srivastava 2005; McMichael 2006; Huber 2007). Despite this and because of knowledge gained from other successful vaccines, the design of a vaccine that will elicit functional antibody responses directed against multiple HIV clades continues to be an important goal in laboratories that are developing and testing vaccines. It has been proposed that, although the virus envelope (Env) proteins have evolved an extraordinary ability to evade neutralizing antibodies, a vaccine that can elicit protective antibodies remains the best hope for developing an HIV vaccine that confers sterilizing immunity. Vaccine strategies that exclusively stimulate T-cell immunity may at best generate persistent and broadly reactive T-cell responses that can suppress virus and limit damage caused by the virus, without preventing infection (McMichael 2006). Characterization of the development and function of HIV antibodies in patients infected with both B and non-B subtypes will therefore be critical for understanding the role of humoral responses in both sterilizing and non-sterilizing immunity. It will also be critical to determine whether or not HIV-1 clade has a direct influence on functional humoral responses, in order to effectively inform vaccine design.

In addition to the viral Env proteins, antibodies may be generated to several HIV antigens. Antibody responses to conserved structural proteins like Gag are thought not to have any antiviral function, but these responses may provide correlates of immunity. For example, declining or absent Anti-Gag antibody responses are associated with disease progression (Forster 1987; Weber 1987). The kinetics of the antibody response also may correlate with overall immunity. In a study of antibody responses during structured treatment interruption, it has been

demonstrated that those who have a robust and quick anti-Gag response showed decreased virus set point. In addition, the magnitude of the Gag antibody response correlated with specific CD4 T helper frequency (Trkola 2004). In human vaccine trials, the correlation between antibodies and CD4 help has been provocative to date and will garner more attention as more vaccines elicit both potent antibody and CD4 responses. Long-lived plasma cells are the ultimate goal for HIV vaccines in order to maintain the vaccine induced antibody responses once the appropriate antibody responses are identified. In recent human vaccine protocols (Goepfert 2007; Johnson 2005; McFarland 2006), durable antibody responses were detected many weeks after the last vaccination. This work has suggested that memory B cell responses may be developed as a result of vaccination. Analyses of the kinetics and strength of this durable antibody response in primary HIV infection could enable more informed vaccine design for elicitation of a strong memory response.

The impairment of development of memory B cell responses and the alterations to the humoral immune system in general, that occur in HIV-1 infection have been recognized since the beginning of the epidemic. One of the first observations was that some patients developed autoantibodies and other autoimmune phenomena (Kopelman 1988; Kaye 1989). Since then many investigators have noted the defective humoral response in patients with poorly controlled HIV-1 infection (De Milito 2004; Moir 2001) and that restoration of immune function through the use of Highly Active Anti-Retroviral Therapy (HAART) may (Moir 2008) but does not necessarily result in repair of the humoral responses (Bekker 2006). There is evidence of both a dramatic loss of memory B cells with infection (Titanji 2006) and a shift in the circulating populations of B cells in HIV-1 disease (Malaspina 2006). Most of these studies have been carried out in patients with chronic HIV-1 infection, and the exact nature of changes in acute HIV-1 infection has not been fully characterized. Some of the critical questions that therefore remain to be answered are: What are the alterations in B cell subsets among control subjects, acutely infected patients, and chronically infected patients, particularly in patients infected with non-B HIV-1 subtypes? What are the circulating B cells that can be detected with HIV-1 antigen-specific B cell reagents in these samples? From which subsets are these cells detected and are there differences in these subsets between epitopes and/or in the time course of these cells found in the periphery? If specific antibody populations are detected in functional assays, can we recover the specific B cells using the newer technologies to recover the antibody genes of interest for molecular analysis?

Related to the questions of B cell subsets are also questions regarding the antigen-specificity of the B cells during acute infection. Since the response to HIV-1 appears to be skewed against the production of broadly neutralizing antibodies until late in infection, if at all, the fate of B cells capable of reacting to epitopes that are targets of these antibodies is an open question. That such cells exist at some stage of B cell development is expected based on the diversity of the antibody repertoire, since 10^{10}-10^{14} potential antibodies can be generated via the genetic machinery of antibody gene rearrangement (Sanz 1991). Understanding the fate of these cells (i.e. whether they are deleted prior to emigration from the bone marrow, are deleted or tolerized in the periphery, or are present and anergic) may allow the generation of strategies to ultimately target these cells for the development of an effective vaccine.

Regarding the production of detectable antibodies in HIV-1 infection, previous studies have shown that HIV-1 seroconversion occurs in a range from 8 days to 8-12 weeks from onset of

clinical acute HIV-1 infection (AHI) (Ho 1985; Carne 1985; Fiebig 2003). IgM reactive with virus-infected cells has been detected during the course of AHI (Cooper 1987), but the antigen specificity of these antibodies and the precise timing of the earliest antibody has not been determined. Early studies performed in cohorts developed by CHAVI indicate that gp41 antibodies develop very early; these studies have been possible thus far using samples from clades B and C infections. Following HIV-1 transmission, it has been estimated that there is a window of opportunity of time between transmission and establishment of the latently infected pool of CD4 T cells (approximately 25 days) for a preventive vaccine to work (Johnston 2007; S.B. Justin Wong 2007). Thus, critical questions are: What are the first antibodies that arise following HIV-1 transmission and are these antibodies the same across clades? The detailed analyses of binding antibodies to several HIV antigens at multiple, closely spaced time points post-HIV RNA detection in peripheral blood will allow us to address this question in cohorts where clades A, C, D, and CRF01_AE are prevalent. In addition, this protocol will incorporate a mucosal immunology substudy to allow us to address these questions in secretions of the genital mucosa.

Globally, infection with HIV-1 is primarily via the mucosal route. Defining the earliest mucosal immune events following HIV-1 infection is of central importance for characterizing precise virus-host interactions that must be altered by vaccine-induced immune responses. However, the kinetics, quality and quantity of mucosal antibody development in acute HIV-1 infection remain to be fully characterized. The protective role of antibodies in the prevention of mucosal HIV-1 infection has been demonstrated most convincingly in the macaque-SHIV model, where systemically administered monoclonal virus-neutralizing IgG antibodies protected the animals from vaginal viral challenge (Baba 2000; Mascola 2000). It is still uncertain whether some of this protection was by HIV-1 specific IgG mediating antibody-dependent cell-mediated cytotoxicity (ADCC). Mucosal antibodies of the IgM and IgA isotype have not been well characterized for their protective capability in HIV-1 infection.

Multiple mechanisms of HIV-1 infection via the mucosa have been proposed (Miller 2003). However, modulation of infection by mucosal immune responses (adaptive and/or innate) has not been fully defined. It has been proposed that HIV-specific IgA in the vagina may play a role in the resistance of highly exposed persistently seronegative (HEPS) subjects to HIV-1 infection (Beyrer 1999; Kaul 1999; Mazzoli 1997). However, other reports have not supported this observation (Belec 2001; Skurnick 2002). Mucosal and systemic compartments of the immune system display some independence. In chronic HIV infection, HIV-specific IgG is readily detected in mucosal secretions, and is the predominant isotype in both serum and mucosal compartments. HIV Env-specific IgG has been reported to be dominant to specific IgA in all compartments (Mestecky 2004). Levels of HIV-specific IgG and IgA were comparable between cervicovaginal lavage (CVL) and semen, although the former were approximately 2 $\log_{10}$ higher than the latter. The ratio of serum and seminal plasma IgA1: IgA2 were equivalent (85:15%), whereas in the CVL, the levels of the two subclasses were similar, with a marginal increase in IgA2 around the time of ovulation (Mestecky 2007). In semen and CVL, secretory (s), polymeric (p) and monomeric (m) IgA are present in roughly equal proportions, unlike serum, where p-IgA and m-IgA predominate. The finding of HIV specific sIgA in CVL and semen suggests local production (Artenstein 1997). There have been extensive studies of the mucosal immunology and virology of HIV-1 infection in subtype B prevalent infections (Mestecky 2007; Artenstein 1997; Wright 2002; Belec 1995); however, little is known about the mucosal

immunology of acute HIV-1 (especially non-B subtypes) infection in humans. Additionally, many previous studies focused on the mucosal response to HIV-Env and not other HIV antigens (Artenstein 1997; Belec 1995; Raux 2000).

Following the detailed characterization of binding antibodies in both peripheral and mucosal compartments, it will be important to investigate the functional consequences of binding of these antibodies to HIV. The functions that have been described include virus neutralization (anti-Env), ADCC (Ahmad 1996; Battle-Miller 2002), opsonization, inhibition of bystander apoptosis, direct virolysis and other possible unknown consequences. ADCC eliminates HIV-infected cells through the action of specific antibodies that bind to target cells expressing antigens on their surface. Effector cells bearing Fc receptors are linked to the infected cells via the Fc portion of the antibody and the target cell is killed. Natural killer cells expressing CD16 and CD56 are the major effectors of ADCC, although a broader range of cells including monocytes (Murayama 1990) and granulocytes (Gale 1975) have been implicated. The precise role of ADCC in HIV infection is unknown, but ADCC activity has been inversely associated with HIV viral load (Forthal 2001) and high ADCC activity has been associated with non-progression in humans (Baum 1996). Cervical fluids collected from women with cervicovaginal ADCC had lower genital viral load than women with no cervicovaginal ADCC (Nag 2004). While there have been studies of ADCC activity in the CVL of chronically HIV-infected women, to date there have been no studies of ADCC activity in either the CVL or semen obtained during acute HIV infection.

To study the breadth and potency of functional antibodies and to model some of the extensive genetic variation of HIV-1, several laboratories have produced panels of virologic and serologic reagents from HIV-1 patients from multiple geographic regions (Brown 2005; Li 2005; Li 2006). Early studies performed in several countries indicated that cross-neutralization of HIV-1 isolates using polyclonal sera showed no relationship between viral genotype and neutralization "serotype" (Kostrikis 1996; Kostrikis 1996b; Moore 1996; Nyambi 1996; Weber 1996; Nyambi 2000). In these early cross-clade neutralization studies, the data were complicated by the use of viruses and sera from subjects infected with recombinants, and by autologous virus/serum pairs, which often yield negative results. In a recent study, using full-length sequenced pure clade reagents, we have demonstrated a trend towards preferential neutralization of homologous clade viruses using pooled clade-specific plasma. These relationships were most evident when the PBMC based neutralization assay was used; the clade C plasma pool also appeared to exhibit superior breadth and potency, especially in the pseudovirus assay (Brown 2008).

Several previous reports highlight the notion that clade-specific differences may indeed have an impact on Env immunogenicity or sensitivity to neutralization (Li 2006; Brown 2008; Derdeyn 2004; Frost 2005). A significant effort has been expended by both cellular and humoral HIV immunologists to obtain envelope (env) clones or sequences of very early, CCR5 coreceptor-utilizing isolates from acutely infected individuals at all Feibig stages (Fiebig 2003). This effort is based on the hypothesis that these viruses will best represent the infecting strains that seed an acute infection, and thus are the isolates that vaccines should be targeted against. While logical, there is no published data to support this hypothesis. For the studies proposed here, envs will be cloned from both peripheral blood and mucosal compartments at the earliest timepoints available and at subsequent intervals. This will allow us to assess pseudoviruses that are from most or all Feibig stages and from the four major clades prevalent at the four study sites.

In addition to studies mentioned above, it is important to examine the antibodies that recognize lipids in serum of HIV-1 infected patients during the course of acute HIV-1 infection. Naturally occurring antibodies to many types of phospholipids are present in normal human sera (Alving1984; Cabiedes 1998), and antibodies to phospholipids are also commonly transiently induced as an epiphenomenon during many infectious diseases (Vaarala 1986), including HIV-1 (Silvestris 1996). The possibility exists that some or many of the antibodies in the plasma pools have the ability to recognize pure lipids. Such antibodies would be analogous to, or similar to, the broadly neutralizing human 4E10 and 2F5 monoclonal antibodies that bind to pure phospholipids (Haynes 2005). The existence of these antibodies would also be analogous to the observation that monoclonal antibodies to phosphatidylinositol-4-phosphate (PIP) neutralize primary isolates of HIV-1, as shown by our demonstration of such activities (Brown 2007). As an illustration of the relevance of such antibodies, it has been demonstrated that the 4E10 monoclonal antibody also binds to PIP (Brown 2007; Beck 2007; Matyas 2009).

Naturally occurring antibodies to cholesterol, both IgM and IgG, are present in sera from essentially 100% of humans (Alving 1989; Avila 1996; Alving 1999; Horvath 2001; Fust et al., 2005). Interestingly, the titers of antibodies to cholesterol were markedly higher in the sera of HIV infected patients when compared to HIV seronegative controls (Horvath 2001; Fust 2005). Introduction of highly active antiretroviral therapy (HAART) resulted in a significant and gradual drop in the anti-cholesterol antibody titers in parallel with a decrease in viral load and increase in CD4+ cell counts (Horvath 2001). Recent studies have demonstrated that a murine monoclonal IgG antibody to cholesterol can neutralize HIV-1 infection of macrophages (Beck 2010). However, it is not clear at present whether the increased titers of antibodies to cholesterol associated with HIV-1 infection were an epiphenomenon, or whether the antibodies actually had some beneficial effects against acute HIV-1 infection.

The Alving laboratory has a long history and considerable experience in the practical measurement of human and murine antibodies to cholesterol, including murine multispecific antibodies that recognize both cholesterol and gp41 from HIV-1 (Swartz 1988; Alving 1989; Alving 1991; Alving 1999; Alving 2006; Karasavvas 2008; Beck 2008).

1.5.5 Host Genetics

The barriers imposed by the host to the establishment of a productive HIV infection are multiple and can be schematically divided into mechanisms of host restriction (Lama and Planelles, 2007), innate immunity (Alter and Altfeld, 2006), and adaptive immunity (Stephens, 2005). Genetic variation in the genes controlling these factors has been documented and used to explain, at least in part, the observation that rates of HIV acquisition and disease progression are not uniformly distributed in human populations (Dean et al., 1996).

Understanding the role of genetically determined responses to HIV infection and HIV treatments or preventative interventions, (e.g. vaccines) is critical to development of new approaches to prevention and treatment of HIV. For example, a vaccine that appears to be unsuccessful in efficacy testing may prove to have been effective in a subset of participants who possessed a particular host immune response gene allele. Exploring this insight may allow for re-design of

the vaccine to achieve that response seen only in the genetically defined subset in the broader, general population as well.

Host restriction can be exerted to prevent viral entry, or through the interference with key post-entry steps in the viral replication cycle. Polymorphic variants of these host restriction factors have been identified in human populations and have been associated with variation in rates of HIV acquisition and disease progression, probably reflecting variation in their intrinsic antiviral activity and their capacity to avoid the viral counter-mechanisms aimed at antagonizing them(Alvarez, Lopez-Larrea, and Coto, 1998; Amara et al., 1997; Bashirova et al., 2006a; Bleiber et al., 2005; Brettle et al., 1996; Carrington et al., 1999b; Cohen et al., 1998; Colobran et al., 2005; Dean et al., 1996; Gonzalez et al., 2005; Gorry et al., 2002; Hendel et al., 1999; Javanbakht et al., 2006; Kaslow et al., 1996; Kijak, 2007; Leslie et al., 2004; Liu et al., 2003; Reiche et al., 2007; Schinkel et al., 1999; Smith et al., 1997; Speelmon et al., 2006; Valcke et al., 2006).

The second line of host defense is represented by innate immunity, whose major cellular effectors against viral infection are the NKs. Their capacity to lyse virally-infected cells without the need of prior antigen sensitization makes them vital elements for the control of the initial spread of incipient viral infections (Khakoo et al., 2004). NK cytolytic functions are mediated by their membrane KIRs, which recognize specific ligands on target cells. The complex KIR locus contains various KIR genes, whose products exhibit an intricate pattern of binding specificity/affinity for their ligands, and they also vary in their ability to generate stimulatory/inhibitory signals (Bashirova et al., 2006b). KIR genes are polymorphic in human populations and so are their ligands, and their epistatic interaction can affect the rate of HIV acquisition or disease progression (Carrington et al., 1999; Jennes et al., 2006; Martin et al., 2002). Polymorphism in DC-SIGN lectins on dendritic cells (DC) can also affect initial viral spread, probably by limiting the trans-enhancement of HIV infection of T-lymphocytes. TLRs, expressed on immune cell rich tissues provide innate recognition of viral nucleic acids and pathogen-associated molecular patterns (PAMPs) (Takeda, Kaisho, and Akira, 2003). TLRs have a crucial role in initiating innate immune responses and determine the secretion profile of cytokines and interferons, thus shaping the subsequent adaptive immune responses (Meier and Altfeld, 2007). Their polymorphism has been associated with different rates of HIV disease progression (Bochud et al., 2007; Ferwerda et al., 2007).

The third line of defense is represented by adaptive immune responses, which require antigen sensitization, delaying their onset until the HIV infection has already been established. While the underlying genetic basis of the elicitation of anti-HIV antibodies has not been elucidated (Haynes and Montefiori, 2006), the efficacy of ADCC is associated with polymorphic variants of the Fc-γ receptors IIa (CD32) and IIIa (CD16a) (Forthal et al., 2007a), which are expressed on the surface of macrophages and NKs, respectively. These polymorphisms affect the specificity and affinity of the receptors towards different subclasses of IgG (Bredius et al., 1994; de Haas, 2001; Koene et al., 1997; Parren et al., 1992) and have been associated with accelerated disease progression (Brouwer et al., 2004; Forthal et al., 2007a; Forthal et al., 2007b). The genetic basis of cellular adaptive immune responses has been long acknowledged (Liu et al., 2003). Class I HLA genes are the most variable ones in the human genome and condition the focus of the responses from cytotoxic T-lymphocytes (CTLs), by restricting the nature of epitopes that can be presented by infected cells. Some HLA alleles have documented delayed courses of infection

whereas other alleles are associated with accelerated disease progression (Brettle et al., 1996; Carrington et al., 1999; Dorak et al., 2004; Dorak et al., 2003; Farquhar et al., 2004; Flores-Villanueva et al., 2003; Gao et al., 2001; Hendel et al., 1999; Kaslow et al., 1996; Keet et al., 1999; Leslie et al., 2004; Liu et al., 2003; MacDonald et al., 2001; Martin et al., 2002; Moore et al., 2002; Tang et al., 1999; Tang et al., 2002; Trachtenberg et al., 2003). By their interaction with KIR on NKs, HLA molecules bridge the innate and adaptive arms of cellular immunity(Alter and Altfeld, 2006).

Our knowledge of the impact of host genetic variation on HIV acquisition comes mostly from predominantly subtype-B settings, and in most cases it is unclear if the same principles apply to other HIV clades or non-Caucasian genetic backgrounds. The host genetic basis of variation in disease progression rates proceeds mostly from the comparison of plasma viral load set-points among chronic HIV infections. A major obstacle for the establishment of the contribution of genetic variation in host restriction, innate and adaptive immunity arises from the fact that the effectiveness of each of these lines of defense is highly dependent on the success of the preceding one.

The current study design allows for the comparison of HIV sero-prevalent individuals, exposed sero-incident cases and matched controls to establish the polymorphisms in factors of host restriction, innate and adaptive immunity that confer varying risks for HIV acquisition in East Africa and Thailand, in the setting of non-B clade infections. This affords the opportunity to determine the principal components of host genetic variation in these lines of defense that can distinguish the different levels of viremic control.

The human host genetic information collected in this study is obtained for the sole purpose of understanding the control of HIV infection as it relates to acquisition of HIV and the long-term prognosis of HIV disease course. None of these associations have any clinical relevance at this time. As an example, the known genetic host factor which reduces susceptibility to HIV infection, the homozygous deletion of a sequence within CCR5 does not prompt genetic counseling to inform the participant that they have a lower risk of HIV infection than others. This is because the risk is lower for these persons than the general population but not zero and the message of behavioral risk reduction is not altered by this information. For the vast majority of genetic markers under consideration in this analysis, no known human disease has been established and the information has no impact upon risk behavior counseling or therapeutic decisions. In the rare instance where a disease association exists, there is no standard for counseling participants who are otherwise healthy and have no manifestations of the disease. Finally, the methods used here to ascertain human genetic information are not licensed or approved medical testing methods for determining medically relevant genetic information. They are research methods designed to permit rapid, high throughput information to address scientific questions but may not meet standards needed to support medical diagnoses. In consideration of these issues, the research team does not feel the host genetic information should be provided on a routine basis to participants.

1.6 Description of Partners

Makerere University, founded in 1922 is Uganda's premier higher education institute. With over 20 schools/faculties/institutes, Makerere University offers day, evening, and external study programs to over 22,000 undergraduate and 3,000 graduate students. Located in Kampala, the capital of Uganda, Makerere is a hub for resources and intellectual resources, making it an ideal nexus for conducting research in many fields of study. HIV infection related research at Makerere University has been conducted in collaboration with a variety of North American and European institutions (including Case Western Reserve University, Johns Hopkins University, University of California at San Francisco, Columbia University, University of Medicine & Dentistry of New Jersey, the London School of Hygiene and Tropical Medicine, etc) for over ten years. Among others, HIV infection related research at Makerere University has included the Partnership for AIDS Vaccine Evaluation (PAVE), the HIVNET program, the HIV Prevention Trials Network (HPTN) and the successful completion of Africa's first preventative vaccine trial – ALVAC vCP205 (HIVNET 007). In addition, Makerere University has been actively participating in initiatives directed at the clinical epidemiology, diagnosis, pathogenesis, treatment and prevention of HIV/AIDS since its identification in East Africa in early 1984.

Makerere University Walter Reed Program (MUWRP): MUWRP is a non-governmental, non–profit HIV research Program that was established in 2002 by Makerere University, The U.S. Military HIV Research Program (MHRP), and the Henry M. Jackson Foundation for Advancement of Military Medicine of the United States. Clinical studies were initiated in early 1999 in Uganda through Rakai Project. The focus of the Project has been on development of infrastructure, definition of vaccine research cohorts, acquisition of appropriate products for evaluation in the region, and clinical evaluation of these products. The core of the Project's efforts is to accomplish all activities required for initiation of phase III trials in the region over the next seven years.

MUWRP's main facility, located at Plot 42 Nakeasero Road in Kampala, Uganda, includes administration, data, logistics and the clinic. The College of American Pathologists (CAP)-certified laboratory is located at the Makerere University School of Medicine, approximately 1.6 kilometers from the main complex.

U.S. Army Medical Research Unit-Kenya (USAMRU-K): USAMRU-K is a Special Foreign Activity of the Walter Reed Army Institute of Research (WRAIR), Washington, DC. USAMRU-K is affiliated through a Cooperative Agreement with the Kenya Medical Research Institute (KEMRI). The unit was activated on a temporary basis in 1969 at the invitation of the Government of Kenya to study trypanosomiasis. The success of that initial venture led to the establishment of a permanent activity in 1973. Over the past 32 years, research has been conducted on malaria, trypanosomiasis, leishmaniasis, entomology, HIV/AIDS and arboviruses, with more than 250 manuscripts published.

In addition to laboratories housed in Nairobi (where anti-malarial drug resistance, trypanosome biology and Global Emerging Infections Surveillance (GEIS) are studied), USAMRU-K has other field sites in western Kenya (Kisumu, Kisian, Kombewa and Kericho) that are mainly concerned with malaria and HIV/AIDS.

The United States Army Medical Research Unit of Kenya (USAMRU-K) HIV program is located in Kericho at the Kenya Medical Research Institute (KEMRI) / Walter Reed Project (WRP) Clinical Research Center. Within Kenya's largest tea plantations in the African Highlands of the southern Rift Valley Province, Kericho is a rural city with a population of approximately 500,000. The center was originally established for malaria research in late 1990s. The WRP HIV Program was established in Kericho and began focusing upon HIV research in early 2000 given the recognized breadth and depth of HIV disease in Kenya. The WRP Kericho HIV Program is led by one Department of Defense (DoD) civilian and Kenyan professionals. As part of the MHRP, USAMRU-K's mission in HIV research is the contribution to the development of a globally effective HIV-1 vaccine and testing and evaluating HIV vaccine candidates. The USAMRU-K HIV program currently has several ongoing, complimentary HIV research and care and treatment activities, all contributing to the MHRP HIV mission of vaccine development.

Mbeya Medical Research Program The Mbeya Medical Research Programme (MMRP). MMRP is a partnership between Tanzanian health authorities represented by the Mbeya Regional Medical Office, the Mbeya Referral Hospital, the National Institute of Medical Research (NIMR), the Department of Infectious Diseases & Tropical Medicine at the University of Munich (LMU) and the MHRP. Recently a Memorandum of Understanding has been signed by all MMRP collaborating partners to enable MMRP to become a NIMR-MMRP research centre. With this Memorandum the new NIMR-MMRP research centre has now full legal status. MMRP has expanded its research into the three major infectious disease challenges for Tanzania: HIV/AIDS, malaria and tuberculosis. The center has a CAP certification of its laboratory acquired in October 2007. The shared mission of MMRP is to evaluate new interventions for these diseases utilising vaccines, drugs or diagnostics.

Royal Thai Army, Armed Forces Research Institute of Medical Sciences (RTA-AFRIMS): RTA AFRIMS is operated under the Phramongkutklao Medical Center of the Royal Thai Army Medical Department. RTA-AFRIMS has collaborated with USAMC-AFRIMS in areas of HIV vaccine research for more than 15 years. RTA-AFRIMS provides support in areas of clinical, field and laboratory research.

U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS): USAMC-AFRIMS is the U.S. Army Medical Component of a Royal Thai Army – U.S. Army joint command on the grounds of the Phramongkutklao Medical Campus in Bangkok, Thailand. It is operated under the Walter Reed Army Institute of Research (WRAIR), Washington, D.C.

The Department of Retrovirology was established in 1992 and serves as the MHRP's forward platform in Southeast Asia for HIV vaccine development. The AFRIMS has conducted six phase I/II HIV vaccine trials, multiple vaccine preparatory cohort studies, and have stewarded the largest HIV vaccine trial to date-the RV144 phase III prime-boost vaccine trial which began in 2003.

AFRIMS capabilities include a highly-experienced clinical and laboratory staff, with a laboratory that is CAP-accredited, and is registered with the NIH AIDS Research and Reference Reagent Program and the Division of AIDS, NIAID. The research lab is capable of cellular and humoral

immune assays as well as viral genotyping and sequencing. Additionally, specimen processing, archiving, and other clinical laboratory support for clinical trials can be done at two specimen processing facilities, one in Bangkok and the other in Chon Buri Province (currently serving the Phase III HIV vaccine trial).

The U.S. Military HIV Research Program (MHRP), is a multi-dimensional research project headed by the Walter Reed Army Institute of Research (WRAIR) in collaboration with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) and sponsored by the United States Army Medical Research and Material Command (USAMRMC). The mission of this project is to prepare and protect the U.S. military forces so they are ready for the challenges and opportunities of deployment and peacekeeping operations in the future. This program strives to develop effective vaccines to protect U.S. military forces from infections and also bring under control the international proliferation of HIV. It is through cooperative relationships that scientific ideas are exchanged and progress made in fighting the HIV/AIDS epidemic.

The Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) is a private not-for-profit organization dedicated to improving military medicine and public health. The mission of HJF is to advance medical research and education in the military medical community by providing scientific and management services to improve health worldwide. HJF supports a wide variety of research programs ranging from small bench top programs to complex multi-site programs. HJF was chartered in 1983 by U.S. Congress to support military medical research and education. HJF was named in honor of Henry “Scoop” Jackson, the late Senator from Washington State, and embodies his long-standing dedication to military medicine and public health.

The Walter Reed Army Institute of Research (WRAIR) conducts research on a range of military relevant issues, including naturally occurring infectious diseases, combat casualty care, operational health hazards, and medical defense against biological and chemical weapons. WRAIR provides an essential link between troops in the field and research in the laboratory with a mission to conduct biomedical research that is responsive to the DoD and U.S. Army requirements and delivers life saving products including knowledge, technology, and medical material that sustain the combat effectiveness of the war fighter. Despite a focus on soldier related research, many non-military medical problems around the world have been solved and lifesaving and life enhancing discoveries made.

Division of AIDS (DAIDS)
As protocol sponsor, DAIDS has direct responsibility for human use protections and oversight, which is accomplished through protocol development by the active involvement of the Vaccine Clinical Research Branch, and particularly the assigned Medical Officer in all aspects of protocol development. The draft protocol is subject to internal DAIDS review at the "Prevention Sciences Review Committee" which provides a comprehensive scientific, medical, statistical, ethical and regulatory review. Subsequently, the final draft protocol is subject to a second level regulatory/ethical review at the Regulatory Compliance Center of Regulatory Affairs Branch, DAIDS prior to submission to the WRAIR and local institutional review boards (IRBs) for approval. Prior to initiation of a protocol at a specific site, the Regulatory Compliance Center (RCC) goes through a protocol initiation review to verify that the final, translated consent is faithful to DAIDS and United States Government (USG) guidelines and the sample consent. In

addition, this step involves verification that all required regulatory documents and approvals are present at the site prior to initiation. All amendments to the protocol will be reviewed on behalf of DAIDS by the Medical Officer (MO) prior to submission to RCC and approved by both RCC and the MO prior to submission to local IRBs.

2.0 HIV PREVALENCE AND INCIDENCE IN MOST AT RISK POPULATIONS IN STUDY LOCATIONS

2.1 Uganda Target Populations

The MARPs in Uganda will target two populations that are believed to have high-risk behavior. The populations to be targeted will include, but are not limited to, sex workers (SWs), individuals diagnosed with sexually transmitted infections who are clinic attendees (STIAs), and bar workers (BWs). These populations and known HIV epidemiology in these populations are described in the site-specific addendum (Protocol RV217a).

During Part A of the study, the investigative team in Uganda will mainly recruit and enroll SWs, but individuals from any of the other three population groups specified above that may respond to the recruitment drive and meet the inclusion criteria may be enrolled.

During Part B of the study, the investigative team in Uganda will recruit and enroll from among both target population groups of SW and BW.

2.2 Kenya Target Populations

Kenya will target two MARPs, SWs and STIAs, which are both estimated to have an incidence of 3% or greater. These populations and known HIV epidemiology in these populations are described in the site-specific addendum (Protocol RV217b). For Part A of the study, only SWs will be recruited while in Part B, recruitment will be from all the groups mentioned (SWs and STIAs).

2.3 Tanzania Target Populations

The Mbeya Medical Research Programme (MMRP) in Tanzania site has one target population. The BW cohort has been the subject of much study and multiple recent publications. This population and known HIV epidemiology for this population are described in the site-specific addendum (Protocol RV217c).

2.4 Thailand Target Populations

Overlapping high-risk groups have been identified in Thailand. SWs, both female and male continue to be a group at high risk, along with transgender individuals (TG) and those men who have sex with men (MSM) who are living a high-risk lifestyle in the entertainment areas. These populations and known HIV epidemiology in these populations are described in the site-specific addendum (Protocol RV217d).

3.0 STUDY OBJECTIVES

The research collaboration outlined in phase I of this protocol will define the epidemiology of HIV in a volunteer cohort drawn from high-risk populations in East Africa and Thailand. The primary objective of this study is to estimate HIV-1 incidence and retention in a volunteer cohort established to test vaccine strategies. This study will also define the prevalence of HIV-1 in this volunteer cohort, determine the distribution of HIV-1 genotypes and different host genetic backgrounds, assess the range of CD4 and viral load in HIV-1 infected volunteers, characterize behavioral and other risk factors associated with HIV-1 infection, and augment HIV-1 prevention and education programs, human resources, and laboratory infrastructure to support future vaccine trials.

Individuals with incident infections during observation will be invited to enroll in phase II, a sub-study to evaluate the dynamics of viral burden, diversity, possible impact of host genetics, and adaptive immune responses in early HIV infection. In order to optimize the scientific value of these observations, phase I of the study will be conducted with frequent, small blood volume sampling of the participants to diagnose HIV infection very early, ideally, prior to the advent of detectable antibody using extremely sensitive diagnostic Enzyme-linked Immunosorbent Assays (ELISAs).

Very frequent surveillance as proposed here has not been conducted in the HIV field. It is possible that participants will either be unavailable this frequently or unwilling to participate. To ensure that protocol procedures are going to be successful in meeting the audacious goals of the study, a pilot study will be conducted (Part A). Only after establishing feasibility of the proposed design in Part A would enrollment open fully in Part B. Should compliance with the demanding study visits regime prove unsuccessful in Part A, the study would be re-designed and modified accordingly through an institutional review board (IRB)-approved amendment.

Thus, broadly speaking, there are two sets of objectives as noted below and two parts of the study: Part A) pilot feasibility study and Part B) full study implementation. All Part A participants and data will be used to satisfy the study objectives along with the Part B participants. Part A has limited objectives pertaining to feasibility but will also contribute to the objectives of the fully implemented study (Part B).

3.1 Part A (Pilot Study)

3.1.1 Primary Objectives

1) Determine the ability to recruit up to 200 high-risk volunteers at each study site in 9 months
2) Determine the ability to achieve at least 50% compliance for study visits
3) Observe an increased incidence with at least 30% of the incident cases identified prior to the advent of detectable antibody

3.2 Part B (Full Study)

3.2.1 Primary Objectives

1) Define the risk behavior, prevalence and incidence of HIV infection and retention of a high risk cohort of adults in Thailand, Uganda, Kenya and Tanzania

2) Obtain approximately 150 acute HIV infections (AHI) with at least 30% captured within Fiebig stages I and II to support the full characterization of host responses and viral dynamics

3.2.2 Secondary/Exploratory Objectives

1) Assess and optimize HIV diagnostic strategies in HIV primary infection across multiple subtypes and risk groups

2) Define the genetic diversity and evolution of HIV-1 in the prevalent and incident HIV cases with particular emphasis on characterization of acute, primary HIV infection

3) Characterize immune activation, innate and adaptive cellular immunity in the early acute HIV-1 infection

4) Characterize B cell responses in peripheral and mucosal compartments arising in early acute HIV-1 infection

5) Characterize genetic polymorphisms in genes controlling host restriction, innate and adaptive Immunity, and their influence on HIV acquisition and early control of HIV infections

6) Characterize clinical events including endemic infection as risk factors for HIV acquisition

4.0 DESIGN AND METHODOLOGY

4.1 Hypothesis Being Tested

Two formal hypotheses will be addressed under objective #1: 1) lower bound of the 90% confidence interval around the aggregate incidence will be in excess of 3 per 100 person years; and, 2) 90% of the aggregate cohort will be retained through one year of follow-up.

4.2 Summary of Methods

The study will be conducted at three locations corresponding to the three East African vaccine research sites of the US Military HIV Research Program. These are Kampala, Uganda (Protocol RV 217a); Kericho, Kenya (Protocol RV 217b) and Mbeya, Tanzania (Protocol RV 217c). A fourth study area has been identified in Thailand (Protocol RV 217d).

This is a multi-center, non-randomized clinical observational study to be conducted in two parts (Parts A and B). The purpose of the study is to characterize recruitment, retention, HIV prevalence, HIV incidence and biological characteristics of acute HIV infection in high-risk volunteers in Africa and southeast (SE) Asia. The first part of the trial, Part A, is a small pilot study to establish and optimize the recruiting, retention and study compliance at each participating site. Based upon this pilot phase of the study, the study will expand to full enrollment as planned (Part B) or operational procedures and study event schedules will be adjusted with a corresponding amendment prior to initiating Part B. The study itself, as conducted in both Part A and B, incorporates two phases. The main study activity, phase I, is the observational cohort or surveillance activity. The evaluation of the incident infections arising during phase I is an intensive evaluation of the interaction of HIV virus and host response and constitutes phase II of the study.

Phase I: Non-randomized, closed cohort, prospective, 24-month observational study to be conducted in two steps. Part A is a pilot study to establish and optimize operations and study design features to meet study objectives prior to opening the study to full enrollment at all sites in Part B. The study will conduct a screening visit and initial follow-up in all enrolled volunteers. This will include both HIV negative and HIV prevalent cases. Prevalence is estimated to be high in these populations and (in Part B) approximately 1000-1500 volunteers will be enrolled and evaluated in the first two visits with an enrollment target of 300 HIV negative, high-risk volunteers in Part B; however, some shifting of enrollment numbers per site might occur in order to complete the study at the same time at all sites. In Part A, the enrollment target of HIV negative, high-risk volunteers is 200. Subsequently, only HIV negative volunteers will be followed for a period of approximately 24 months, except for a small number of HIV positive individuals for masking to minimize risk of stigmatization and to provide positive controls for laboratory assays. After the initial two visits, volunteers will be seen at the research clinic approximately every 3 months. In addition, at locations that are as convenient to the volunteers as feasible, small blood volume samples will be collected using microvettes twice weekly. These samples will be analyzed and results returned to the site within 48 hours to permit new HIV

positive participants to be identified while they remain antibody negative, HIV nucleic acid test positive. All newly infected participants will be referred to phase II of the study.

Phase II: study of HIV incident cases arising within phase I. HIV incident volunteers identified at any follow-up visit will be asked to consent to participate in phase II to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of approximately 50 months from the time of entering phase II. Very frequent visits using a more intensive collection of samples initially will occur to characterize acute HIV pathogenesis and then at regular 3-month intervals for the duration of the study.

4.2.1 Part A (Pilot Study)

Part A will be a feasibility study, which will include up to 200 participants per site for a total of 800 participants overall.

At each site approximately as many as 300-450 volunteers will undergo a briefing presentation describing the trials and its risks and benefits provide written informed consent and undergo other activities as noted in Part B below. The endpoint for enrollment is identifying 200 HIV uninfected, high-risk participants per site who are willing to be followed for the next 24 months to determine the ability to recruit in these high-risk groups as well as retention of volunteers. In addition, approximately 20 HIV infected participants will be recruited to a) provide masking of HIV status for the population as a whole and b) provide HIV positive samples to serve as controls for study assays.

HIV incident volunteers identified at any follow-up visit will be invited to participate in phase II, to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of at least 50 months from the entry into phase II.

All Part A volunteers will contribute to analyses for Part B and to total enrollment goals.

4.2.2 Modified Transition to Part B

In view of the success of the pilot phase of the study (Part A) we propose to transition to Part B permitting full enrollment to 500 participants per site. However, in discussion with DAIDS, the primary funding agency, the transition to part B must be made on a site-by-site basis based upon an increased incidence and at least 3 acute infections identified in Fiebig stage 1 or 2. To manage work flow, logistics, and costs each site will only enroll new volunteers in part B as existing participants enrolled under part A complete the intensive phase of surveillance at one year. For the purpose of protocol management we will define the visit at 60 weeks, visit G, as the end of a full year of intensive surveillance. All participants completing visit G remain in the protocol but the frequency of SBV visits change from twice weekly to once per month, but they will continue to come in for all other visits as scheduled until study termination. As volunteers complete visit G, more volunteers can be enrolled under the expansion of enrollment in Part B. This will permit a gradual expansion of the overall enrollment yet maintain a population of approximately 200 participants in the intensive, twice-weekly surveillance phase.

4.2.3 Part B (Full Study Implementation)

At each site approximately 1000-1500 volunteers (total includes those enrolled in Part A) will undergo a briefing presentation describing the trial and its risks and benefits. The volunteers will then complete a written informed consent. The endpoint for enrollment is identifying 2000 HIV uninfected, high-risk participants (ideally, up to 500 at each site; however, some shifting of enrollment numbers per site might occur in order to complete the study at the same time at all sites), (participants in Part A will contribute to the total) who will be followed for the next 24 months to establish incidence and retention rates. Recruitment methods and activities are directed at each group separately to minimize stigma risks and for operational efficiency. Volunteers will receive HIV risk reduction counseling, a briefing regarding HIV and HIV vaccine research in general, a medical history and physical, a behavioral risk questionnaire, and provide a blood sample to characterize general health (e.g. complete blood count (CBC), chemistries and liver function tests (LFTs)), diagnose HIV status and characterize both host genetics and viral characteristics. All volunteers will be seen in follow-up within approximately 6 weeks of Visit A to review laboratory and clinical findings from the screening visit. Those testing HIV positive or indeterminate at the first visit will provide a blood sample to verify HIV status at the second visit and PBMC and serum to contribute as positive controls for other study Aims. The extent of participation in the study is determined by HIV status at Visit A. Those enrollees who test HIV positive at Visit A will be discontinued from study participation after visit B, with the exception of a small number of HIV prevalent cases who will continue follow-up to afford some masking to the HIV infected participants and decrease the risk of stigmatization. In addition, the positive samples derived from their participation contribute to quality control of the diagnostic and other assay platforms.

Incidence and retention rates among this cohort will be defined at each site and for the study population as a whole for the 24-month period of follow-up. Participants will be seen at 3-month intervals for HIV post-test and risk reduction counseling and every 6 months standard HIV diagnostic labs, and the presence of co-infections will be assessed. Exposure risk will be carefully documented by a questionnaire administered at baseline and every 6 months.

To ascertain acutely infected volunteers in a timely fashion and begin their intensive observation in the AHI phase, it is necessary to obtain at least weekly and preferably twice weekly samples for diagnosis. This will be accomplished by collecting up to two microvettes of blood via capillary puncture or venipuncture. The serum sample will be analyzed for RNA and any individual reactive for RNA (and previously known to be negative, seronegative volunteers) will be counseled at the next encounter (planned twice weekly) and entered into the AHI phase. The microvettes collected on each newly infected individual prior to the first RNA reactive sample will be evaluated for HIV specific DNA to define the eclipse phase of infection. We expect to detect 150 acute infections during the study with at least 30% of those being Fiebig stage I or II using this approach to diagnosis. Seroincident infections will be defined as acute HIV infection cases while the remainder of the volunteers completing 24 months of follow-up will be defined as exposed uninfected controls. This will afford the ability to capture the ascending and peak portions of plasma viral RNA in these participants. Approximately 1,850 exposed uninfected controls will be available for comparison to the AHI population as well as approximately 2000 seroprevalent cases acquired at study entry.

The study will employ an open cohort design to accommodate loss of participants due to death, pregnancy, and departure due to non-compliance. For the primary objectives of the study, the initial enrolled population will be treated as a closed cohort for the purposes of analysis.

4.3 Laboratory Methods

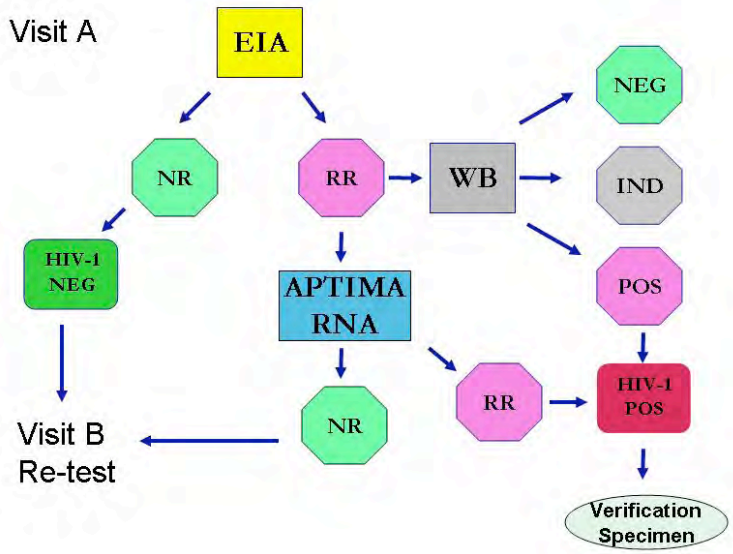
4.3.1 HIV Diagnostics

All clinical/translational research diagnostic support will be conducted in CAP accredited diagnostic laboratories using performance verified instrumentation/platforms and assays validated in accordance with Clinical Laboratory Standards Institute (CLSI) standards (formerly the National Committee for Clinical Laboratory Standards (NCCLS)).

Blood will be drawn for: 1) hematological evaluations (CBC, differential, platelets) and for clinical chemistry (Creatinine and alanine transaminase (ALT)). In addition, co-infecting pathogen screening (syphilis, hepatitis B, Herpes Simplex Virus Type 2 (HSV-2) and other endemic infections with viral vectors), eosinophil determination as a surrogate for helminth infestation and plasma lipopolysaccharide (LPS) determinations will be performed to assess the influence of co-infection and microbial translocation on immune activation. Assays will be performed per manufacturer's specifications.

HIV testing for cohort enrollment will be by anti-HIV antibody using a 3rd generation EIA, Genetic Systems HIV-1/HIV-2 Plus O EIA (BioRad Laboratories, Redmond, WA), or equivalent, or 4th generation methodology. Repeatedly reactive EIA samples will be tested using Genetic Systems HIV-1 Western Blot (WB) (BioRad Laboratories, Redmond, WA). Samples may be subjected to Aptima HIV-1 RNA Qualitative Assay (Aptima: Gen-Probe) (see Figure 6 below) to detect acute HIV infection. Diagnosis will be made based on detection of serological markers of infection. Diagnostically challenging cases may be resolved by supplemental confirmatory testing for HIV proviral DNA. The enrolled HIV prevalent participants will also be concurrently screened using the capillary microvette sampling method described below as concordant detection of HIV-1 prevalent cases by microvette sampling. Standard EIA/WB testing will be employed as a critical internal control for the small blood volume sampling methodology proposed.

Figure 6 HIV Screening Algorithm*



*NEG = negative, POS = positive, IND = indeterminate, RR = repeatedly reactive, NR = non-reactive, WB= Western Blot

Participants non-reactive by both EIA and/or by qualitative RNA assay will be considered non-infected for purposes of the study and will enter AHI surveillance by twice weekly small volume capillary blood sampling. Rapid identification and transition of participants to the AHI protocol is essential if eclipse and early Fiebig stage I-II infections are to be captured. A volume of 600 ul of blood will be collected at each visit and then tested for the presence of HIV-1 RNA by Aptima HIV-1 RNA Qualitative Assay (Aptima: Gen-Probe). This assay was chosen over mini-pooling RNA detection strategies (Pilcher 2005), or detection by p24 antigen EIA (Fiscus JCM 2007) due to lack of lower limit analytical sensitivity achieved by these approaches, and in the case of min-pooling, time/labor constraints imposed by construction/deconvolution of positive pools. Aptima RNA detection at ~90-100 cp/ml (98.5%, confidence interval (CI) .973-.992) to as low as ~20 cp/ml (82.6%, CI .796-.853) can be effected (personal communication, Dr. Tom Nugent, Gen-Probe). This approach will mark a critical advancement in AHI surveillance as it will permit detection of the lower level viremia (LLV) phase of primary infection, defined as viremia of <100 cp/ml which occurs post infection, but prior to the RNA ramp-up phase (Fiebig 2005). This study evaluated 69 samples from 15 patients that were collected before the period of steadily rising viremia (SRV) above 100 cp/mL. Using an ultrasensitive assay (2 mls plasma) with an analytical sensitivity of 1.4 RNA cp/mL (50%) and 4 RNA cp/mL (95%), 23/69 (33.3%) samples had detectable LLV with a median of 18 days (range 9-25 days) prior to SRV. More over, this approach will allow us through associated studies (viral genetics, host genetics, immunological, genomic) to redefine the window period in which pre-existing immunity controls the virus prior to expansion – a critical period for vaccine intervention. The EDTA microvette sample will be processed for plasma (~150-300 ul) and peripheral blood mononuclear cells

(PBMCs) (~0.15-0.3 x 10^6) and will be archived for look back studies for proviral DNA and LLV associated with the eclipse phase (Fiebig 2005).

The first Aptima RNA reactive sample will immediately transition participants into the AHI (Phase IB) study where multiple large volume blood draws will be acquired at 3-4, then 7-14 day intervals (see SOE #2 and #3). This approach will provide valuable highly exposed uninfected (EU) and exposed infected (EI) samples with well defined lineages for construction of matched panels of negative, pre-seroconverter, and seroconverter sample sets of which ~50% are anticipated to be in eclipse to early Fiebig stages I-II infection. These panels will provide material for laboratory staging, characterization of incident estimation/recent infection methodologies, assessment of novel technology advances in molecular and serological diagnosis, and the associated studies referenced herein. Laboratory staging studies and novel technology assessments will be conducted in the MHRP Reference Laboratory within the Department of Laboratory Diagnostics and Monitoring (DLDM).

Laboratory staging assignments of AHI are dependent upon the sensitivity of assays employed. While staging by Fiebig et. al., (2003) employed the most sensitive/specific United States Food and Drug Administration (USFDA) approved screening methods available at the time, state-of-art screening/diagnostic platforms are now available which further narrow the detection window for RNA and HIV antigen/antibody with higher sensitivity and specificity for diverse subtypes (Swanson 2006; Schumacher 2007; Guendin 2007; Weber 2004). Staging will be performed on large blood volume samples (LBVs) using 1) ultrasensitive HIV-1 RNA qualification (Aptima HIV-1 Qualitative Assay, Gen-Probe) and quantification (Abbott HIV-1 Real Time, Abbott Diagnostics) nucleic acid amplification technologies (NAAT), 2) HIV-1 DNA polymerase chain reaction (PCR), and 3) serological assays (p24 antigen EIA [Perkin Elmer or equivalent] 3rd generation IgM sensitive EIA (Genetic Systems HIV-1/HIV-2 Plus O [BioRad Laboratories], 4th generation p24 antigen, anti-HIV antibody EIA, HIV-1 WB [BioRad Laboratories; Abbott Laboratories], 4th generation Rapid Diagnostic Tests [RDTs; Determine HIV-1/2 Ag/Ab Combo; Alere, Inc and equivalent], HIV WB [BioRad Laboratories] and, SMART plasma/cells, SMARTube HIV/HCV blood enhance device; [SMART Biotech, Ltd]. Estimations of the emergence of HIV viremia, p24 antigenemia, anti-HIV antibody, and WB reactivity, and proviral DNA in these subtype diverse cohorts will be generated.

Alternative NAAT or serological assays may be employed if data suggests that subtype diversity impacts detection sensitivity/specificity or if technology advances occur during the course of this study.

A 2ml blood sample will be collected as a heparin specimen or directly into a SMART tube containing 2ml of anti-coagulant and reagent. Specimens will be acquired on Visits 1 and 3 within the stutter phase and in conjunction with the proposed ACD blood draw post a reactive Aptima result and prior to transition to stutter phase. Additional specimens will be collected at visits 13 and 14 for evaluation of this product as a recent infection detection device. Samples will be incubated in a 5% CO_2 humidified incubator (or candle jar if feasible) at 37^{0}C for 3-5 days. Post ST stimulation, the diluted plasma from the SMART tube will be screened using standard EIA and subsequently reflexed to HIV Western Blot if EIA reactive. The approximate remaining 700ul diluted plasma from the SMART tube will be reposed for future studies. While ST stimulated diluted plasma has been examined by standard serological methods an analysis of

the ~ 1 million stimulated PBMC for integration of proviral DNA has not been conducted (personal communication, Dr. Jehuda-Cohen) nor have there been attempts to sequence infecting virus. The PBMCs will be preserved as dry cell pellets or isolated by ficoll hypaque methods for proviral integration studies and, if possible, sequence analysis. Results will be compared to nucleic acid testing results and standard serological HIV diagnostic testing. Sample numbers will be small and the intent is to provide descriptive analysis of the comparisons.

Identification of the first RNA reactive will trigger a look back of archived small blood volume samples (SBV) PBMC for detection of proviral DNA for an estimation of the duration of the eclipse phase and of SBV plasma to investigate the dynamics of low level viremia preceding RNA ramp-up (Fiebig et. al 2005). This effort will leverage against Viral Genetics Section studies: whole genome amplification (WGA) for detection of low-proviral DNA and single genome amplification (SGA) for full-length (FL) viral sequence analysis. Samples will be scored for presence of proviral RNA and detection/quantification of RNA. If the WGA approach fails, ultrasensitive nested PCR will be employed, which can detect as few as one proviral integration events of 1/100 – 1/100,000 cell.

As qualitative and quantitative NAATs for detection of HIV RNA and HIV Ag/Ab tests are continuously advancing, panels constructed from laboratory staging studies will be used to assess the most promising. Several promising technologies are advancing which include, but are not limited to:

1. IQuum Lab-in-a-Tube (LIAT). This assay integrates plasma specimen processing (200 ul), nucleic acid extraction, reverse-transcription, and real-time PCR in a single closed-tube format. The LIAT analyzer provides an interpreted result in less than 60 minutes. A recent evaluation of product against gold standard Abbott m2000sp/Real Time HIV-1 RNA assay demonstrated that HIV-1 RNA levels were over quantified approximately 1.5-fold higher and with 1.3-fold less precision than the gold standard platform over a analytical range from 1.5 $x10^3$ to 1.5 $x10^6$ that is suitable for monitoring HIV-1 infection (Coombs et al. Abstract).
2. BioHelix Corp IsoAmpTM “On Demand” Manual Molecular Analyzer- a manual platform for HIV viral load determinations consisting of a manual sample-prep, isothermal amplification (30-60 min), and instrument-free rapid detection (5 min) reported as a visual qualitative result. This technology, which is in beta development, shows great promise for deployment in resource-constrained settings. We will also assess the Biohelix IsoAmpTM “Quan” Realtime Molecular Analyzer - low cost and portable instrument that integrates sample-prep, isothermal amplification, and quantitative RNA detection steps for plasma specimens, whole blood, dried blood spots and serum specimens. Primers are targeted at the gag gene. Preliminary results are promising for HIV-1 subtype performance post primers redesign for detection of C, A and A/G subtypes. The current lower limit of detection is ~500 cp/ml. We have developed collaborations with Bertrand Lemieux, Director Technology Development, BioHelix Corp, to assess both of these technologies. Biohelix's IsoAmp MRSA Screening Assay has a sensitivity of 5 copies of MRSA. The Ebola RNA Rapid RT-HDA can detect 50 copies in 30 minutes.

3. The Simple Amplification Based Nuclei Acid Test (SAMBA) (Diagnostics for the Real World, Inc, Sunnyvale, C) is qualitative point of care (POC) HIV viral load platform which leverages sample preparation, isothermal nucleic acid amplification (< 1 hour), and dipstick based rapid visual detection of nucleic acid (<0.5 hour). Amplification and detection steps are combined in a disposable cartridge which is processed on the POC device permitting containment of amlicons. An extraction module is under development. Detector probes labeled with multiple hapten moieties form lattice-like structures by binding to anti-hapten antibodies conjugated with multiple colored particles. In the presence of target RNA or DNA, visible lines develop in the capture zone of the test strip. Studies with Group M subtype panels (A-K), Groups N and O, and clinical samples demonstrated sensitivity to 200 copies/ml **(Lee et al. 2010).**
4. Wave 80 BioSciences (San Francisco, CA) EOSCAPE HIV platform is based on continuous-flow microfluidic, microchip technology, with on board lyophilized reagents requiring no fluid exchange. The product employs thermostable branched DNA-like amplification with luminescent readout, is disposable, and has a flexible instrument design. The product is scheduled for beta testing in 2012

Studies to evaluate methods for estimation of incidence/recent within populations of diverse subtype/circulating recombinant forms will be conducted in collaboration with Drs Steven Ethridge and Bernard Branson, CDC. This work will be conducted under an investigative new device (IND) application (USFDA IND 8193). Assessment of newer assay platforms in diverse non-B populations is critical if refinement of current methods is to be accomplished. Matched seronegative and seroconverter panels from samples acquired at 3, 6, 12, 18, and 24 months post detection of the first RNA positive sample will be constructed. Panels will be used to evaluate 1) IND BED-Capture EIA (Trinity Biotech, PLC), 2) two 3rd generation sensitive/less sensitive (detuned) EIAs, the fully automated enhanced chemiluminescence Vitros ECi HIV1/2 (Ortho Clinical Diagnostics) and the automated GS HIV-1/2 Plus 0 (BioRad Laboratories), and 3) one 3rd generation automated avidity assay, the Vitros ECi HIV1/2, 4) SMARTplasma, and newer methodologies.

Serum LPS levels in LBV plasmas will be assessed using the QCL-1000 Chromogenic LAL Endpoint Assay (Lonza) which has an analytical sensitivity of 0.1 EU/ml-1.0 EU/ml. Co-infection induced immune activation is known to influence HIV acquisition and disease progression. Presence of malarial infection will be assessed by standard giemsa stain smear microscopy. Archived EDTA anti-coagulated whole blood samples or will be screened for the presence of low level parasitemia by Real-Time PCR using modifications of methods of Mangold et. al., 2005. Analytical sensitivity of 1-5 parasites per microliter of blood can be attained by this approach (Snounou 1993; Mangold 2005; Boonma 2007).

HCV serology status will be determined using Ortho Diagnostics HCV EIA 3.0 (Ortho Diagnostics, Raitan, NJ) or following followed by supplemental confirmatory testing (Chiron RIBA HCV 3.0 SIA (Novartis Vaccine and Diagnostics, Inc, Emeryville, CA), and/or HCV viral load (COBAS AmpliScreen HCV version 2.0; Roche Diagnostics, Indianapolis, IN). HCV genotyping may also be performed.

HSV-2 serology status will be determined using HerpeSelect 2 enzyme-linked immunosorbent assay (ELISA) IgG (Focus Technologies, Cypress, CA) or equivalent.

Syphilis testing will be performed using a non-treponemal screen (Rapid Plasma Reagin (RPR), Wampole RPR, Wampole, Inc.) followed by treponemal supplemental confirmatory testing.

Malaria testing will be performed by DNA PCR on thawed whole blood lysates using modifications of Johnston et al (2006) and Snounon et al (1993b).

Samples will be reposed for potential testing of antibodies to HIV vaccine vectors.

Among participants who are known to be on anti-retroviral therapy or have demonstrated control of HIV viremia without a history of anti-retroviral therapy, drug levels will be ascertained to verify treatment status.

4.3.2 Viral Evolution

Figure 4 shows the timing of samples to be used for viral genetics (Fiebig 2003) and describes the nature and scope of the analyses proposed. The earliest attained viral RNA positive sample will trigger a look-back into earlier plasma samples for the presence of low-level viral RNA (Fiebig 2005) and in the primary PBMC, collected twice weekly for the previous 3 months, for the presence of proviral DNA, in order to retrieve viral variants that were transmitted but never replicated to a detectable threshold in the viral RNA population. The approach will be random, WGA of the extracted DNA, which may derive from as few as 100,000 cells, followed by nested PCR for the presence of viral genome. Using very short, well-conserved target gene segments, conventional first round PCR will be followed by second round, real-time PCR in the presence of a specific fluorescent probe within the amplicon. This exploratory study will be pursued on the first 10 seroconverters and, if any are positive, a second stage will be initiated to recover the viral defense genes *vif* and partial *gag*, which interfere with apolipoprotein B-editing catalytic polypeptide-like subunit (APOBEC) and (TRIpartite Motif 5 (TRIM5α), respectively. This will help to determine if the successful viral variants represent escape variants from host defense genes. If no sample is DNA positive, or of insufficient material is present to recover viral defense gene sequences, this approach will not be pursued further.

The first RNA positive small-volume sample will transition the participant to the AHI study, where a large volume blood draw schedule begins 3-4 days later. The first large RNA positive blood sample will be the source of several crucial materials: viral RNA for 5-15 complete genome sequences, depending on the complexity of the quasispecies observed, a complete viral proteome sequence for the synthesis of autologous peptides to be used for identification of recognized CTL epitopes, reagents for production of an infectious molecular clones representing the earliest attainable virus, the first 20-30 envelope sequences, and an in-depth study of the later identified CTL epitopes. Full-length (FL) viral sequences will be attained by single genome amplification (SGA) strategy. Five FL genomes will be generated initially and, if two or more distinct variants are observed, ten additional genomes or more will be generated. We expect 80% of samples to the homogeneous and 20% heterogeneous, based on current reports (Salazar-Gonzalez, Bailes et al. 2008) and Keele, Giorgi, et al. 2008. A consensus sequence of the viral proteome will be generated and provided to the cellular immunology group for use in the

synthesis of autologous peptides for evaluation of CTL epitopes. The full genome PCR products will be used to generate an infectious molecular clone and chimeric/ IMC with swapped envelope. These reagents will be used for biological characterization of the earliest viral strain, for studied of virus neutralization, and to generate autologous infected target cells for certain cellular immune studies. Envelope sequencing will be performed at closely spaced intervals in very early infection. We will again use SGA of RNA extracted from plasma, generating approximately 20-30 envelope sequences from the first RNA positive sample and at 3, 8, and 24 weeks post infection, starting with 10 acute infections initially to explore the utility of the frequent sampling approach. Functional envelope clones will be generated from every sample for evaluation of the evolution of neutralization susceptibility during the elaboration of innate and adaptive humoral effector mechanisms. From the study of recognized CTL epitopes and their early escape variants, we will also perform 5-10 genomes at 3 and 8 weeks post-infection. From the sequence diversity obtained comparing to the transmitted/founder (T/F) viruses we will select approximately 5 regions across the viral genome to perform in-depth longitudinal analysis for each study subject. Viral RNA from the first RNA positive sample, and from 3 and 8 weeks post-infection, will be subjected to pyrosequencing to interrogate the viral quasispecies in great depth. Other timepoints may be added for pyrosequencing. This technology provides a more complete description of the population of viral genomes present in a given sample, and can permit identification of variants present as low as 0.1% of the quasispecies. Envelope SGA will be performed to study the earliest viral quasi-species identified in mucosal compartments arising during acute HIV infection. The specimens used will be semen and vaginal/rectal swab collections and sigmoid gut biopsies (collected via approved protocols RV 254 and RV 304) at the earliest positive time point. Sharing between RV 217, RV 254 and RV 304 is planned to optimize use of controls and minimize overall risk to volunteers.

Additionally, several sequences from the first sequencing timepoint to the viral peak, before onset of immune pressure, will be analyzed for mutation rates due to reverse transcriptase misincorporation. Sequences will also be analyzed in order to refine models currently available on the LANL website for estimate timing of infection, most recent common ancestor sequence and onset of host selection. (Giorgi 2010).

In addition, the Multi-region hybridization assay may be conducted on the plasma samples in order to determine HIV subtype. This will likely be performed on all acute cases.

4.3.3 Cellular Immunology

The aim of this study is to take advantage of a unique opportunity to comprehensively evaluate the early and pre-seroconversion HIV-1-specific $CD4^+$ and $CD8^+$ T cells and NK cell repertoire in the setting of four different genetic subtypes of HIV-1 in Uganda (subtype D, A, A/D), Tanzania (subtype C, A, A/C), Kenya (subtype A), and Thailand (subtype CRF_01 AE, B). Previous studies of the role of cellular immunity in the earliest time-points after HIV-1 infection have focused upon only single parameters of the cellular immune response such as cytolytic capacity or cytokine expression and secretion, have usually relied upon sporadic capture of acute seroconverters, have suffered from a small sample size and have concentrated upon subtype B and to a lesser extent subtype C. The proposed study is designed to take a systematic approach to studying early and pre-seroconversion cellular immunity by first identifying the earliest

emergent HIV-specific T cells using multiparametric flow cytometry and then use novel and exploratory assay platforms to reveal potential cell-mediated correlates of initial viral control. Perturbations in the innate immune response will be studied through assessment of the NK cell repertoire using multiparameteric flow cytometry to measure expression patterns of activating and inhibitory surface receptors. Pre-existing and generalized immune activation will also be studied throughout the course of early infection and correlated with clinical parameters such as the serum LPS evaluation. The comprehensive analysis of viral diversity that is proposed in the viral genetics study will be used as a guide for all reagents and peptide sets used to screen for adaptive cellular immune responses, while the KIR-typing, HLA-typing and other host genetic factor assessed in the host genetics study will provide a genetic background for studies of both innate and adaptive cellular immune responses. Taken together this study will provide a unique opportunity to evaluate the adaptive and innate cellular immune response at the earliest possible time-point after infection in the setting of four different genetic subtypes of HIV-1.

Adaptive Immune Response Analysis:
Guided by the full-length sequencing data from the earliest time of detection of plasma virus peptide sets based-upon the autologous virus sequence will be designed. Between 5 and 10 subjects will be selected for this analysis depending upon the reliability of patient follow-up. Peptide sets will consist of approximately 400 peptides, 16-18 amino acids in length, overlapping by 11-12 amino acids and spanning each ORF of the entire HIV-1 proteome. Peptides will be: 1) kept as individual aliquots; 2) pooled into master pools representing each parent protein (e.g. Gag or Env), and 3) pooled in a matrix format for epitope mapping. According to SOE #2, PBMC drawn at 2 weeks post the first RNA-positive SBV will serve as the source of cells for detecting the earliest emergent adaptive T cells. Instead of relying solely upon the IFNγ Elispot to detect antigen-specific T cells, we propose to use multiparametric flow cytometry to detect 5 important functional markers of T cell effector activity – intracellular trapping of IFNγ, TNFα, IL-2 and MIP1β, and CD107 surface translocation. Bulk responses against a particular gene product will first be identified using the master pools of peptides, and the individual epitopes identified using a matrix approach and screening in an Elispot format assay. 10×10^6 PBMC will be required for each of these screening steps. The epitope recognition data (also the CD4 and CD8 lineage information) will be co-analyzed with the sequencing data from later time points to identify potential epitope escape variants. The data will be further stratified according to the viral load during this period. We plan to rank epitopes according to their "viral pressure index" and "escapability index". Epitopes that mutate rapidly will be assumed have placed selective pressure upon the virus but have readily escaped immune recognition - these epitopes would therefore have a high "viral pressure index" and a high "escapability index". Epitopes that remain unchanged in the presence of high viral load would have a low "viral pressure index", while those that remain unchanged in the presence of low viral load would have a high "viral pressure index" and a low "escapability index". We predict this analysis will reveal the epitopes, and importantly the variants of these epitopes, which might be the best to include in a vaccine formulation. In addition, we will gain direct insight into the breadth and depth of cellular immune responses that control initial peak viremia.

Analysis of HIV Antigen Specific Immune Responses in Uninfected Volunteers:
These analyses will only be conducted when feasible based on arrival time in the laboratory. Heparinized fresh whole blood from exposed uninfected HIV-1 individuals will be activated with

several different HIV-1 peptide pools for 6 hr at 37°C in the presence of anti-CD28/49d, BFA and monensin. To maintain cell integrity, the assay will be performed within 8 hr after blood is drawn. After stimulation, red blood cells will be lysed by FACS lysing solution and remaining cells permeabilized with FACS Perm2 buffer. Samples will be stained with monoclonal antibodies against the relevant lymphocyte, cytokine and degranulation markers. Samples will be analyzed by FACS-Canto II or LSR-II and data analyzed by FlowJo.

Functional and phenotypic characterizations of immune cells:
Efforts will focus on the comprehensive detailed phenotypic and functional characterization of immune cells in AHI with and without HIV specific stimulation, including but not limited to, B cells, T cells, dendritic cells, neutrophils, and NK. Importantly, we will identify the cellular sources of some of the key cytokines involved in the acute phases of HIV infection. PBMC samples collected at the pre-stutter time point and during the acute and chronic phases of infection will be assayed for each volunteer. At a minimum, 3 samples will be included for study: those drawn within 1 week after the detection of viremia (where early peak cytokines are expected), within 2 weeks and within 1-3 months after the detection of viremia (for late peak cytokines). Multiparameter flow cytometry will be used to identify and characterize immune cell populations isolated from whole blood. Fluorescence conjugated antibodies against the markers for lymphocytes, chemokine receptors, activation, apoptosis and other functional markers will be used. Markers may be added or substitutes as new reagents become available. All antibodies are titrated prior to use to determine the optimal concentrations for staining. Samples will be acquired on an LSR-II flow cytometer equipped with 4 lasers and 18 detectors. Flow cytometric data will be analyzed using FlowJo (Treestar). In addition, PBMCs in complete media will be stimulated with heat-inactivated influenza virus as a positive control, aldrithiol-2 treatment of HIV-1 particles (AT2-HIV) as a stimulus, or Jurkat cell-derived microvesicles (MV) as a control for 18 h at 37°C. After stimulation, supernatants will be collected for cytokine detection. Cells will be stained for various phenotypic and activation markers and analyzed by flow cytometry. Similarly, in order to detect cytokines intracellularly, PBMCs in complete media will be stimulated with heat-inactivated influenza virus as positive control, AT2-HIV, or control MV for 4 h at 37°C followed by an additional 4 h in the presence of protein transport inhibitor (Brefeldin A and/or monensin). Surface staining will be performed and cells will be fixed and permeabilized and intracellular cytokine staining will be performed. Samples will be analyzed on an LSR-II flow cytometer and data analyzed with FlowJo software. Plasma cytokine levels will also be quantified using a custom Q-Plex™ Human Cytokine–IR Array according to the manufacturer's protocol. Images are taken on an Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE) and analyzed using Quansys Q-view™ Plus software (Quansys Biosciences). At this time the Type 1 interferons are not yet optimized in the multiplex array. As such, standard cytokine ELISAs will be utilized for the quantitation of IFN-α and IFN-β (PBL Biosciences / R&D Systems). In addition, other markers of immune activation and inflammation will be studied.

Regulatory T cells:
Regulatory T cells may be examined on cryopreserved PBMC samples, when cell yields allow, using multiparameter flow cytometry on the LSR-II. Potential markers that may be analyzed in combinantion with some of the core markers described in the above sections will be CD25 (IL-2 receptor) bright expression, CD62L (L-selectin) high, and Foxp3 (forkhead box protein 3 transcription factor), CD69, CD122, CD127 and CTLA-4. Tregs will be functionally interogated

by screening for IL-10 and TGFbeta production or other cytokines. In addition a4b7 expressing cells will examined to look at the effects of gp120 binding to these cells and their cytokines.

NK cell Immunophenotyping and Immune Activation Marker Analysis:
Cryopreserved PBMC will be thawed and used for measurement of NK cell frequency, phenotype and activating and inhibitory receptor expression. The cells are washed and stained in flow panels consisting of core antibodies to differentiate NK cell and subsets. The core panel will distinguish NK cells based on CD16 and CD56 expression. Each panel will also look at different NK cell activating and inhibitory receptor expression. For study of immune activation, a core panel will be used and will have a selection of the following markers added to it: CCR5, CD25, CD38, HLA-DR, CD69 and Ki67. Other markers may also be added as necessary. Samples will be acquired on an LSRII and analyzed using Flow Jo software. For functional analysis of NK cells, samples will be thawed, washed and activated using media, MHCnull K562 cell line, or PMA/ionomycin, or other stimuli. These samples will be incubated with CD107a, Brefeldin A, and monensin overnight (18 hours). The samples will then be stained for expression of CD3, CD4, CD19, CD14, CD16, and CD56 to determine NK cell frequency and subsets. Additionally, MIP1β and IFNγ will be stained intracellularly and compared to other functional markers of interest. The samples will be acquired on an LSRII and analyzed using FlowJo software. We anticipate that immune activation markers will be generally higher in the populations studied (especially east African) and that perturbations in the NK cell repertoire will be detectable early in infection.

Pilot Studies of Gene Expression Profiling and Novel Assay Platforms:
Samples for gene expression analysis will be taken at the earliest stages of HIV infection in order to elucidate those host gene expression patterns indicative of the status and duration of the Fiebig stages. PBMC samples for expression analysis will be identified in close co-ordination with those used in the Host Genetics sections and from those generated by flow cytometric analysis and sorting of subsets of cells of interest with respect to assessment of the cellular immune response. In the first year of the study we will perform several small pilot experiments to determine the feasibility of gene profiling of small numbers of cells. Ideally we would like to sort or purify only the antigen-specific T cells. However, these will be limiting in number and we would need in the order of 20,000 cells at least, to perform the analysis. In collaboration with other groups we will be exploring novel assay strategies that may reveal underlying, and as yet undefined, mechanisms of T cell mediated inhibition of HIV propagation in *in vitro* culture systems. A modified assay for direct measurement of antiviral $CD8^+$ T cell activity against autologous $CD4^+$ T cells infected with select strains of HIV-1 will be applied. This assay has the advantage of directly measuring antiviral activity, without the need for a pre-determined surrogate marker of immune function. $CD8^+$ T cells displaying effective inhibition of viral growth can be further studied using gene profiling and multiparametric flow cytometry in an attempt to identify the function, or pattern of functions associated with antiviral activity. Gene array profiling will be used to identify signaling and transcriptional signatures of innate immune cells as well as that of responding T cells during acute HIV-1 infection and a systems biology approach may be taken to elucidate signaling pathways and transcriptional networks.

Proteome Analysis of Activated CD4+ T cells Infected with Different Clades of HIV-1:
PBMCs from HIV-1 clade A and clade C infected individuals at different time points will be used for the isolation of $CD4_+$ T-cells. A small aliquot of the PBMCs from each of the

individuals will be analyzed on an LSRII to determine the % of CD4 infected T cells by intracellular p24 staining. PMBCs will be stained for CD3, CD4, and MHC class I and sorted based on the surface expression of these markers using a FACS ARIA cell sorter. $CD4^+$ T- cells will be sorted into two populations: (i) $CD3^{hi}$, $CD4^{hi}$ and MHC class I^{hi} (uninfected), and (ii) $CD3^{hi}$, $CD4^{low}$ and MHC class I^{low} (HIV-1 infected). The proteome profile of HIV-1 infected and uninfected $CD4^+$ T-cells will be analyzed by 2-D isoelectrophoresis followed by image analysis. Protein spots that are absent or have a greater than a twofold difference in spot intensities between HIV-1 infected and uninfected $CD4^+$-T cells will be identified. The appropriate proteins will be excised from the gel, digested with trypsin and peptides will be subjected to mass spectrometry.

4.3.4 Humoral Immunology

Binding antibody assays

The antigens utilized in the assessment of binding antibodies are versatile and can mirror the diversity of the vaccine immunogens for the clade of the infection cohort studied. HIV-1 Env and HIV Gag Proteins are the predominant antigens utilized to characterize the antibody response. In addition, peptides specific for certain regions of the HIV proteins can be utilized to fine map the specificity of antibody responses.

A customized multiplex assay (using Luminex technology) for the detection of IgM, IgA1, IgA2, IgG1, IgG2, IgG3, IgG4 antibodies with specificities against HIV Env gp140 and gp41, Gag p55, Tat, Nef, RT, p31 and Env epitopes (ie. MPER) will be assessed in longitudinal samples. Autoantibody responses will be assessed including a rheumatoid factor test. Samples will be separated into IgG and non-IgG fractions for analysis. Positive and negative controls are included in every assay. Fully characterized samples in terms of antibody type and specificity will then be assessed for functional activity. The formation immune complexes (antibody-virion) will also be measured using developmental antibody capture assays to assess the possible role of antibodies complexed with virus in acute infection. Capture assays will be done for both IgG and IgA by measuring the capture of infectious virions (in addition to total virions). This work will be performed in collaboration with Dr. Georgia Tomaras at Duke University.

Mapping the epitopes for the early antibody responses:

Broad epitope mapping may be done at select timepoints using a peptide microarray that covers multiple clades and CRFs (Tomaras). In addition, early immunodominant non-protective epitopes (IDNPE) may be mapped through use of binding to a series of engineered mutant proteins (Nara). These initial studies will be a follow-up to ongoing immune refocusing efforts using the CM235 CRF01 AE env. Dr. Tomaras has under development the peptide microarray technology predominantly used for mapping linear and non-conformational epitopes. This method may be applied to select samples of interest to determine the binding epitopes. This may be of interest for both IgGs and for IgAs. Dr Peter Nara at Biological Mimetics, Inc. has requested samples to identify the earliest immunodominant non-protective epitopes; these may be mapped through use of binding to a series of engineered mutant proteins that are produced in a vaccinia expression system.

ADCC, Neutralization, and other functional assays.

These assays will be performed on frozen plasma or mucosal samples collected at all indicated timepoints post-HIV-1 RNA detection. Assays for a single subject will be tested in the same assay to reduce inter-assay variability and the same positive and negative controls will be used for all assays to allow normalization of the data. The positive and negative controls will be sera from HIV infected and uninfected subjects, and in the case of neutralization assays, additional specific reference mAbs. The plasmas will be assessed first in the TZM-bl assay using autologous and well characterized heterologous env clones, and later, available IMC and infectious isolates will be characterized in a subset of PBMC assays for comparisons and to assess clade-specific relationships. There will be no paucity of reagents for neutralization studies; however, antigens available for ADCC studies may be a technical challenge. The laboratory at AFRIMS in Bangkok has extensive experience performing ADCC using subtype B and CRF01_AE gp120, and an assay optimized at Duke University will also be performed by Guido Ferrari. We will purchase gp120 of subtypes A, C, and D from Immune Technology, Inc, and will also test vaccinia-expressed o-gp140 from the strains used in our A, C, D and CRF01_AE vaccines. An ADCVI assay may also be used to assess antibody-dependent, cell mediated virus inhibition; this assay would be performed in the Rockville labs using donor PBMC infected with IMC as targets for suppression.

The systematic evaluation of both binding and functional antibodies directed against autologous and heterologous viruses will contribute to our knowledge of the cross-clade responses within peripheral and mucosal compartments and against non-B HIV-1 subtypes from East Africa and Thailand, and specifically at potential vaccine cohort sites.

Characterization of HIV envs and isolates from acute infection

Envelope clones will be prepared from both the mucosal and plasma samples at multiple early time points, and these envs will be tested for function (formation of infectious pseudovirus through transfection of 293T cells and infectivity assessment in the TZM-bl cell model). Functional envs will be assessed for neutralization phenotype using reference panels of poly- and monoclonal antibodies. The env clones will be assessed first in the TZM-bl assay, and later, available IMC and infectious isolates prepared from samples obtained at or near the first RNA positive sample, will be characterized in a subset of comparative PBMC assays. These data will provide extensive characterization of the biotype of envs from acute infection for subtypes A, C, D, CRF01_AE and some novel recombinants. The A3R5 neutralization assay may also be employed.

Characterization of Antigen-Specific B Cells

The laboratory of Dr. Tony Moody, a CHAVI investigator at Duke University School of Medicine has developed a B cell phenotyping panel that is based on a series of core markers for positive and negative selection of B cells and the definition of core subsets, as well as a series of rotating markers for the characterization of additional B cell subsets. The core set is based on early work by Banchereau (Pascual 1994) and additional work by Bohnhorst (Bohnhorst 2001). The core panel consists of negative markers (CD3, CD14, CD16, CD235a) to eliminate cells not of interest and the B cell marker CD19. The CD19+ve population is then further subdivided by into six subsets that divide cells into pre- and post-class switching and into mature and naïve groups. Circulating plasma cells are then grouped into this scheme. A set of several additional markers are rotated in different FACS channels are to identify and enumerate numerous cell populations. A set of epitope-specific B cell tetramer reagents is also available to identify

antigen-specific B cells. Comparisons of cells that express immunoglobulins reactive with epitopes of well known broadly neutralizing antibodies, or epitopes to which antibodies are commonly made, will inform our understanding of the fate of these cells and potentially offer clues as to the rescue of these cells by vaccination strategies.

Determination of the TCID50 of infectious virus present in plasma throughout acute HIV infection

Unpublished data indicate that there may be a fall-off in the infectious titer of plasma virus during acute HIV infection, suggesting that there could be an immune basis to the drop in viral load that is observed in early infection. This would indicate a potential role for HIV-antibody immune complexes, or an as yet unidentified antibody population. A standard TCID50 assay to determine infectious viral titers in plasma will be employed using PHA-stimulated donor PBMC as viral targets and a p24 readout for infection. This will be done in collaboration with David Montefiori at Duke University.

Analysis of antibodies binding to proteins and lipids

ELISA and Biacore assays will be utilized to analyze the serum samples during the course of an HIV-1 infection for the presence of antibodies to lipids. We will initially screen the sera for antibodies to four representative lipids, cholesterol, phosphatidylinositol-4-phosphate (PIP), cardiolipin, and galactosyceramide (GalCer) using a modified ELISA procedure (Swartz 1988; Matyas 2006; Matyas 2009). If antibodies to cholesterol, GalCer, cardiolipin and PIP are found, the sera may be screened for additional antibodies to other lipids described in Matyas (2009). Biacore assays (Beck 2007; Karasavvas 2008) will also be used to analyze the serum samples to HIV-1 proteins (env, p17, and p24). The subclass of the captured anti-HIV IgG antibodies will be determined by sequentially injecting through the Bioacord anti-IgG1, anti-IgG2, anti-IgG3, and anti-IgG4 to allow binding and isotype determination. Serum samples will also be analyzed for their ability to bind primary human macrophages by confocal microscopy and flow cytometry. Supernatants from macrophage cultures incubated with serum samples will be analyzed for the induction of chemokines, MIP-1 alpha, MIP-1beta, RANTES, and IFN-gamma by ELISA or by flow cytometry.

Mucosal Specimen Collection

Mucosal secretions for this study are defined as CVL, cervical cups, rectal sponges, and semen.

CVL collection will be conducted on those participants who have already previously donated this way. As longitudinal comparisons would be difficult if a participant were to switch from CVL to cup, cervical cup collection will only be used for new participants. This will allow comparisons between the two collection devices and also bridge the gap between those studies that used only CVL collection and those studies that use the cervical cup for collection.

Rectal secretions will be collected by rectal sponge, and will be collected in addition to the CVL/cervical cup and semen collections. The rectal secretions will be offered to both male and female participants.

We propose to assess the following in mucosal secretions from subjects with acute HIV infection:

1) Qualitative and quantitative determination of the evolution HIV-specific binding antibody to include, but not limited to Gag and Env and characterization of the antibody isotype using techniques described below.
2) Measurement and evolution of HIV-specific neutralizing antibody to primary isolates of HIV-1.
3) Determination of the immunoglobulin isotype(s) of neutralizing antibodies and their temporal evolution.
4) Correlation between HIV-specific binding and neutralizing antibodies.
5) Determination and evolution of ADCC activity against HIV Env from diverse subtypes and the isotype(s) of the ADCC antibody if present

HIV-1 acutely infected study participants will be asked to provide mucosal specimens as indicated on SOEs #1 and 3. A pregnancy test will be performed prior to mucosal collection for safety considerations. At the time an incident case arises, specimens will be collected on HIV seronegative subjects to assess specificity of the assays and ensure that their performance is comparable across the different sites. For each HIV acute infection in the study, an HIV seronegative will be matched on age and gender, and specimen collection will follow the schedule for HIV acute infection (Phase II). Should that seronegative convert during the study, another HIV seronegative will be asked to enroll in the mucosal study and follow the same visit schedule as the HIV acutely infected subject.

In addition, each site will be permitted to collect baseline samples (as noted on SOE#1) on 20 women and 20 men. If the participant does not move out of phase I, their sample may be used as a control or discarded, based on the PI's discretion.

CVL and semen will be collected according to the procedures detailed in "The Manual for Collection and Processing of Mucosal Specimens; AVEG Mucosal Immunology Laboratory, University of Alabama at Birmingham, Alabama; Feb 1999). Mucosal collections from females via CVL must be accompanied by a serum tube collection (3ml) for hormonal testing as this may affect levels of immunoglobulin in the CVL.

Collection of CVL:
Briefly, 3ml sterile saline will be flushed several times over the cervix and around the external cervical os. Material will be collected and placed into a test tube on ice, aliquoted (0.4ml) into cryovials, and protease inhibitors added at 1/10th the specimen volume. Samples will be stored at $< -70^{o}C$. Samples will be excluded for any of the following criteria; abnormal vaginal discharge, sexual intercourse without a condom, douching within 72h prior to collection, or blood contamination. CVL will be tested for Occult blood using the Hemoccult test.

Collection of Cervico-vaginal Secretions Using the Cervical Cup:
The collection of mucosal samples will be subject to the volunteer's acceptance and tolerability and laboratory constraints. Detailed sampling technique will be as per SOP. All specimens will be processed within 8 hours of collection.

We will evaluate the concentration and specificity of antibodies in vaginal mucus using a new collection method called *Instead Softcup*. The Instead Softcup is an FDA approved feminine hygiene alternative to pads and tampons for use by women during menses. The cup is a disposable single use device that can be placed by the woman in the vagina for up to 12 hours to

collect blood, or in this case, vaginal secretions. An advantage for this collection method is that the woman can insert the cup intra-vaginally herself so no pelvic exam is required. It also eliminates the dilution factor as the mucus will is collected in its natural state and normalization of the samples will not be needed.

Softcups will be used according to manufacturer's instructions. Specifically, use of the Softcup is not recommended for those using an IUD, for those with a history of Toxic Shock Syndrome (TSS), and during the period immediately following childbirth, miscarriage, or termination of pregnancy. For this protocol, this period will be defined as within six weeks.

For preservation of cervical sample integrity, participants should be advised not to have vaginal intercourse or douching for the 72 hours preceding collection. Female volunteers enrolling in the mucosal secretion substudy will receive an initial pelvic examination by clinic staff with PAP smear. If the volunteer requests, clinic staff may assist and instruct the volunteer on insertion of Softcup device during this pelvic examination process. Volunteers with abnormal results on the PAP smear will be referred for further care and treatment as clinically indicated. They will be referred for further care and treatment and deferred from enrollment into the mucosal substudy.

Additionally, they will be queried for signs and symptoms for sexually transmitted infections (such as gonorrhea or Chlamydia), abnormal discharge, and date of last menses. Additionally, urinalysis will be conducted to look for the presence of white cells (leukocyte esterase). Women with signs and symptoms of an active STI will be referred for care and treatment and deferred from giving mucosal specimen. Menstruating women will have their mucosal collection deferred, up to the end of the study visit window, after which the mucosal collection time point will be missed. The stage of the menstrual cycle will be determined by checking LH and FSH levels at the time of each mucosal collection.

Collection of Mucosal Samples by Rectal Sponge:
The inclusion of rectal sponges to collect rectal secretions is a new methodology that has not been tested yet with our assays. It is being included so we can compare against other mucosal collection methodologies. The advantage of using the sponge is that the amount of mucosal secretions collected should increase dramatically. The process should only take 1 minute (retention of sponge, not including preparatory work). Mucosal secretions would be undiluted. Since samples will be undiluted, we could avoid normalization and have more confidence in the results. The product we are planning to use is the Schindler Ear Packing Ear Wick (Merocel hemoX). It has to be introduced using a disposable anoscope, and retained for 1 minute before it is retrieved. The anoscope is kept in place until the sponge is retrieved to prevent the fluid from squeezing out. Detailed sampling techniqe will be as per SOP.

Collection of Semen:
Briefly, the volunteer is asked to masturbate without lubricant, and the ejaculate collected into a sterile specimen container. Ejaculate is stored at 4 ^{o}C for 1h, diluted with an equal volume of PBS and centrifuged at 1200g/10 minutes. Protease inhibitor solution is added at 1/10th the final volume, the supernate aliquoted at 0.2ml and stored at < -70^{o}C, and the cell pellet frozen in a single vial at < -70^{o}C. The protease inhibitor solution contains PMSF, aprotinin, leupeptin, antipain and pepstatin. Samples will be excluded for any of the following criteria; symptoms of

infection – e.g. abnormal penile discharge, semen samples do not liquify following refrigeration, any ejaculation within 48h prior to collection.

4.3.5 Host Genetics

We propose both the targeted analysis of genetic polymorphisms within genes controlling host restriction, innate, and adaptive immunity factors that can influence HIV acquisition and disease progression and GWAS-derived associations with peak and set-point viral load. We will, respectively, apply a real-time PCR platform at our Rockville laboratory and the Illumina Infinium Whole Genome Human1M BeadChip within the Duke IGSP genotyping facility to accomplish these goals. The Human1M BeadChip combines an unprecedented level of content for both single nucleotide polymorphisms (SNP) and copy number variation (CNV) analysis, along with additional unique, high-value genomic regions of interest, as the MHC region. This chip is therefore especially promising in a population with African ancestry. A series of quality control procedures will be carried out to check for: 1- Infinium BeadStudio Raw Data Analysis quality, 2- minor allele frequency consistency to assess data handling accuracy, 3- mismatches between clinical and genetically inferred specification of gender, 4- cryptic relatedness, 5- pattern of missing genotypes, 6- low minor allele frequency, 7- Hardy-Weinberg Equilibrium violation, and 8- visual inspection of genotyping quality for top SNPs. Core association analyses will focus on single-marker genotype trend tests of the quality-control passed SNPs using linear regression. To control for the possibility of spurious associations resulting from population stratification, a modified EIGENSTRAT (Price, 2006) method will be used. Other covariates considered in the regression model will include age and gender. To assess significance, the suitability of a straight Bonferroni correction will be evaluated by comparing this to the results of permutation testing to assess the appropriate cutoff. If a straight Bonferroni correction is used, the conservative P cutoff will be $5 \text{ X } 10^{-8}$.

The targeted analysis of pre-identified host restriction factors will be directed at those polymorphisms that occur within HIV co-receptors, the natural cytokine ligands of these co-receptors, and host defense systems interfering with crucial post-entry events of the viral replication cycle, that have been reported to be associated with different rates of HIV acquisition and disease progression. Commercial reagents will be used when available, and otherwise, reagents published in the literature will be adapted to the real-time PCR platform.

The analysis of innate immunity factors will be targeted at KIR genes and their ligands, and polymorphisms in DC-SIGN and TLRs, that that have been reported to be associated with different rates of HIV acquisition and disease progression. The targeted analysis of humoral adaptive immunity factors will be directed at polymorphisms in Fcγ receptors that affect binding specificity and affinity for the diverse subclasses of IgG. The targeted analysis of cellular adaptive immunity factors will be directed at alleles in the Class I HLA-A, -B, and –C loci, which restrict CTL responses. Population-based comparative analyses of frequencies of the targeted polymorphisms will be conducted between exposed HIV sero-negative vs. HIV sero-prevalent individuals derived from the same socio-demographic setting. The comparison between exposed HIV sero-negative and HIV sero-incident cases will be performed in a case-control format.

Among HIV sero-incident cases, the following associations with targeted polymorphisms will be explored:

- patients' genotypes vs. the duration of the different Fiebig stages
- patients' genotypes vs. timing and level of peak HIV viremia
- patients' genotypes vs. timing and level of set-point HIV viremia
- patient's host restriction genotypes vs. dynamics of HIV genetic variation (e.g., APOBEC3s vs. *vif*, TRIM5α and Cyclophilin A vs. *gag*, co-receptors and their ligands vs. *env*, tetherins vs. *vpu*)
- patients' KIR/ligand genotypes vs. dynamics of HIV genetic variation, to identify early NK-escape viral variants
- patients' Fcγ receptor genotypes vs. dynamics of anti-HIV ADCC responses

The HLA genotyping information from HIV sero-incident cases will support the work of the functional cellular immunology sub-project, and will be used to design reagents to ascertain CTL responses to autologous and near-autologous viral sequences, identify immunodominant and subdominant epitopes, design tetramer or pentamer reagents.

The HLA testing that will be used in this study is a research tool that has not yet been validated for clinical use. The HLA typing will be essential for the analysis of adaptive cellular immune responses in early HIV-1 infection and their subsequent effects on viral evolution and they will be among the host factors that will be evaluated for their contribution to protection from HIV-1 infection and early control of the virus. The methodology that is proposed for HLA typing is adequate for these purposes, but is intended for research use only. To provide this information to study volunteers, the new HLA typing approach would have to be validated, and the testing would have to be done by an accredited clinical lab. Further, we are advised by colleagues in the field that even the strongest HLA effects are not used in current practice to dictate treatment or other clinical care options, because they represent population-level probabilities, not necessarily predictive for any given individual. To meet the research needs, the HLA typing that is proposed would be done unlinked to any personal identifiers, and results reported in aggregate for the populations studied.

The GWAS approach will be limited to associations with viral load peak and subsequent viral load set-point in the anticipated 150 subjects who develop incident infections. Associations with peak viral load will be completely novel while associations with viral load set-point, while not completely novel, will provide substantial new information from individuals with subtype A, C, D, CRF01_AE, and intersubtype recombinant infections. Exploration of associations between incident infections and the anticipated 1850 subjects who are exposed and uninfected using GWAS would be of great scientific value, but are beyond the scope of this current proposal. Additional funding will be sought from other sources to execute this specific aim.

4.4 Acute HIV Infection Phase Design

All HIV positive volunteers identified at any time after a negative HIV test at the first follow-up visit will be asked to participate in an HIV incident follow-up phase IB. This is integral to phase

I of the study and does not require re-consent and allows definitive diagnosis of HIV status. Those who are confirmed as HIV infected incident volunteers will be asked to consent to further participation in phase II. Those who decline to participate in phase II will continue follow-up within the main protocol if they so wish. The HIV incident follow-up schedule in phase II will assess viral diversity, host immune responses, host genetics and early expression makers, which will be related to viral burden and characteristics of host immune response. AHI cases will be followed intensively with blood collection for cellular and acellular specimens in the first several months following infection and will then be followed every 3 months for at least five years (see attached Schedule of Evaluations #3). All HIV positive volunteers will be referred to a President's Emergency Plan for AIDS Relief (PEPFAR) funded clinic or to an appropriate medical facility for care and treatment. CD4 counts and viral burdens derived from the research will be available to the clinic at the direction of the volunteer at no charge.

4.5 Susceptibility to HIV Infection from Co-Incident Infections Design

Study volunteers will be tested for malaria if febrile; for syphilis and HSV-2 at Visit B and also at Visit 1 of stutter; and Hepatitis B (if appropriate), eosinophilia, and LPS upon study entry, within 42 days (Visit B) and subsequently every 6 months during the course of the study. If volunteer becomes HIV(+) they will be tested at the time of phase II enrollment for malaria (if febrile), syphillis, HSV-2, LPS, and eosinophilia. Self-reported STIs will be assessed at every scheduled visit and queried every 6 months by questionnaire. EDTA whole blood will be reposed to assess subclinical malarial infection by PCR. Self reported STIs will be confirmed by voluntary physical exam and syphilis will be diagnosed by RPR followed by confirmatory testing. Full CBC differentials will be obtained to quantify absolute eosinophils. Plasma will be collected to assess LPS levels.

4.6 Overview of Volunteer Activities (Phase I - Surveillance)

Recruitment methods and activities are directed at each group separately to minimize stigma risks and for operational efficiency. Volunteers will receive HIV risk-reduction counseling, a briefing regarding HIV and HIV vaccine research in general, a medical history and physical, a behavioral risk questionnaire, HIV testing and provide a blood sample to characterize general health (e.g. CBC, chemistries and LFTs), and HIV status. All volunteers will be seen in follow-up no later than six weeks after visit A (within 7 to 42 days of Visit A) to review laboratory and clinical findings from the screening visit.

Those testing HIV positive or indeterminate at visit A will provide a blood sample to verify HIV status at visit B. Only healthy and non-HIV infected volunteers will continue follow-up after the second visit, with the following two exceptions:

1. Participants will be enrolled contemporaneously and consecutively in a 1:10 ratio of HIV positive to HIV negative participants.

2. For those volunteers who are considered acute seroconverters based on testing indeterminate at Visit A and positive at follow-up can be enrolled into the RV 217 phase IB at the discretion of the site PI.

All other volunteers who are not eligible for participation due to HIV infection or other chronic illness will be referred to a PEPFAR funded care and treatment activity or to an appropriate medical facility for care.

Eight follow-up visits will occur at 12-week intervals after the second visit and include a directed medical history and physical and post-test counseling for all HIV results and risk reduction counseling. At every other visit (every 24 weeks) a behavioral and knowledge questionnaire, and blood draw to ascertain HIV status will be conducted. All volunteers will be asked to return to a site as convenient to their work place or home as feasible for small blood volume collections collected in a microvette. These samples will be delivered to the lab for analysis. Study volunteers will be tested for syphillis, Hepatitis (if appropriate), HSV-2 LPS, and eosinophilia according to the SOE. Self-reported STIs will be assessed at every scheduled visit and queried every 24 weeks by questionnaire. In East Africa sites, malaria will be tested if the volunteer is febrile. Malaria will be diagnosed by a thick and thin blood smear. Self-reported STIs will be confirmed by voluntary physical exam and syphilis will be diagnosed by RPR followed by confirmatory testing. Full CBC differentials will be obtained to quantify absolute eosinophils. Plasma will be collected to assess LPS levels.

Swab Collection

All participants entering twice-weekly surveillance after visit B will be asked to consent to the twice-weekly collection of vaginal and/or rectal swabs (according to the most recent sexual practice of the individuals) in order to identify the presence of HIV and determine quasispecies evolution at the site of infection prior to the advent of and after systemic viremia. Any volunteer within the first 2 months of commencing twice-weekly surveillance is eligible for vaginal/rectal swab collection.

Volunteers will self-administer these swabs and place them into collection tubes provided by the site. This collection may be done in the clinic in conjunction with their SBV visits or at home. The intent is to begin these mucosal swab collections early after enrollment, when transmission rates appear highest, and continue the collection for four months. The volunteers will be compensated for the vaginal/rectal swab collection.

At the end of the 4-month swabbing period, the swabs collected during the first 2 months can be discarded. At the end of 6 months since the beginning of collections, all retained swabs on an individual can be destroyed. The timing of discarding the swabs relates to the fact that the swabs can be kept at room temperature for only 60 days. The swabs should not be discarded until the analyses stipulated below are accomplished.

Receptive Risk Questionnaire

In addition, at the time of SBV, a questionnaire will be completed by the volunteer if literate or administered with staff assistance to document the frequency and type of sexual exposure occurring since the last SBV visit. The purpose of this brief questionnaire is to accurately measure the frequency and type of sexual acts to link to the “at-risk” exposure data.

The questionnaire will be independently collected from all volunteers who are undergoing SBV visits, regardless of whether they choose to consent to the swab collections.

Swab Analysis
The swabs will be analyzed using the APTIMA analysis platform.

For all volunteers participating in swab collection, prior to swab discard, 10-20 samples (roughly one per week of observation) will be analyzed for HIV RNA. Any positive samples will be frozen and transferred to Rockville, MD for sequence analysis.

If a volunteer participating in swab collections develops a reactive APTIMA plasma sample during the four months of swab surveillance or within 2 months after the last swab collection all of the swabs collected (that have not been discarded) will be analyzed. This analysis will occur even if the participant does not enter or remain in phase IB, i.e. analysis of all collected swabs will occur for an individual with a false reactive APTIMA as well as true reactive.

All individuals with a reactive APTIMA plasma sample, regardless of their participation in the prospective collection of vaginal/rectal swabs, will have a vaginal or rectal swab collected at each phase IB visit. A volunteer with a reactive APTIMA that has not been participating in the swab collections will be consented to allow swabbing during phase IB. Such a volunteer, if at visit 1 is no longer APTIMA reactive, will return to surveillance in Phase I with no additional swabs.

If a volunteer participating in the collection of vaginal/rectal swabs returns to surveillance after Phase IB (they were determined not to be infected), swab collection only continues if they remain in the four-month window of swab surveillance.

Should compliance with collections be under 50% after offering enrollment to 20 participants at a given site or analysis of the swab samples show no HIV nucleic acid signal among these first 20 participants, the collections will be stopped.

4.7 Entry into Phase Ib (Stutter Phase)

Very early HIV infection may have low amounts of HIV RNA or intermittently negative RNA tests. In addition, some HIV infected individuals control HIV infection very effectively and have very low levels of HIV RNA or may be negative even with the most sensitive tests. Hence, negative test results do not exclude the possibility of HIV infection. Positive tests are not conclusive evidence of HIV infection either. Some false positive results may be due to mis-labeling, contamination or other error. Hence, diagnostic testing and post-test counseling are provided using larger blood volume sample test results at the 24-weekly clinic visits. HIV uninfected participants with a new reactive Aptima test from a valid run will prompt a return visit at the earliest opportunity to either enter stutter at Visit 1 or collect a single, 8.5 mL tube of ACD blood. In addition, a 2ml heparin tube can be collected. ACD cells and plasma will be processed in a single cryopreserved PBMC vial and plasma as 1 mL aliquots (3 to 4). The heparin anti-coagulated whole blood will be used to inoculate a SMARTube. The diluted plasma from the SMART tube will be processed as 1 ml aliquots (3) and PBMCs as a single

cryopreserved vial. An SBV will be drawn at the same time as the visit 1 stutter SOE blood collections or the 8.5 mL ACD either by finger stick or from the venipuncture. An APTIMA test will be performed on this newly collected SBV plasma using the 1:5 dilution in PBS schema. If the follow-up sample APTIMA test is reactive, the participant is placed into phase IB. If the result is non-reactive, the participant returns to surveillance. If the volunteer returns to surveillance and has a second reactive APTIMA within 2 weeks, the volunteer is placed into phase IB. If the volunteer has a second reactive APTIMA after 2 weeks, then they are treated as if this were a first new reactive result, i.e. a single 8.5ml ACD tube is collected as soon as possible (as above).

However, if the participant is western blot positive at visit 1 or 2 (Fiebig IV, V, VI), the participant will move directly to SOE 3 and will skip remainder of stutter phase.

All confirmed HIV incident cases will be referred for entry into phase II. The evolution of antibody response will eventually confirm all assignments as HIV infected and its long-term absence will confirm an assignment of HIV negative.

Should a volunteer at any stage of participation indicate their desire to leave the study due to re-location or other reason, the team will attempt to arrange an exit visit to establish HIV status at the time of discontinuation.

4.8 Overview of Volunteer Activities (Phase II – Incident HIV Infections)

Volunteers who are confirmed to be newly infected with HIV are referred to the clinic at the next visit or as soon as possible to offer enrollment in the HIV acute infection (phase II). Confirmation of HIV status will be completed through phase IB.

Among volunteers who consent to participate in phase II, the blood volume and sample collection schedule specified in Appendix VI-SOE #3 will be applied and serve to provide all samples needed to confirm the diagnosis. Participants in phase II will have an initial assessment of safety labs, HIV diagnostic labs and collection of PBMC and plasma to meet the scientific discovery objectives of the study. Upon entry into phase II the participant will be administered a short questionnaire (Appendix XI), which asks about symptoms, treatment, if any, received and the circumstances under which the participant thinks they were infected with HIV. However, this information could be collected from interviews and exams, documented in the source docs, from phase IB visits.

The first visit in phase II will follow the last visit in phase IB by one week and relative to the first visit in phase II, subsequent visits will occur one week, two weeks, four weeks and then every 12 weeks for at least 50 months.

Should a volunteer at any stage of participation indicate their desire to leave the study due to re-location or other reason, the team will attempt to arrange an exit visit to establish HIV disease status at the time of discontinuation.

All HIV positive participants will be referred to an affiliated PEPFAR clinic or other care provider to manage HIV infection including provision of HAART as indicated.

5.0 STUDY POPULATION

5.1 Study Population

A description of the sites' study populations can be found in section 2.0. Part A (pilot phase), will enroll primarily SW and BW in Africa and SW, TG and MSM in Thailand. Each site will enroll up to 200 volunteers. Part B (full study) will enroll all of the MARPs to attain a total enrollment of 300 HIV negative volunteers at each site. However, some shifting of enrollment numbers per site might occur in order to complete the study at the same time at all sites.

It is customary to provide the exact inclusion and exclusion criteria in the volunteer consent. In order to minimize the risk of stigmatization of participants who must engage in high-risk behaviors to enter the study, the specific behavioral elements for inclusion will only be summarized in the consent as follows: "Based upon information provided in the questionnaire, your behavior places you at greater risk for HIV infection than the general population."

5.2 Inclusion Criteria*

A participant must meet all of the following criteria†:

1) Able to demonstrate understanding of the study by passing score of 90% on test of understanding within 3 attempts to take the test
2) Ability and willingness to sign/mark/thumb print the informed consent form
3) Man or woman aged 18-50 years
4) Available for follow-up for a total study duration of 24 months
5) Willingness to consent to HIV counseling and testing
6) Willingness to receive HIV test results
7) Willing to provide location or contact information
8) Willing to have picture taken for the study ID, or provide a photo ID or fingerprint
9) Must understand English or local language as approved by IRB
10) In the last 3 months prior to inclusion in the study, the volunteer must meet one or more of the HIV risk criteria as follows:
    a. has provided vaginal or anal intercourse in exchange for money, goods, or services
    b. has had unprotected vaginal or anal intercourse with one or more known HIV-positive partners

* Any modification to the three African sites enrollment criteria will be described in the site-specific addenda.

† A known or suspected HIV positive partner of an eligible participant may be permitted enrollment into phase II. Enrollment will be for 1 visit only (Visit 11), and will be at the discretion of each site. There will be no contact and tracing of the participant's partner. It is up to the participant to notify the partner and bring them in, if they so choose.

c. has had unprotected vaginal or anal intercourse with three or more partners of known or unknown HIV status

5.3 Exclusion Criteria

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

1) Any significant condition (including medical and psychologic/psychiatric disorder) which in the opinion of the study investigator might interfere with the conduct of the study
2) Unwillingness to give social history, medical history, undergo medical examination and provide blood specimen
3) Has ever received an HIV vaccine

Note: When the protocol is submitted to a local regulatory agency for approval, the site may note that it will be limiting enrollment to a subset of the risk criteria based upon relevant epidemiological data or prior site cohort research.

6.0 STATISTICAL CONSIDERATIONS

6.1 Power and Sample Size

The study is expected to generate approximately 150 seroconverters over the course of 24 months of follow-up, and 1850 are expected to remain seronegative as shown in Table 1 (assuming no loss to follow-up). On average therefore, at least 37 seroconverters are expected to be observed at the end of the 24 month follow-up, at each of the four participating country sites. We expect roughly 66% of infections (100 AHI) would be observed in the first 12 months (Riedner 2006; Baeten 2000) and of these at least 50% would have been compliant with surveillance and therefore identified in the first 2-3 weeks of HIV infection (Fiebig stages 1 and 2).

Of primary importance will be the ability to determine the incidence rate of HIV-1 infection and the rate of participant retention during the approximately 24-month study. With regard to the incidence rate, based upon prevalence and incidence estimates from previous research with these and related populations, and given the approximate sample size proposed for this study, we estimate an aggregate HIV-1 incidence rate of 3.7/100 person-years of follow-up, with 90% confidence intervals around this estimate of 2.9/100 person-years to 4.6/100 person-years. The sample size anticipated for this study also provides sufficient power (>90%) to detect a retention rate that is + 3% or more of 90%.

The main body of Table 1 shows the power to detect given rate ratios (RRs) between HIV-1 sero-converters (SCs) with a given risk factor of interest at baseline ranging in frequency from 10% to 50%; versus HIV non sero-converters (SNs) with the same risk factor of interest.

The following assumptions were made in the calculations:

- A total of at least 150 HIV incident cases would be observed over the 24 month follow-up period
- A loss to follow-up of 10% by the end of the 24 month follow-up period, thus 150 seroconveters, and 1650 non sero-converters by the end of the 24 month follow-up
- A level of significance (α) of 5%

Table 1 Power to detect a given Rate Ratio

Risk Factor proportion at baseline, among non sero-converters (SNs)	**Rate Ratio***				
	1.8	**1.9**	**2.0**	**2.1**	**2.2**
0.1	77.8%	85.3%	90.7%	94.4%	96.7%
0.2	98.5%	99.0%	> 99%	> 99%	>99%
0.3	>99.0%	> 99%	> 99%	> 99%	>99%

0.4	> 99.0%	> 99%	> 99%	> 99%	>99%
0.5	> 99.0%	> 99%	> 99%	> 99%	>99%

**Rate Ratio = rate of HIV infection among participants with risk factor of interest at baseline, versus the rate of HIV infection among participants without the risk factor of interest.*

We note from Table 1 that the study will have more than 80% power to detect a rate ratio of at least 1.9 if the baseline prevalence of any risk factor of interest among HIV non sero-converters (SNs) is at least 10%. All study power calculations were performed using the PS (Power and Sample Size Calculations) Software Version 2.1.31 (Dupont and Plummer 1990, 1998).

6.2 Analysis of Phase I Primary Objectives

Brief descriptions of statistical analysis methods to be applied to address the objectives of this study are as follows:

Prevalence - Baseline prevalence will be defined as the proportion of individuals testing positive at baseline divided by the total number of individuals who are test for HIV.

Incidence – Aggregate and interval-specific HIV-1 incidence rates will be calculated using person-time analysis, with the numerator being the number of seroconverters over a given time period and the denominator being the cumulative number of person-years contributed by the total number of individuals in the study at the beginning of the same time period. HIV-1 negative individuals will contribute person-time to the denominator beginning with the time they enter the study until the study concludes or they are censored (lost to follow-up, die). The respective 90% CI of the HIV incidence rate will also be calculated. We will then establish if the overall lower bound of the 90% CI of the HIV incidence rate is greater than 3.0%, as hypothesized.

Seroconverter case definition: for purposes of this study, incident HIV cases/seroconverters will be defined as any volunteer testing HIV positive at any encounter (scheduled or unscheduled) after enrollment by a standardized testing algorithm consisting of sequential ELISA and confirmatory WB testing who also test positive on a subsequent, repeat phlebotomy and verification HIV testing. Volunteers testing HIV positive and defined as seroconverters as above who are not able to return for verification HIV testing (i.e. refuse to return; lost to follow-up) will also be considered in analyses as incident HIV cases/seroconverters due to the high positive predictive value of the ELISA, ELISA, Western Blot algorithm.

For HIV incident case/seroconverters, the date of seroconversion will be calculated as the midpoint between the visit dates of the last negative test and first positive test. Seroconverters will contribute zero person-time from their calculated date of seroconversion to the visit corresponding to their first positive test. Ninety-percent confidence intervals will be calculated around point estimates.

Retention-Retention over approximately 12 and 24 months of follow-up will be calculated as the number of all study participants completing the approximately 12 and 24 months of follow-up at all of the country sites, divided by the total number of participants enrolled in the cohort at baseline at all of the four country sites. From this, we will establish if the overall retention rate is greater than 90% as hypothesized.

Risk Factors –Chi-square or Fisher's Exact Test will be applied to compare differences in proportions for categorical variables. Student t-test will be used to compare differences among continuous variables. RRs and their 95% CIs relating individual risk factors to incident HIV-1 infection will be calculated. Univariate and multivariate Poisson regression analysis will be used to assess the associations between these risk factors and HIV-1 infection. P-values for all tests will be two-sided, with a p value <0.05 considered statistically significant.

6.3 Data Analysis for Secondary/Exploratory Objectives

Host Genetic Factors- Phase I and II: Descriptive statistics will be generated to summarize the frequency and distribution of polymorphic variants at loci for host restriction, innate and adaptive immunity, among both uninfected and infected subjects at baseline. Allele frequencies will be determined by direct counting of the occurrences of each variant and statistics on genetic analyses will be computed using modules implemented in the Pypop version 0.6.0 (Lancaster, Nelson et al. 2003) and Arlequin version 3.1 (distributed by L. Excoffier, CMPG, University of Berne, Switzerland) software packages. Deviations from Hardy–Weinberg (HW) proportions will be tested using chi-square or the exact tests of Guo and Thompson (Guo and Thompson 1992). The Ewens-Watterson homozygosity test of neutrality (Ewens 1972; Watterson 1978) will be conducted as implemented by Slatkin (Slatkin 1994; Slatkin 1996). The significance of linkage disequilibrium (LD) among loci and the corresponding haplotype frequencies will be assessed by a permutation test using an expectation-maximization (EM) algorithm (Slatkin and Excoffier 1996). Two measures of overall LD will be determined: D′, which weights the contribution to LD of specific allele pairs by the product of their allele frequencies (Hedrick 1987), and Wn, which is a re-expression of the chi-square statistic for deviations between observed and expected haplotype frequencies (Lancaster 2004). For each inferred haplotype, individual D′ values will be calculated as previously reported (Cao, Moormann et al. 2004) to investigate deviations from random association of alleles, and to account for differing allele frequencies at the loci.

Phase II: The frequency and distribution of host genetic factors will be compared among the following groups: subjects who are uninfected and remain uninfected for the duration of the study, subjects who are HIV infected at baseline, and subjects who become HIV infected during the course of the study. Exploratory descriptive analyses of association of host genetic factors with susceptibility to HIV infection will be performed. We will compute unadjusted odds ratios for HIV infection in the presence of different alleles and extended haplotypes of host restriction factors and HLA, using variance estimates and 95% confidence intervals to evaluate the significance of any associations observed

HIV Viral Genetic Analysis- Descriptive statistics will be generated to summarize both baseline prevalent and incident HIV infections including frequency and distribution of each viral subtype.

Exploratory descriptive analyses including unadjusted univariate analyses will be performed to explore potential associations between risk factors and subtype. The study of viral genetics will be performed in a descriptive manner, as the anticipated number of HIV infections accrued during the study will be too low to perform any meaningful statistical test. However, any associations or significant trends will be followed up.

Humoral Immune Responses- ELISA and Neutralizing antibody titers will be compared at various stages of disease using non-parametric statistical analyses. The evolution of antibody responses will be characterized through a comparison of ELISA titers for different Ig subclasses (against several HIV antigens) at various time points post-seroconversion and between the various Feibig stages at which the patients enrolled in the AHI protocol are captured. Differences in the magnitudes of titers in early versus late Feibig stages, as well as in chronic infection, may be compared by Wilcoxin-Rank sums analysis, the Mann-Whitney U-test, or other appropriate measures. The agreement between titers derived from different assay platforms will be analyzed by linear regression to assess comparability of different methodologies and to identify antibody populations for which discordance exists.

HIV Diagnostics Analysis –Descriptive statistics and group comparisons to include stratified Mann-Whitney and stratified Kruskal-Wallis tests will be calculated for Fiebig Staging. Samples will be stratified, and the intervals of assay specific window periods will be projected using a parametric model. Analyses will evaluate 1) aggregate analysis of reactivity of participant panels, 2) relationship between HIV laboratory stage and HIV-1 RNA quantification, 3) model relationship between HIV-1 RNA level and p24 antigen signal-to-cutoff ratio, estimate interval of window period closure relative to Fiebig staging, 5) estimate probability of presentation due to high risk exposure, and 6) multivariate/univariate analysis of stage vs results of viral genetics, host genetics, immunological, and genomic studies.

Cellular Immunology Data Handling and Analysis- All flow cytometric studies will utilize a Becton Dickenson LSRII flow cytometer equipped with three or four lasers for simultaneous acquisition of data in 12-15 channels. Datasets will be analyzed using FlowJo™ software. The 12-parameter panels for the surface immuno-phenotyping will utilize a subset of the following surface markers: CD3 (or Tetramer), CD8, CD27, CD28, CD45RA, CD45RO, CD62L, CD127, CCR5, CCR7 and PSGL-1. A viability marker will be used in a discrete channel and a "dump" channel will be used to gate out non-T cells from the datasets. The 12-parameter panel for the functional immuno-phenotyping will utilize the following panel: CD3, CD8, CD28, CD95, IFNγ, TNFα, IL-2 and MIP1β expression, while degranulation will be studied by analysis of CD107 surface expression. Hierarchical clustering analysis will be used for the phenotypic analysis and definition of the different T cell subsets. Defined T cell subsets will be assessed in terms of absolute numbers and enrichment for HIV-1 antigen-specific cells. Boolean gating strategies will be used, particularly in the case of the cytokine gene up-regulation assays, to discern the different patterns of cytokine gene expression. Data will first be analyzed in terms of descriptive statistics, which will include calculation of the median and interquartile (25^{th} and 75^{th} percentiles) and extreme values (highest and lowest values). Non-parametric statistical tests (e.g. Mann-Whitney and Kruskal-Wallis Tests) will be used to compare and compute statistical differences among the data sets. The epitope mapping studies will utilize the interferon-gamma Elispot assay where the widely accepted cut-off for a positive response of 55 spot forming cells per 1 million input PBMC is to be applied.

7.0 PROCEDURES

7.1 Volunteer Recruitment

Volunteers will be recruited using methods in accordance with 32 US Code of Federal Regulations (CFR) 219 and all other policies and regulations of the United States Government and host nation. In addition, host national guidelines will be adhered to for conduct of the research study in each country.

Recruitment will be done using a combination of methods after approval by the WRAIR Institutional Review Board (IRB) and local IRBs. IRB-approved flyers/posters and public announcements will be used to recruit potential volunteers. The recruiting efforts will depend heavily on direct interaction with members of the target community, their community leaders, support groups and NGOs active in these communities. Information seminars will be organized in strategic locations within the targeted communities. At the end of each information seminar, potential volunteers will be referred to the clinical research sites for screening and enrollment. Details on recruiting and advertising are included in each site-specific protocol addendum.

In addition, sites may employ a respondent driven survey (RDS) method that is known to be effective in "hidden" populations subject to legal or cultural opprobrium. In this setting, initial "seed" respondents recruited by the methods noted above are given two coupons to recruit at most two individuals to the study. The new recruits may obtain appointments for study briefing and enrollment with presentation of the coupon. The coupon will identify the "seed" using a code known only to the research staff and if the recruit is qualified for enrollment, the participant providing the recruit via the coupon will receive a very modest compensation. The recruiting participant and the recruited participant will not have further interaction, i.e. the participant who referred the new recruit is not in any way responsible for the recruited volunteer's compliance.

7.2 Study Visit A

Potential participants will receive a briefing, consent, "test of understanding" (TOU) and questionnaire. Visit A commences with a detailed briefing provided by the site principal investigator (PI), the associate investigator (AI) or their designee (qualified staff such as, a study medical officer, study nurse, or counselor). The briefing will review some general information about HIV, HIV research, and vaccines as well as the study purpose, design, risks, benefits, compensation, volunteer rights and other topics included within the written consent form. A question and answer period will follow the briefing.

Interested participants will then undergo a detailed review of the consent form (found within Appendix I: Site-Specific Addendum) by research study staff and will be asked to provide consent to participate in the study by signing the Informed Consent Form (ICF). In addition to the consent to participate, participants will be asked to provide consent to store their samples for future use on a separate "future use" consent (also found within Appendix I). Volunteers will receive a verbal, brief TOU (Appendix II) that is used as a tool to insure that key elements of

consent and participation are understood. Volunteers will be permitted three successive attempts to pass (passing score defined as 90%) and those not passing will not be permitted into the study.

After volunteers have given consent to participate in the study, volunteers will proceed for study number assignment and preparation of a study identification card that may be used for identification in study follow-up. The identification card may contain the volunteer study number or contain the photograph, but not both together. Further details of volunteer identification can be found in the site-specific addenda.

After successful completion of the consent and TOU, the volunteer is administered a questionnaire using the Audio-Computer Administered Self Interview (ACASI) or if preferred by the participant, the questionnaire will be administered by research study staff. Behavioral risk information will be collected through ACASI to determine eligibility for continued study participation. All consenting participants will undergo a full questionnaire. Only those participants whose behavior categorizes them as members of a MARP will continue the study. To provide masking, i.e. to obscure the stigmatization of participants who continue the study due to high-risk behaviors, a small percentage of eligible volunteers may be randomly excluded. All volunteers who are categorized as MARP will then undergo an entry medical history and physical and blood collection. This visit may be conducted over two days to allow the time necessary to complete the study procedures. Study procedures by visit can be found in the SOE #1 (Appendix IV).

Contact information will be collected from the volunteer at either study visit A or B. The volunteer may be asked to provide their contact information using a contact information form. This form may include the volunteers' name, phone numbers, if applicable, date of birth, age, address of residence (or best description), review of national identification and/or personal identification card, fingerprint and acceptable person(s) to contact including address and phone number, if applicable. The volunteer will be asked to specify which methods of contact are acceptable and will sign giving approval for acceptable methods. The personal identifiers are included on the contact information form for purposes of verifying the identity of the volunteer at each study visit. Furthermore, the contact information will be useful for contacting volunteers who may not return for their scheduled visits, or those who may move away from their places of residence.

7.3 Study Visit B

The second study visit will provide an opportunity to review initial test results with the volunteer. Those who are found HIV negative who remain otherwise eligible have blood drawn as described in SOE#1 and are then scheduled to begin their twice-weekly small blood volume collections (SBVs) within the next seven days after Visit B. They are also scheduled for Visit C.

Individuals found HIV positive at Visit A will have a full Visit B blood draw. They will be scheduled for a test result session (see Section 7.6) later for result disclosure and counseling. If they are confirmed infected, they will be referred to care and treatment programs without further follow-up in the study. A small number of HIV positive volunteers will be retained in phase I to provide both masking to minimize risk of stigmatization and to provide positive controls to study

assays. The retained HIV positive participants will be selected at random as each group of ten seronegative volunteers are recruited and consented.

7.4 LBV and SBV Visits

Phase I of the study, the surveillance of HIV uninfected volunteers for early infection, is comprised of two types of visits: 1) clinic visits with large blood volume collections (LBV) and surveillance visits with small blood volume collections via microvette collection (SBV). To easily distinguish activities and locations they will be referred to as LBV and SBV even though counseling, questionnaires and other activities may occur at each.

The purpose of the SBV visits which occur twice weekly at locations as convenient as feasible to the volunteer, is to identify HIV infection prior to the advent of detectable levels of HIV antibody. The project plans to analyze these samples within 48 hours and deliver results to the study team to allow a volunteer to be referred to the clinic for entry into phase IB of the study and once HIV infection is confirmed to enter phase II of the study for acutely infected individuals. The currently recognized diagnostic criteria for HIV world-wide depend upon antibody responses.

The study will identify individuals in advance of detectable antibody. A positive test detecting HIV nucleic acid is also an acceptable standard for establishing a diagnosis and is commonly employed in the diagnosis of HIV infection in infants. In the study proposed here, individuals are contributing diagnostic samples very frequently and may have an initial positive HIV nucleic acid test which is not positive again for a few weeks (Fiebig 2005). This era of low level, intermittent viremia is referred to as a "stutter phase" and has been documented up to 21 days after an initial positive sample. Thereafter, the nucleic acid test signal is generally strongly positive. Therefore an initial positive sample from SBV is not diagnostic but prompts entry into an accelerated schedule (SOE #2) where samples will be collected that will verify the initial sample results and lead to definitive diagnosis.

At each LBV clinic visit after and including visit B (occurring at six-month intervals), blood is collected for HIV testing in an attempt to identify individuals using standard diagnostic methods to as soon after infection as possible.

7.5 Exit Visit

The exit visit is performed for cases that are not willing or able to continue in the study and do not withdraw their consent. For those who have recently (within the last six weeks) had an LBV, the "Exit Visit" column in SOE#1 is followed. If the LBV occurred within 2 weeks prior to the exit visit, no blood will be collected and no ACASI will be taken during the exit visit. For all other cases, the procedures for their upcoming LBV may be performed as their "exit visit". For those participants who withdraw their consent, no further research procedures may be conducted (see Section 9.10 for more information).

7.6 Test Result Sessions and HIV Test Counseling Visits

During phase I, HIV tests are done on all LBV visit samples (Visit A, B, D, F, H, J and Exit). For each of these, pre-test counseling is done at the time of the visit. Post test counseling for Visit A is conducted at Visit B. All other test result sessions for LBV are done in "test result sessions" following the LBV, conducted outside of the protocol schedule of events. These visits collect no study data and are not counted as visits (unless it coincides with an SBV) although they are documented in source documents. The site may give compensation up to half the amount of a regular visit.

HIV tests on the SBV samples are done twice weekly. HIV pre and post-test counseling visits take place every three months. More frequent counseling sessions would be time-consuming and would potentially dilute the counseling message.

Volunteers who are found HIV positive will be handled as described in Section 9.1 on HIV Test Results.

All counseling is done by research study staff with training in HIV test counseling, and routine supervision. The post-test counseling sessions will be conducted in a private setting, with great attention paid to the preservation of volunteer confidentiality. To receive their HIV test results, volunteers must identify themselves to the research staff as outlined in the site-specific addenda. Counseling guidelines for each country will be applied.

All HIV sero-positive volunteers will have in-depth counseling regarding the nature of HIV disease progression and the availability of interventions, support groups and follow-up counseling. Follow-up counseling and support will be available.

7.7 Transition to Phase II

All participants who have a positive test on the RNA assay (as described in Section 4.7) will be transitioned to the IB schedule (SOE #2) as soon as possible and ideally on the day of notification. They will receive LBV twice weekly for one month. At the end of this period, if they are determined to be HIV negative, they will return to the phase I schedule. If they are determined to be infected, they will be offered participation in the phase II schedule as well as the mucosal sample collection arm of phase II. Those phase II volunteers who agree to participate in the mucosal sample collection arm will complete the mucosal specimen collection consent and follow collections as indicated in SOE #3.

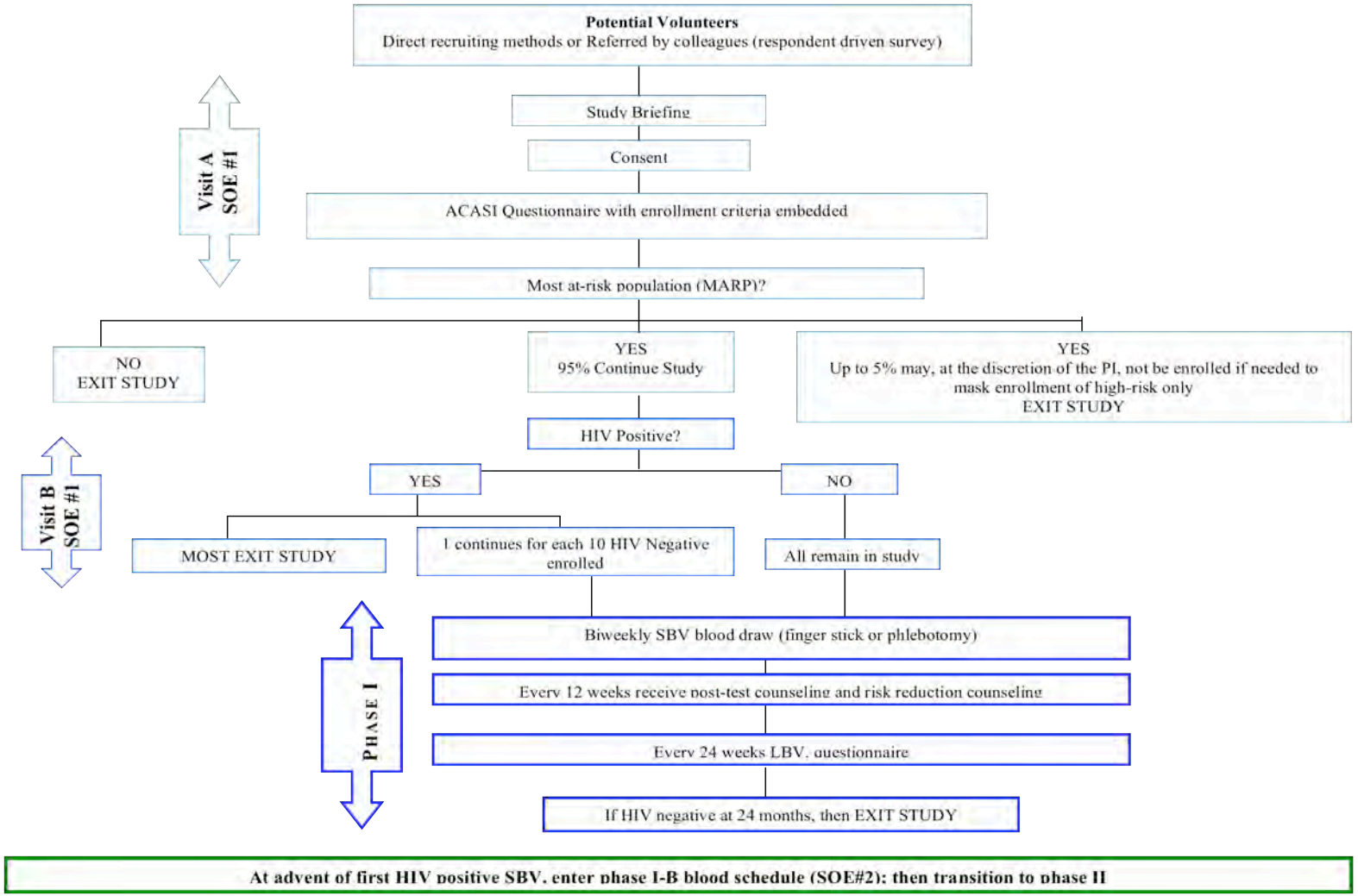
Those who agree to participate in phase II will stop all activities in phase I and enter the incident infection cohort phase II (SOE #3). Phase II participants may continue in phase I surveillance for masking purposes. Any HIV incident volunteer who refuses to enter phase II may continue follow-up in phase I of the study for the remainder of their visits. All HIV incident participants are referred to HIV care and treatment programs regardless of their decision to participate in phase I or phase II of the study.

7.8 Phase II Visits

Those volunteers who are determined to be infected (incident) in phase I are given post-test counseling and the opportunity to enroll in phase II. On the first phase II visit they receive a briefing about the procedures and sign the ICF for phase II. Thereafter, they follow the procedures as described on SOE #3.

Figure 7

RV 217 Flow Chart



7.9 Behavior Data Collection

A standardized questionnaire will be administered to each study volunteer using ACASI. If the volunteer is unwilling or unable to take the questionnaire using the ACASI system, a trained interviewer will administer the questionnaire and record the answers in the ACASI system.

Volunteers will have a choice of taking the ACASI interview in English or the local language. Volunteers needing assistance or having questions during the ACASI interview will be aided by a trained staff member. An SOP and interviewer instruction manual will be available on the site and used for reference and training. The volunteer's identifying information will be dissociated from the completed questionnaire and kept separately in the volunteer's file. The questionnaire will have only the volunteer's study number (but no personal identifiers) on each page.

The questionnaire will seek information on basic social demographic information, including occupation and income, knowledge about vaccines including HIV vaccines, willingness to participate in a trial of preventive HIV vaccine, as well as questions on general health, HIV/AIDS knowledge and behavioral risk factors for HIV. A modified questionnaire will be administered at the follow-up visits. The demographic, general health, HIV knowledge and behavioral risk factor questions have been adapted in large part from: the 2000-2001 Uganda Demographic and Health Survey instruments (UBOS 2001), the 2005 Uganda HIV/AIDS Sero-Behavioral Survey (UHSBS) instruments (MoH 2005) and the 1998 Demographic and Health Survey conducted by the Republic of Kenya National Council for Population and Development instruments (Mwidu 2004).

In addition to the ACASI questionnaire, two questionnaires will be administered to participants. One questionnaire will ask questions pertaining to vaginal and anal washing methods (Vaginal/Anal Practices (VAP) Questionnare--Appendix IX) and another will be ask questions pertaining to frequency and type of sexual acts (Receptive Risk Questionnaire--Appendix X).

7.10 Mucosal Specimen Collection

All acutely infected individuals, including those in the intermittent, low level viremic or "stutter phase" (SOE #2) of HIV infection, will be offered the opportunity to participate in the mucosal sample collection arm. Mucosal specimens will be collected as indicated on SOEs #1 and 3. Other mucosal collections, if appropriate, can be collected including breast milk, oral secretions, urine and semen.

For each site, at the time of each incident case that is identified, an HIV seronegative volunteer will be matched to the incident case for age and gender, and will be offered the opportunity to provide mucosal specimens. For this, they will sign a separate consent form and they will receive extra compensation, as the visit will take longer than regular visits. In addition, they will continue to be followed in their phase I schedule, including SBV. If any of these become infected, they will be replaced with another control.

In addition, female participants will be offered a Papanicolaou smear. Abnormal results will be referred for further evaluation and treatment.

8.0 SPECIMEN COLLECTION, LABELING, HANDLING, STORAGE AND SHIPPING

Trained clinic staff using sterile techniques will collect blood specimens. Up to 67 mls of total blood per visit will be collected by venipuncture for testing in vacutainer tubes as defined in the SOE. SBVs will be collected twice weekly for the first year of phase I and then, if the volunteer so chooses, monthly during the 2nd year of phase I.

8.1 Labeling

On blood collection, all blood tubes will be labeled on site using pre-printed barcode labels with only the study identification number of the volunteer, developed by the clinical data and specimen management system. The study identification number will be bar-coded onto tubes to facilitate tracking and processing of the specimen. On arrival at the site laboratory, all specimens will be logged on a reception database.

8.2 Handling

After collection, all specimens will be transported to the site laboratory according to SOPs. Samples will be packaged in isothermal containers for transportation.

For distal sites, blood samples will be transported in sealed boxes on the day of collection with appropriate packaging to control temperature within limits, per SOP. On arrival at the laboratory, all specimens will be logged into a sample reception database before distribution to the different assay benches for processing.

All blood processing will be undertaken using Good Laboratory Practices (GLP), with protective material including lab coats and gloves. Processing will be conducted using sterile techniques and under laminar flow hoods. All specimens will be labeled with a unique barcode, sample type, date and study visit number and will be indexed and cross-referenced in a specimen-tracking database. The processing lab will be blinded to volunteer identifiers except for the study number to ensure confidentiality.

8.3 Storage

EDTA plasma will be separated from the cells by centrifugation of the lavender top tubes after they have been used for the hematology, CD4 counts and HIV screening. The plasma and packed cells will be aliquoted and stored at –70°C. Serum will be separated from coagulated blood in the red top tubes and aliquoted as per visit requirements. One fraction will be kept at 4°C for HIV, syphilis and HSV, and serum chemistry tests while the remainder will be stored at –70°C. PBMC will be isolated from the acid citrate dextrose (ACD) yellow top tubes and cryopreserved in aliquots of 2 to 10 million cells per vial and stored in the vapor phase of a liquid nitrogen freezer. ACD plasma will be separated, aliquoted, and stored at –70°C. All

specimens will be labeled with bar code of: study numbers, sample type, date and study visit number and will be indexed and cross-referenced in a specimen-tracking database.

Each study volunteer will be asked to separately, and voluntarily consent to their blood samples to be stored for other research studies that may be done after this study is completed. As stated above, the sample will be labeled with bar code of the volunteer study number that can be linked to their study information. In case the volunteer is unwilling to have their blood samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples for which consent has been obtained and for which additional material is available after study specified testing is complete will be stored for future testing at the site. However, WRAIR IRB and local IRB approval will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB.

8.4 Shipping

All samples for HLA typing and host genetic characterization, viral sequencing, viral isolation, neutralization, and Fiebig staging assays will be shipped to and stored at the MHRP laboratories in Rockville, Maryland, United States of America (USA) by dry shipper or on dry ice accompanied with all the required export and import documentation. Alternatively, if technology for the above assays becomes available in one of the MHRP field laboratories, testing may take place in the local laboratories. It is the intent of the study team to ship only samples as needed to provide uniform scientific data across samples derived from all sites. Some assays will be deployed to the sites for analysis and capacity development of these and other assays will be critical for program sustainability.

All other investigations such as CD4/CD8 count and diagnostic testing will be conducted at the local laboratory.

8.5 Laboratory Testing

Specimens collected for this study will be used for diagnostic testing, exploratory laboratory evaluations corresponding to the study secondary objectives and research assay validation. See the SOE for a breakdown of specific testing to be conducted.

9.0 MANAGEMENT OF SUBJECTS

9.1 HIV Test Results

Prior to the blood draw, pre-test counseling will be provided on an individual basis to each volunteer, as described in section 7.6. Test results will be obtained and recorded and will only be identified by the study number. Written results, only identified by the study number, will be

issued to research study staff. The data linking study number to personal identifiers will be stored on a password-protected database accessible only to the study data management officer.

At the time of specimen collection, the volunteers will be informed of the expected dates for the post-test counseling sessions for giving the HIV results and other clinical results. The goal will always be to provide volunteers with diagnostic data as quickly as feasible. To receive their HIV test results, volunteers must identify themselves to the research staff as outlined in the site-specific addenda. The research study staff will use the volunteer study card or fingerprint to retrieve the results and provide post-test counseling.

All volunteers who test HIV reactive on nucleic acid testing (Aptima) for the first time will enter phase IB (SOE 2) and undergo repeat testing twice weekly for 4 weeks to verify the diagnosis. At the end of phase IB the acutely infected participant will be asked to transition to phase II.

Individuals who test positive for HIV will be counseled about their HIV status and the available options for care before being referred for further medical evaluation. If female and pregnant, volunteers will be referred for services to prevent mother-to-child transmission of HIV. Referral to other health facilities in nearby areas that offer similar services, away from their local community will also be made on request, in order to retain confidentiality and avoid stigma.

Informal support group sessions and/or focus groups may be utilized by the study participants to further discuss their concerns and provide a support network for those testing positive for HIV.

9.2 Management of HIV infection

All HIV infected persons identified through the study in Africa will be referred to PEPFAR funded clinics, which are co-located with, or in close proximity of, our research activities. In Thailand, HIV positive participants will be referred for care at the Thai National HIV care and treatment program. More information is provided in the site-specific addenda.

9.3 Positive malaria smear

All smear positive febrile or symptomatic individuals with a history consistent with malaria will be treated with anti-malarial therapy, using appropriate first-line anti-malarials in accordance with the host country's Ministry of Health guidelines; and referred to the health center personnel for follow-up. The clinical study staff will be notified of all volunteers with positive malaria smears to insure that treatment was provided. Malaria smears will be conducted in Africa only.

9.4 Management of STIs

All individuals will be tested for syphilis and if infected, the volunteer will be treated according to the national standards of care in the host country either by the study site or referred for local care in settings where available. All individuals with clinical STIs will be treated according to the national standards of care of their host country (see site-specific protocol for details).

Volunteers with chronic and/or severe illness will be referred to the local hospital for further evaluation, treatment and care.

Volunteers testing positive for any STIs, including HIV will be advised and encouraged (through health education) to take their sexual partners to get tested. Such sexual partners of study volunteer who are not participants in this study will be assisted with appropriate referral.

9.5 Other/Opportunistic Infections

Volunteers who are diagnosed with other chronic infections will be referred to a PEPFAR-funded care and treatment facility or to an appropriate medical facility for care.

9.6 Management of Other Medical Conditions

Volunteers will consult study staff for treatment and/or referral to the appropriate health facility during the study period.

9.7 Unanticipated Events and Social Harms Reporting

Unanticipated events and social harms may occur during the course of the study. When such events are related to study participation, the host country and WRAIR IRB will be informed. The study staff, informed of these events, will inform the PI or his/her designee. The PI or designee will then prepare a narrative summary of the event and report to the IRBs.

All unanticipated problems related to the study and involving risk to subjects or others and all subject deaths should be promptly reported by phone (+1 301-319-9940) or by facsimile (+1 301-319-9961) to the WRAIR IRB. A complete written report should follow the initial notification. In addition to the methods above, the complete report will be sent to the Director, Division of Human Subjects Protection (DHSP), Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910-7500.

9.8 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) is seldom employed for monitoring of an observational study. This is because there is no need for an unblinded group to compare safety, toxicity and efficacy data between two or more study arms receiving experimental interventions or controls. It is important to recognize that the IRBs would serve as the logical review point for safety concerns arising in the conduct of RV 217 as there is no blinded data and this group is empowered to review this subject area on behalf of the participants and as an oversight element for the investigative team.

9.9 Follow-Up

Participants enrolled in Phase I will be followed for a period of approximately 24 months after screening. HIV incident volunteers identified at any follow-up visit will be invited to participate in phase II to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of at least 50 months beyond the time that they enter phase II. All individuals recruited, whether HIV positive, negative, or a new HIV diagnosis, will be seen and undergo the same study procedures at each visit.

9.10 Termination Of Study Participation

Volunteers are free to withdraw from the study at any point without prejudice. A volunteer's study participation may be terminated for various reasons, to include:

1. Sponsor terminates the study for unforeseen reason. All participants will be informed of this possibility in the consent process.

2. Lost to follow-up (LFU)/non-compliance to visit schedule: every three months, a volunteer's participation is evaluated. If more than two clinic visits have been missed, and greater than 75% of the SBVs have been missed the participant will be considered LFU and no further tracking will be done. Volunteers with a single missed clinic visit and/or more than 50% missed SBV will be counseled and evaluated for consideration of continued participation. The PI may exercise some discretion in assessing compliance as the intensity of surveillance is not consistent with some activities of daily life to include vacations, family and personal medical leaves, etc.

3. Refused further participation:

Refusal of all study activities: volunteer decides not to continue

Partial refusal of study activities, e.g. refuses SBV but willing to continue LBV.

The "lost to follow-up" and "refused further participation" group may be re-contacted to the extent the volunteer permits and may resume follow-up should obstacles or other issues resolve. Participants who refuse SBV will be terminated for non-compliance and will be replaced as noted above.

4. Withdrawal of Consent: volunteer actively withdraws consent, either in writing, in person, or by phone. When a volunteer indicates to the investigator that they are withdrawing consent, the communication should be recorded in the volunteer source documents (documented per SOP). It will be understood that no further interaction by the study team will occur except to provide the volunteer with any IRB-directed information related to participant safety. The "withdrawal of consent" (WDC) group will not resume follow-up. The volunteer's samples and data accrued during participation prior to "withdrawal of consent" will be retained and used only for the analyses approved under the protocol. No samples will be retained for "future use" and no additional data or samples will be collected.

10.0 COMPENSATION OF VOLUNTEERS

In accordance with common practice of the local sites, there may be compensation for lost time, travel expenses and inconvenience, for each scheduled visit, including the mucosal collection visits. The amount of compensation will vary according to visit to account for the differential lost time and inconvenience of longer visits (LBV and initial visit A and B) compared to shorter visits (SBV). For sessions to collect test results, volunteers will be compensated with half the amount compensated for a clinic visit. For those who have moved further away from the site, there may be indirect costs over and above the compensation (e.g. transportation, lodging if necessary) to accommodate study follow-up and retention.

A volunteer is compensated for the time and inconvenience associated with the type of visit he or she is completing. If, by coincidence, the volunteer completes study activities for more than one type of visit on the same day, the volunteer will be compensated for each type of visit completed.

Any applicable guidelines by IRBs/ERCs (ethical review committees) for compensation will be sought and followed and described in the site-specific addenda.

11.0 LANGUAGE

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood. Accordingly, the consent and all other written materials will be translated into the site-specific local language in addition to English.

12.0 DATA MANAGEMENT AND ANALYSIS

12.1 Hardware and Software

Data will be entered on secure computers with access limited by individual usernames and passwords. Data will be stored using a system that is limited by a firewall with access available only to authorized users physically logging on from authorized internet protocol (IP) addresses. Data analysis will be performed using SAS® software (version 8.0 or higher).

The Data Coordinating and Analysis Center (DCAC) will manage data into a validated password-protected data management system. For this study, CRFs will be maintained and data entry will be conducted at the clinical sites unless technological or other issues preclude this option. If data entry by any site(s) is not feasible, then CRFs (either in hard copy or electronic images) will be sent to DCAC for entry, until such time as those requirements are met.

Study subjects will complete a risk behavior questionnaire on a microcomputer using ACASI software. The questionnaire will be offered in English and in local languages as approved by local IRBs. Each question and response option is visible on the screen as well as read aloud using the audio portion of the software. Subjects use headphones to listen to the questions and response options. Responses are obtained using touch screens. Subjects may decline to use the computer-administered questionnaire. If this happens, a trained interviewer will read the questions and response options to the subject and record the answers in the ACASI software.

ACASI is being offered in this study for several reasons: to reduce site staff and data entry time and potential errors in recording subject responses, and to provide a consistent administration of the questionnaire. For example, skip patterns can be programmed into the software, and individual interviewer biases can be eliminated by using a recorded voice. In addition, there is evidence in the literature that subjects being interviewed about high-risk behavior are more likely to give truthful answers to a computer interview than to a human interviewer. Subjects may feel that the administration of the questionnaire using headphones and computer is more private than a traditional interviewer-based approach. Subjects may also gain experience using computers that gives them a more positive attitude towards study participation.

12.2 Data Entry

Study site staff or data entry personnel at DCAC will perform double data entry of case report forms (CRFs) into a relational database. DCAC will not receive any fingerprints or personal identifiers such as name, national identification number (if applicable), or house number. Identifying information will be kept locally at each site in a secure database. The local database will include a master file containing personal identifiers such as name, national identification number (if applicable), and house number. This file with identifiers will only be accessible by the data management supervisor (DMS) and data management officers (DMO) or designee through password access. During all data entries and subsequent data and laboratory analysis, only the study number will be used as the identifier. All volunteer folders will be stored in locked filing cabinets and/or rooms accessed only by DMS, DMO or designee.

It is the policy of DCAC and each site data management team to limit availability of personal identifying information to only those individuals with a requirement for the information. All research data and samples are only managed using the coded study number when used for research activities including data or samples analysis. Personal identifying information and links to the study number are limited to those clinical staff who work directly with the participants and must have this information to fulfill their role in the conduct of the study.

12.3 Data Entry of Other Laboratory Assay Results Not Conducted On Site

The results of other specialized laboratory assays not immediately available will be maintained by the individual investigators and merged, as needed, with the analysis file.

12.4 Data Storage and Monitoring

All source documents such as contact information forms and laboratory record sheets will be maintained at each participating site. CRFs will also be maintained at each study site. If CRFs must be transmitted to DCAC for data entry, they will be sent either via hard copy or electronic image on a regular basis. Paper CRFs will be stored for 5 years post study completion (database lock). Data will be stored on dedicated servers (real-time) and tapes/CD-RWs (weekly). Tapes/CD-RW backups will be stored in a secure fireproof cabinet at the on-site storage, and on a duplicate tape/CD-RW set at an off site location (weekly).

All aspects of the study will be carefully monitored by the sponsor or authorized representatives of the sponsor, with respect to current Good Clinical Practice (GCP) and SOPs for compliance with applicable government regulations. These individuals will have access, both during the trial and after trial completion, to review and audit all records necessary to ensure integrity of the data, and will periodically review progress of the study with the principal investigator.

Case report form (CRF) data obtained in the conduct of this study will be housed in a secure database maintained by DCAC. These data will be entered in a secure database, using a double-data entry process, with standardized quality assurance review procedures.

DCAC serves as the central data management facility for MHRP research protocols. Data managed by DCAC are entered into and maintained in a password-protected database. Data are accessible only to authorized users, including appropriate site study staff and those DCAC and Information Technology staff authorized to work on the protocol. The database is located at the Rockville campus of the MHRP and is protected by a firewall and a wide range of other security measures.

This data do not contain participant names or Social Security Numbers (or any equivalent national identifiers) but is referenced only by the study number.

Questionnaire data and some other data such as laboratory research results will be reported to DCAC electronically, identified only by the study number.

12.5 Data Analysis

Data analysis will be performed using SAS® version 8.0 or higher. DCAC in Rockville, MD, USA will support the analyses.

All datasets (without the personal identifiers) are stored individually and merged as needed for specific analyses. These datasets will only be made available to personnel performing analyses.

Analysis files are created on a periodic basis and made available to the PI. Data may be made available as a listing, external file, or through a query program.

13.0 ETHICAL CONSIDERATIONS

13.1 Risks

The proposed study is unique in several respects. It is the only study with a concerted effort to acquire most HIV incident infections in Fiebig stages I/II. Volunteers will need to briefly encounter the research team on a very frequent basis. This raises the specter of stigmatization and interference with work. Aside from monetary compensation, the rewards for this investment by the volunteer are primarily altruistic as they contribute to our understanding of HIV disease and transmission pathogenesis.

Frequent surveillance visits for uninfected participants and the intense, clinic based visits for those who become infected raise a potential risk to stigmatize the participant. To mitigate this risk, we will follow both HIV infected and uninfected participants so the volunteers and their peers will not be able to determine or assess HIV status based upon the schedule of activities an individual or group undertake. To minimize risk in the work place, visits may be conducted using mobile platforms, vehicles or rented establishments, convenient to the participants at their homes and/or work place and all surveillance visits in these locations will be extremely brief to minimize disruption of the volunteer's normal schedule. In some sites, the research clinic is sufficiently convenient and non-stigmatizing an environment to permit all study related visits. Details are provided in the site-specific addendum. Prior to initiating the proposed studies, we will conduct focus group discussions with potential participants, employers, and key opinion leaders (inclusive of CABs) to a) inform them of the studies goals and value to optimization of vaccines and therapies for HIV and b) identify means of minimizing volunteer harms.

Stigma is the greatest risk for participants but also, simple disruption of work and other activities of daily living must be considered. As noted above, every effort will be made to make the surveillance encounters both brief and convenient to minimize this concern without engendering a further risk of stigmatization.

Although the forgoing represent the greatest potential harms, the intense phlebotomy schedule among the AHI participants is also a potential hazard. Blood volumes will be reduced by 50% if a participant's hemoglobin (Hgb) at entry into the AHI phase is under 10.5 and will be suspended if the Hgb is under 9.5. Anemic volunteers will be offered iron supplementation.

Thus far, no safety issues have arisen from the blood volumes collected during the conduct of ECHO. Hemoglobin is monitored throughout the period of blood collection and if anemia develops or is worsened, we will adjust phlebotomy accordingly. In view of the safety experience to date and the great scientific value of samples collected early during acute viremia, the total volume for the 8 week period during phase IB has been increased modestly from 448 mL to 472 mL.

Site study physicians will also assess overall health prior to phlebotomy and may reduce blood volumes as clinical circumstances require. All women who become pregnant during participation will be carefully evaluated by the PI to determine if they may continue participation with SBV

and LBV per protocol. They will have a reduction of LBV to only samples needed for HIV status determination and SBV may be deleted if Hgb is less than 10.5.

Many consider the advent of an HIV diagnosis a social harm in the context of reactive depression, potential family disruption and work place stigmatization. The field teams employ trained counselors who have experience with post-test counseling of newly infected individuals through other cohort development studies conducted in each of these countries. Although harms may nevertheless occur, every effort will be made to minimize these. A benefit to volunteers who are diagnosed with HIV through their participation in this study will be ready access to a care and treatment program, funded by the United States Government (USG) through PEPFAR in Africa and the Thai government in Thailand, and empowerment through knowledge of their diagnosis to avail themselves of prevention interventions to reduce risk to sex partners and, if they become pregnant, maternal-child prevention measures. It must be recognized that the era of early HIV infection is one that poses great risk for HIV transmission.

13.2 Post-exposure prophylaxis (PEP), Pre-exposure prophylaxis (PrEP) and early initiation of HAART

The current US recommendation for individuals seeking care within 72 hours of a sexual or needle sharing exposure with a known HIV positive person is to provide HAART for 28 days. (Smith 2005). WHO PEP recommendations do not include chronically exposed individuals (Joint WHO/ILO PEP Guidelines). The high-risk participants in this study may not know the HIV status of their partners, endure multiple high-risk exposures with partners of unknown status and would not be appropriately placed on HAART for the purpose of post-exposure prophylaxis in ordinary circumstances according to either the US or WHO guidelines. Should a volunteer present with a known exposure to an HIV positive person which meets the standard for intervention as defined in these guidelines, PEP will be discussed (as appropriate) with the volunteer and sought via PEPFAR or other resources.

More applicable is the use of PrEP in these high-risk groups. Currently, PrEP is the subject of several clinical trials. Should these interventions prove safe and effective they will be promptly incorporated in the proposed studies clinical management of these volunteers. The study would require re-design and re-consent in this circumstance as one may expect fewer observed infections. It is recognized that highly effective PrEP may well reduce transmission rates in high-risk populations to such an extent that the proposed study is no longer feasible.

Current US guidelines for treatment of HIV infected persons do not recommend routine use of HAART in acutely infected individuals but rather suggest that it be considered optional based upon expert opinion and enrollment in a clinical trial should be considered (Panel on Antiretroviral Guidelines for Adults and Adolescents). The WHO recommendations on HIV treatment have no comment upon this subject (WHO 2006). Nevertheless, many US and European physicians offer HAART in the setting of acute infection. As summarized recently (Hirsch 2008), the clinical data to support this approach is inconclusive, but there is a theoretical basis for this intervention. This is based upon evidence of substantial injury to the immune system occurring within days to weeks of initial infection and the possibility of very early use of HAART could durably influence the long-term course of disease. Thus far, most studies with

HAART intervention in acute infection are acquiring participants after the peak of viremia and may therefore have little opportunity to modify the critical early events of acute infection. In addition, many studies are uncontrolled. In general, those studies, which have evaluated either a short course of HAART or repeated treatment interruptions following initial HAART among acutely infected individuals, have not found sustained benefit. (Hecht 2006; Streek 2006; Hoen 2005) Care providers in the developed world who offer HAART in the acute infection setting typically commit patients to sustained therapy.

In the developing world, the current standard of care initiates therapy based upon symptoms and CD4+ T cell count criteria. The limited availability of second line agents to cope with resistance and the costs associated with life long therapy are considerations which thus far have compelled the national bodies responsible for treatment guideline policy to adopt a more conservative approach. Diagnosis of acute infection in the developing world is a challenge due to the limited availability of nucleic acid testing. Consequently, there is virtually no experience with HAART administered during acute infection. Currently, other studies conducted in acutely infected participants in Africa and China are not routinely providing access to HAART (Streek 2006, Myron Cohen, personal communication).

Ongoing research in this arena may provide compelling data to alter the treatment practice at the sites of the proposed study. The RV 217 team would welcome such an advance and facilitate provision the new paradigm to our participant population as quickly as possible and accordingly alter the study design and goals. Although some study objectives might become infeasible, those most novel questions focusing upon early peak viremia and/or eclipse phase provirus and outcome would be preserved. At this point, the feasibility of capturing individuals in the earliest stages of acute infection is not known. Should RV 217 prove that an appreciable number of volunteers in Fiebig stages 1-3 can be acquired, the opportunity to evaluate either drug or immune based interventions in these populations would be pursued.

The current observational study design is ethically defensible only in the circumstance that the available clinical data fail to provide a convincing basis for adopting early highly active antiretroviral treatment in the setting of acute HIV infection. Similarly, pre-exposure prophylaxis (PrEP), which is under active clinical investigation, may impact on the ethical considerations of conducting RV 217 as currently designed as the population enrolled in RV 217 would be candidates for PrEP. In the settings contemplated for RV 217, a paradigm shift to initiating either PrEP or early HAART in acute infection may be difficult to implement. Nevertheless, it is anticipated that research activities would be suspended to permit integration of these interventions into the study design with a likely alteration in objectives.

The well being of participants is the foremost priority of the RV 217 investigators. There is no extant clinical evidence to inform the routine use of HAART in acutely infected individuals, nor to use PEP or PrEP, in MARPs who will participate in RV 217. Ongoing dialogue with community advisory boards (CABs), national regulatory authorities, ethical review boards and key opinion leaders will guide the study team.

13.3 Benefits

Although study volunteers may benefit from clinical testing and physical examination, management of STIs and malaria, health education, HIV counseling and reproductive health counseling, they may receive no direct benefit from participation.

13.4 Community Advisory Board

CABs at each site will serve as a link between the research team and the community, and therefore be able to provide a feedback to the research team on issues about the study that might be of concern to the volunteers or the community.

13.5 Informed Consent

Informed consent will be obtained from each volunteer before enrollment in the study. The Consent Form will be used as the informed consent document for adult volunteers. Consent forms will be available in both English and site-specific local languages, and can be found as attachments to the site-specific protocols. Volunteers may take as much time as needed to decide if he/she wants to participate. A copy of the protocol, proposed informed consent form, other written participant information, and any proposed advertising material will be submitted to the appropriate ethical and scientific review committees in each country where enrollment will occur. In addition, the protocol will undergo review and approval by the country-specific IRBs and the WRAIR IRB as part of the U.S. Medical Research and Materiel Command.

The investigator must submit and, where necessary, obtain approval from the country-specific IRBs and the WRAIR IRB for all subsequent protocol amendments and changes to the informed consent document.

Volunteers may withdraw from the study at any time point. In most cases, volunteers simply disappear (loss to follow-up) or express a desire to discontinue participation. Rarely, a volunteer will not only stop participation but also explicitly withdraw consent. We intend to work cooperatively with volunteers and support their participation constructively with a view to their well-being and respectful of their autonomy. We do not expect withdrawal of consent will occur or will occur only rarely. We use the term "withdrawal of consent" to indicate a declaration by the volunteer that no further interaction with the study team is permitted. Only data and samples already obtained will be analyzed according to protocol but no additional data or samples will be collected. The study team will engage in no further communication with the volunteer except as directed by an IRB on behalf of participant safety. The study team will not utilize samples or data from this volunteer for any future use and will discard residual samples when the study is completed. When a volunteer indicates to the investigator that they are withdrawing consent, the investigator or staff member will insure that the communication should be recorded in the volunteer source documents.

13.6 Volunteer Confidentiality

Volunteers will be assigned a study number that will be used as a personal identifier for volunteer identification. For further identification each volunteer will be issued a Study-ID-card that contains either a photo, or a number that links the study ID card to his or her photo ID card. No card will be prepared if the site adopts the finger print identification system, and no name will appear on it. Further details of volunteer identification can be found in the site-specific addenda.
A database of the volunteer name and study number will be only accessible to the study coordinator and the local PI. The data management personnel will prepare barcode labels that will be fixed on data record/collection forms with only the contact information form bearing identifying information for use by the field staff in data and blood collection. Contact information may be collected from the volunteer at either study visit A or B. Other than the contact information form, the rest of the data record/collection forms will not bear personal identifiers but only the study number. HIV testing will be performed on samples that are identified only by study number. Every effort will be made to maintain confidentiality of records within the limits of the law. All data and medical information obtained about volunteers as individuals will be considered privileged and held in confidence. Research and clinical information relating to volunteers will be shared with other investigators and the scientific community through presentation or publication; however, volunteers will NOT be identified by name or other personal identifying information. Further, all study personnel will undergo training on various aspects of the study, including ethics in human research studies, and the need and importance of protecting the confidentiality of the participants.

Representatives of the MHRP, DoD, USAMRMC, local IRBs/and or Ethical Committees (ECs), NIAID and the Office for Human Research Protections (OHRP) are eligible to review records from this study as part of their responsibility to protect human subjects in research.

13.7 Participation of Children

Healthy children may be involved in research when the study is determined to be “no greater than minimal risk” (45 CFR 46, subpart D, 401-409). No site plans to enroll minors.

13.8 Management of Vulnerable Volunteers

In the event that the status of an enrolled volunteer changes during the course of their enrollment in the study and that the volunteer’s ability to exercise free choice could be limited in some way, the volunteer is recognized as a vulnerable participant. A vulnerable volunteer is any individual whose willingness to volunteer in a clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation; or of a retaliatory response from senior members of a hierarchy in case of refusal to participate.

The volunteer that is likely to be vulnerable to coercion or undue influence might include individuals such as minors, pregnant women, prisoners, soldiers, the physically handicapped, or mentally incompetent persons. Other vulnerable volunteers could include persons in an

emergency situation like refugees, persons living on streets, and very sick persons who are incapable of giving consent or providing continuing consent.

If a change in status of a volunteer already enrolled in the study should occur, it is the responsibility of the investigators to assure that appropriate safeguards are in place to protect the rights, safety and welfare of all study subjects. The principal investigator shall notify all IRBs and/or ECs associated with this study in the continuing review report (CRR). The IRB/EC must decide what types of special protections are required and provide direction to the investigator. Participation of prisoners is not planned and any volunteer will be suspended from study visits while incarcerated. The IRB will be notified of the period of incarceration.

Volunteers who have returned to the clinic after a period of incarceration will be counseled again about the potential social risks of being identified as a participant in the study. A note to the effect that the counseling was done will be written in the progress notes in the volunteer's binder. Any volunteer who is incarcerated for more than 6 months will be re-consented to include taking and passing the TOU.

14.0 POLICY REGARDING RESEARCH-RELATED INJURIES

If a participant is hurt or gets sick because of this research study, emergency medical care will be provided at a hospital or clinic free of charge. The US Federal Government will not provide long-term care (over 6 months) for any injuries resulting from study participation. The participant will be given information on where to get further treatment if needed for non-emergency care, and will receive short-term care for study-related injuries. The participant will be responsible for any future medical treatmemts, and will not receive an compensation for illness or injury. If the participant has questions about this medical care, they may talk to the principal investigator. If the participant pays out-of-pocket for medical care elsewhere for injuries caused by this research study, they should contact the principal investigator. If the issue cannot be resolved, the principal investigator will contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

15.0 PROTOCOL DEVIATION REPORTING

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

The timeline for reporting protocol deviations to the Division of Human Subjects Protection/ WRAIR IRB/DAIDS Medical Officer is determined by the categorization of the deviation: (1) emergent/significant or (2) non-emergent/minor. Unanticipated problems should be reported in the appropriate timeframe according to the seriousness of the event as a significant deviation or a minor deviation.

Emergent/significant deviations are departures from protocol that have a significant impact on the welfare or safety of a volunteer or on the integrity of the study data and will be reported within 10 working days to the DHSP/WRAIR IRB to the following address

wrairdhsp@amedd.army.mil. Example: providing the wrong lab result to a volunteer. Such deviation reports may be initiated without prior IRB/ ethical review committee (ERC) approval, only in cases where the change (s) is /are necessary to eliminate an immediate apparent hazard.

Non-emergent/minor deviations are routine departures that typically involve a volunteer's failure to comply with the protocol. Examples: missing scheduled visits; failing to complete required questionnaire. Minor deviations will be reported to the sponsor and the Division of Human Subjects Protection/IRB in a summary report with the annual continuing review report.

A cumulative deviation report will be submitted to the DHSP/WRAIR IRB with each protocol continuing review report or with the closeout report, whichever comes first.

16.0 PROTOCOL MODIFICATIONS

Any amendments to the protocol, consent form and/or questionnaires, including a change to the principal investigator PI, must be submitted to the WRAIR IRB and local IRB for review and approval. Any change or amendment to the protocol affecting study volunteers, study objectives, study design, study procedures, or significant administrative aspects will require a formal amendment to the protocol. The protocol must be revised to concur with the amendment. Such amendment will be submitted to the WRAIR IRB and local IRBs for review and approval.

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

Administrative changes to the protocol are corrections and/or clarifications that have no effect on the way the study is to be conducted. Such administrative changes will be submitted to both the WRAIR IRB and local IRB/ERCs for review and approval prior to implementation.

17.0 CONTINUING REVIEWS /CLOSEOUT REPORT

A CRR will be submitted to all ERCs/IRBs prior to the anniversary date determined at initial IRB review. If the continuing review is not approved by the local ERC/IRB and WRAIR IRB by the anniversary date, all protocol activities must stop at that site until such time as the approval is obtained. A copy of the approved CRR and local IRB approval notifications will be submitted to the WRAIR DHSP as soon as these documents become available. A copy of the approved closeout report and local IRB approval notifications will be submitted to the WRAIR DHSP as soon as these documents become available.

18.0 STRATEGIES FOR IMPROVING ENROLLMENT AND COHORT RETENTION

A number of strategies are planned in order to enhance recruitment into the cohort and retention of volunteers over time. Recruitment methods and activities are directed at each group separately to minimize stigma risks and for operational efficiency. These are outlined in the site-specific addenda.

In accordance with the protocol objective of defining retention, and to look at issues regarding participation and retention, data will be collected on the outcome of tracking activities. This data will include type of discontinuation (withdrawal or lost to follow-up), and reasons for discontinuation (such as family, job, prison, relocation, invalid contact information, etc).

19.0 RESOURCES AND COORDINATION

This study is funded and sponsored by MHRP and DAIDS and coordinated locally by MUWRP, USAMRU-K, MMRP and AFRIMS.

Laboratory: Each study site has an established laboratory that is capable of handling most of the tests required. Quality assurance programs will be conducted consistent with the College of American Pathology Standards prior to study initiation. Every site has established a quality assurance (QA) program, which will allow its laboratory to comply with GLP standards of the U.S. FDA.

Staff: The research team to implement this study at each of the participating countries will include the country-specific Principal Investigator who will be responsible for implementation of this study protocol in their country and timely fulfillment of all study activities. The site-specific PI will work together with the co-investigators listed in this protocol to oversee the successful implementation of this study in his/her country.

20.0 USE OF INFORMATION AND PUBLICATION

It is expected that data from this study will be reported in both scientific journals and international scientific meetings. Confidentiality of subjects will be maintained by the fact that no individual results will be reported or published, only group/aggregate results. All research data will be identified by the study number. The linkage between personal identifiers and study number will only be available in a confidential database at the respective sites. The local health authorities will be informed of all scientific outcomes of the study and general prevalence and incidence data however, confidentiality will be maintained, and participant identities will not be released. Only aggregate information will be released. All publications resulting from this study will be cleared through the collaborating partners to this study.

WRAIR recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. Any results of medical investigations and or publication/lecture/manuscripts based thereon, shall be exchanged and discussed by the investigator, the sponsor representative(s) and the U.S. Army Medical Research and Materiel Command 60 days prior to submission for publication or presentation.

Results from investigations shall not be made available to any third party by the investigating team outside the publication procedure as outlined previously. WRAIR will not quote from publications by investigators in its scientific information and/or promotional material without full acknowledgment of the source (i.e., author and reference). All publications written by WRAIR investigators must be reviewed and approved by WRAIR Office of Research Technology and Applications (ORTA).

21.0 CONDUCT OF THE RESEARCH STUDY

This research study will be conducted in accordance with GCP and the revised CFR. Additionally, it will follow the local site guidelines for the conduct of health research involving human subjects. Copies of all the above documents and any other information and/or guidelines that are applicable for the safe and legal conduct of the study will be available at each clinical site.

21.1 STATEMENT REGARDING POTENTIAL CONFLICT OF FINANCIAL INTEREST

The Principal Investigators and the Co-Investigators have no financial interest in any component of this study.

22.0 SIGNATURE OF PRINCIPAL INVESTIGATORS

WRAIR Institutional Review Board (IRB)
Signature Page for Studies Not Exempt Under 32 CFR 219

Principal Investigator Agreement:

1. I agree to follow this protocol version as approved by the IRBs/ERCs.
2. I will conduct the study in accordance with applicable IRB/ERC requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I will not modify the protocol without first obtaining an IRB/ERC approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants.
5. (For Greater than Minimal Risk studies or studies of public interest) In accordance with Command Policy 2008-35, I will ensure that the Commanding General receives a pre-brief (or Executive Summary) and approves the study prior to execution.
6. I will ensure that the data (and/or specimens) are maintained in accordance with the data (and/or specimen) disposition outlined in the protocol. Any modifications to this plan should first be reviewed and approved by the applicable IRBs/ERCs.
7. I will promptly report changes to the research or unanticipated problems to the WRAIR IRB immediately via the WRAIR Division of Human Subjects Protection at (301) 319-9940 (during duty hours) or to the WRAIRDHSP@amedd.army.mil and submit a written report within 10 working days of knowledge of the event.
8. I will prepare continuing review reports at an interval established by the IRB/ERC, and a study closure report when all research activities are completed.
9. I will immediately report to the WRAIR Division of Human Subjects Protection knowledge of any pending compliance inspection by any outside governmental agency.

I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

Overall Study Director, RV 217	Date (dd/mm/yyyy)
Site Principal Investigator, Kampala, RV 217a	Date (dd/mm/yyyy)
Site Principal Investigator, Kericho, RV 217b	Date (dd/mm/yyyy)
Site Principal Investigator, Mbeya, RV 217c	Date (dd/mm/yyyy)
Site Principal Investigator, Thailand, RV 217d	Date (dd/mm/yyyy)

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APPENDIX I: SITE SPECIFIC ADDENDUM

<<Enter Site Here>>
In Collaboration with the
US Military HIV Research Program
And the
U.S. National Institutes of Health (NIH)

CONSENT TO BE A RESEARCH VOLUNTEER
MAIN CONSENT FORM

1. TITLE

RV 217: "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand"

Short Title: Early Capture HIV Cohort (ECHO)

2. ENTER SITE INVESTIGATOR CONTACT INFO

3. INFORMED CONSENT

Thank you for your interest in this study. <<Enter site info here>> and the U.S. Miltary HIV Research Program (MHRP) are conducting a research study to find out how many people become infected with HIV in the study at this site, how they might have become infected, and to determine retention rates and willingness to participate in future HIV vaccine studies in this area. We are also conducting this research to learn how the body responds to HIV very early in infection and how the HIV virus changes over time. This study will take place in Kampala, Uganda; Kericho, Kenya; Mbeya, Tanzania; and Pattaya, Thailand. You are being asked to take part in this research study because you are between the ages of 18 and 50, a resident <<enter site info here>> and may be at risk for HIV infection. Before you decide whether or not to take part in this study, we would like to explain the purpose of the research study, how it may help you or others, any risk to you, and what is expected of you. This process is called informed consent.

It is important that you know the following:

a) Taking part is of your own free will (entirely voluntary).

b) You may choose not to participate or to withdraw from the study at any time without being treated unfairly or discriminated.

YOUR PARTICIPATION IS VOLUNTARY.

Please ask questions about anything you do not understand. The clinic staff will talk with you about the information in this form. The study investigators encourage you to ask questions about this study at any time. You can take as much time as you need to review this form and discuss your study participation with your family, friends, and related community as you feel comfortable and appropriate, in order to decide whether or not you would like to participate. A copy of this informed consent will be provided to you.

4. EXPECTED LENGTH OF TIME IN THE STUDY

This study will last about 24 months or longer, depending on how long it takes to enroll volunteers. The study will require 10 clinic visits: One visit for screening (Visit A) and enrollment (Visit B), and nine follow-up visits (Visits C-J)) over a 24-month period for each volunteer. Visit A takes the most time and can be completed over two days. If you choose to withdraw from the study, you will be asked to complete an exit visit. However, it is your right to stop participating in the study at any time, for whatever reason, even if you agree to take part in the study now. In addition to the ten study visits, you will also be asked to return to the clinic approximately one to three weeks after the last scheduled visit, to find out the results of the HIV test and for post-test counseling. You will be compensated for all study visits and post-test counseling visits. If you become HIV infected, we would like you to participate in a sub-study, and there will be additional information provided and consent forms to participate in that portion of the study.

5. NUMBER OF VOLUNTEERS PARTICIPATING IN THIS STUDY

This study is also being conducted in Kenya, Tanzania and Thailand with up to 500 volunteers enrolled at each site. In <<Enter country>> at this project, about 500 adult residents will take part in this study. Thus, a total of 2000 volunteers from all four sites will be involved in this research study.

6. ELIGIBLE VOLUNTEERS

In order to participate in this study, you must be:

1) Able to demonstrate understanding of the study by passing score of 90% on test of understanding within 3 attempts to take the test
2) Able and willing to sign, mark or thumb-print this informed consent form
3) Man or woman aged 18-50 years
4) Available for follow-up for the total study duration of 24 months.
5) Willing to consent to HIV counseling and testing
6) Willing to receive HIV test results
7) Willing to provide location or contact information
8) Willing to have picture taken for the study ID, or provide a photo ID or fingerprint
9) Must understand English or <<local language>>
10) Based upon information provided in the questionnaire, your behavior places you at greater risk for HIV infection than the general population

You will be denied participation in this study if one or more of the following apply:

1) Any significant condition (including medical and psychologic/psychiatric disorder) which in the opinion of the study investigator might interfere with the conduct of the study
2) Unwillingness to give social history, medical history, undergo medical examination and provide blood specimen
3) If you have ever received an HIV vaccine

7. PURPOSE OF THE STUDY

Over the past decade, AIDS has been a major problem worldwide. AIDS is a disease caused by HIV. HIV is transmitted through unprotected sex, by intravenous drug abuse, transfusion of unscreened infected blood, sharing sharp piercing instruments such as needles, knives, razor blades and safety

pins and from an infected mother to child during pregnancy, childbirth and/or breast-feeding. This research study seeks to find out how many people become infected with HIV over 24 months and how they might have become infected. It will also determine how frequently volunteers miss their scheduled study visits (retention rate) as well as volunteer willingness to participate in future HIV vaccine studies. The study will also evaluate individuals who enter the study with HIV infection and those who become HIV infected during this study.

8. PROCEDURES

You will be given a briefing on HIV and HIV vaccine research in general. Those who attend the briefing sessions will sign a briefing log. If you are willing to take part in this study, you must sign the consent form before any other tests or procedures are done. You must complete a test of understanding (10 questions that test your comprehension of this information) and answer at least 9 out of 10 questions correctly. You may take this test up to 3 times. If you do not pass this test by the third time you take it, you cannot participate in this research study. Upon successful completion of the test of understanding, you will have an interview, physical exam and have your blood collected to determine if you are eligible to continue participation. Many volunteers who are HIV infected and all volunteers who have other chronic illnesses will not continue in the study but will be referred to appropriate medical facilities for care or told about other HIV related research studies if available. The first visit will take about 2 to 3 hours and, if longer, investigators may have you complete this visit on another day. All other visits will take about 1 to 2 hours. Procedures included in the visits are described below.

Visits C-J will be done every 3 months and will include the following activities:

	Visit C	**Visit D**	**Visit E**	**Visit F**	**Visit G**	**Visit H**	**Visit I**	**Visit J**
HIV Counseling	√	√	√	√	√	√	√	√
Questionnaire Interview		√		√		√		√
Additional Questionnaire on washing practices		√		√		√		√
Blood Collection		√		√		√		√

You will receive HIV risk reduction counseling. You will be issued a photo identification (ID) card that you must bring to each appointment or be indentified by a fingerprint identification system on a computer. We will be storing your information generated by your fingerprint along with your name and address in a secure database for the purpose of identifying participants.

Interviews: You will answer some questions about yourself and some questions about your sex life and other possible risk factors for HIV infection. The answers to these questions will be recorded on a behavioral and knowledge questionnaire. In addition to the behavioral and knowledge questionnaire, you will be asked to answer question on two other, brief questionnaires. The first additional questionnaire asks question on your vaginal and/or anal washing practices. This questionnaire will be given to you during the same visits that the behavioral and knowledge questionnaire is done. The second additional questionnaire will be collected twice a week during the SBVs (these are known as small blood draw visits). This questionnaire asks questions on your most common method of sexual intercourse.

If you are uncomfortable with some of these questions, you may choose not to answer them, but the value of your participation is more worthwhile if you answer all of the questions as truthfully as possible. Your answers will be kept confidential.

Medical History and Physical Exam: You will be asked questions about diseases or illnesses you have had in the past. You will have your height, weight, temperature, pulse and blood pressure taken. You will also have a physical examination done by a doctor.

Blood Specimen Collection and Tests: Your blood will be tested for HIV and to assess your general health. We will take 86.4 ml (about 17 teaspoons) of your blood (this is much less than if you were donating blood at a blood bank) from a vein in your arm at the first visit. An additional 3 mL will be drawn at your next visit for verification, if the first HIV test result was positive. We will also do some additional specialized testing to study why some people are more resistant to HIV infection or better control HIV when infected, than others. Most of the tests will be done at the laboratory on-site; but some of the specialized test will be done at the MHRP Laboratory in the United States of America (USA) and to their research partners.

The amount of blood that you will give will depend on the type of visit: for Visit B, about 72.9 mls (about 5 Tablespoons) and for your large blood draw (LBV) visits about 33.7 mls (about 2 Tablespoons) from a vein in your arm at each study visit.

The totoal amount of blood that you will give in this phase I of the study will depend on the period of time you will participate in it. Those who will participate in only visits A and B will give a total of 159.3 mls only (about 11 Tablespoons) over the 2 visits (4 weeks apart).

Participants who will complete all visits for Phase I will donate a total of 379.3 mls for the LBVs and about 67.2 ml for the SBVs (the small blood tests done twice weekly) to make a total of 426.5 ml over the 24 months (this is less than the amount of blood you woud donate at a blood bank).

Participants who may get HIV infected (at any time during the 24 months) will continue with twice weekly blood draws (but at larger volumes) to confirm the HIV infection. After the first positive research-only test result, using the SBVs above, we will draw blood twice weekly as follows for the next 9 visits (over 4 weeks): 60.1ml (about 4 Tablespoons) at the first visit, between 31 and 47.2 ml (2-3 Tablespoons) at visits 2-4, 105.2mls (a little more than 7 Tablespoons) at the 5th visit, then between 10-35mls (less than 1 Tablespoon to a little more than 2 Tablespoons) for visits 6-8 and then 45.2mls (about 3 Tablespoons) at the last visit.

This would make a total of 391.2 mls of blood (26.5 Tablespoons) over 4 weeks for all persons that test HIV positive using the new research tests. Tests done on this blood will help investigators achieve the goals of this study and you will be invited to join Phase II of the study.

For those who are suspected to be HIV positive, your saliva will also be collected in a cup after chewing on dental wax.

HIV Testing: The HIV testing will be done at the laboratory on-site, and the results will be kept confidential and privately informed to you.

HIV Test Results: Before your blood is drawn for HIV testing you will receive pre-test HIV counseling. Post-test counseling will be provided to you in the subsequent (follow-up) visit. If the HIV test result is positive, you will be counseled on what you can do to help yourself and your partners and what we can do to help you. We may suggest that you need further examination and treatment and you will be referred to an appropriate health facility or the doctor of your choice using your rights under the <<enter local info>>.

If your test is negative you will be counseled on how to stay negative.

HIV Diagnostics: A portion of the sample taken today will be used for additional HIV diagnostic tests that the investigators believe may work better than the currently used tests. These test results will also be kept confidential.

STI (Sexually Transmitted Infection)-Test Results: Your blood will be tested for syphilis and herpes simplex virus (HSV) which are sexually transmitted diseases and if during your visit, the investigator determines that you have a sexually transmitted disease, you will be treated according to the <<Enter local info>>

Other: If you test positive for malaria, you will also be treated according to the current national standard of care, and referred to a health center/ hospital for follow-up.

You may have the opportunity to provide vaginal and/or rectal secretions. If you are interested in participating in this part of the study, then a separate consent document will be given to you.

Taking part in this study will require 10 clinic visits (screening (Visit A, which may be completed over 2 days), and 9 follow-up visits (Visits B-J)) over a 24-month period. The second study visit (Visit B) will occur 7 to 42 days after Visit A, and the rest of your follow-up visits will be every three months. Some of your follow-up visits are considered LBVs. The amount of blood that you will give will depend on the type of visit as noted above.

In addition to your follow-up visits above, you will be asked to give a very small amount of blood twice each week for at least the first year of the two-year study (these are known as Small Blood Draw (SBV) visits). The amount of blood collected at these visits is about 0.6 milliliters (about one tenth of a teaspoon). These small frequent blood samples are used to do special tests to detect very early HIV infection. The frequent small blood tests done twice weekly add up to about 3 teaspoons every 12 weeks. The total blood drawn for the LBV and SBV is much less than if you were donating blood at a blood bank.

If you test positive for HIV after Visit B, you will be asked to complete a series of additional visits (phase IB). The phase IB visits are needed to verify your HIV diagnosis and assess HIV disease stage. This study may identify HIV infection very early, before standard HIV tests can be reliable. Special testing done twice weekly for one month in phase IB will determine your HIV status. You will be asked to collect vaginal or anal swabs at each of these visits. You will have the opportunity to review, ask questions and provide consent to these swab collections on the "Phase Ib Swab Collection Consent".

If you are confirmed HIV positive, you will be requested to enter phase II of the study. You will enter phase II after a thorough explanation of the study and again providing informed consent. In phase II, the amount of blood taken at each visit will vary, but we will take no more than 107.2 mls (about 7 Tablespoons) of blood at a single study visit. We will find out how much HIV virus is present in your blood and will determine your helper T cell count. The amount of virus and helper T cells in your blood are useful to measure HIV disease progresssion and will help your doctor know when to give you the best treatment. These will be given to you so that you can provide them to the doctor that will take care of your HIV infection.

Exit Visit

The exit visit will occur if you choose to withdraw from this study during phase I, phase IB or phase II. The exit visit will include the following activities: the questionnaire interview, medical history and physical exam, and the collection of blood specimens.

Returning to the clinic for HIV test results

Within three weeks of each LBV study visit, you will be expected to return to the clinic to receive your HIV test results and receive post-test counseling. These visits are not considered study visits, because no blood specimens will be collected and no questionnaires will be administered. If you

should test positive for HIV at any time after Visit B, you may be eligible to join phase II of the RV 217 study. If you choose not to participate in phase II, you will still be followed for the duration of the main study (up to 24 months). If you test positive for HIV at any time after Visit B, you will receive counseling and be referred to appropriate medical facilities for care.

9. RISKS

Blood Drawing Risks: Drawing blood may cause pain and bruising. On rare occasions, it may cause bacterial infection at the part of your body where the blood is taken. Sometimes, drawing blood causes people to feel dizzy or to faint. Some people, especially women, may become anemic (have a low red blood cell count). We will monitor your blood count and alter your blood draw schedule if a low count occurs.

Social Risks: There is a risk of having your HIV status revealed to others. If you learn that you are HIV positive, it might cause you to become depressed and suicidal. If others learn you are HIV positive, it could cause you problems like stigmatization in your community, with your family members, and/or with your job. Others might judge you for the reasons you are in this study. If you test HIV positive, you may need to come back to the clinic more frequently. Social harms may also occur during the course of the study. These may include physical assault, relationship issues, suicidal attempts and may or may not be related to the study, The study staff will take appropriate action to assist you with any discrimination you may experience by being in this study. However, the study staff will take great care to minimize this risk occurring. To ensure this, your personal identifying information like your name and age, will be collected at the time of enrollment and stored in a lockable cabinet to which only key study team members will have access. You will then be allocated a study number, which will be used on all subsequent visits, and not your name. Only the study team members, and no one outside of the study team will know this study number. These steps will ensure confidentiality of your personal information and minimize the chances of your HIV status, and other information of yours becoming known to others.

Risks from Data and Stored Samples:
The greatest risk is the unplanned release of information from your study records. The chance that this information will be given to a person outside the study team without your permission is very small. Possible problems with the unplanned release of information include problems with family, friends, and your community or refusals when applying for insurance and employment. Similar problems may occur if you disclose information yourself or agree to have your medical records released.

You will not be personally identified in any publications of the results of these other research studies.

If in the course of the study you do not want your blood stored, you can inform the study team member who will provide you with a "withdraw of consent for sample storage" form, which you will have to sign to document your withdrawal.

10. BLOOD SPECIMENS

Some of the blood drawn from you as part of this study will be used for genetic tests. <<Uganda only: You will have the opportunity to review, ask questions and provide consent on having genetic tests performed on your blood samples in the "Informed Consent for Genetic Testing Form.">>

For specialized tests that are not available at this site, some of the blood samples will be shipped to and stored in the United States for HIV and Adenovirus (a common virus that causes colds) immune response testing as well as determination of your HLA type and other genes. There is a chance that blood samples that you are donating for this study may be used in other research studies within the scope of the objectives in this study but with prior approval from all related IRBs (institutional regulatory boards). You will have the opportunity to review, ask questions and provide consent on the storage and use of your blood samples in the "Future Use Blood Sample Consent Form."

Although you may withdraw from the study at any time, the samples and data collected up to that time will be used in accordance with the protocol. You may choose to restrict your samples or data from future uses which are not currently in the protocol. These future uses are not anticipated or known at this time and must be reviewed and approved by the ethical and scientific review boards before use.

11. BENEFITS

Although you may benefit from clinical testing and physical examination, you may receive no direct benefit from participating in this study.

During the study you will be provided education about HIV, counseling, and risk- reduction education to help you protect yourself from becoming infected with HIV. In addition you will have free periodic HIV testing to keep you informed about your HIV status. You and others may benefit in the future from the information that will be learned from this study. <<Include, if going to offer>> Cervical cancer screenings will be offered to all women participants.

12. COMPENSATION

You will be compensated (given) <<Enter Site info>> for your time, transportation, and lost wages for the first two visits (visits A and B), the subsequent scheduled 6 monthly visits, and the final exit visit. You will also be compensated <<Enter Site info>> for the twice weekly small blood volume visits for your time, transportation and lost wages. You will also be compensated (given) <<Enter Site info>> for post-test counseling visits in which you receive your HIV test results or unscheduled visits. Visits for other reasons, which under the investigator's judgment are unrelated to the study will not be compensated.

13. ASSURANCE OF CONFIDENTIALITY OF VOLUNTEER'S IDENTITY:

The Principal Investigator at this clinic <<Enter Site info>> , will maintain all research records of your taking part in this study. Every effort will be made to keep these records as confidential as possible within the limits of the law. All study volunteers will receive study numbers that are known only to the study team at the clinic and used to insure the confidentiality of research information. All blood tests will only bear the date, your study number and study visit number.

Your clinical and research records may be reviewed by representatives of the U.S. Military HIV Research Program, U.S. Army Medical Research and Materiel Command (USAMRMC), the US Department of Defense (DoD), Local Institutional Review Boards/Ethic Committees, the National Institute for Allergy and Infectious Diseases (NIAID), the U.S. Office for Human Research Protections (OHRP), the local regulatory authorities, and people who work for these organizations, and other regulatory agencies as part of their responsibilities for ensuring the protection of research volunteers.

Complete confidentiality cannot be promised but every effort will be made to keep the records as confidential as possible within the limits of the law. All data and medical information obtained about you as an individual will be considered important and held in confidence. All the above representatives are bound by rules of confidentiality not to reveal your identity to others.

Research and clinical information relating to you will be shared with other investigators and the scientific community through presentation or publication; however, you will not be identified by name or other personal information, which could be used to identify you.

14. MEDICAL CARE FOR RESEARCH RELATED INJURY

Should you be injured as a direct result of taking part in this research study, you will be provided emergency medical care only, at no cost to you, for that injury. The US Federal Government will not provide long term care (over 6 months) for any injuries resulting from study participation. You will be given information on where to get further treatment if needed for non-emergency care and you will receive short-term care for study-related injuries. You will be responsible for any future medical treatments. You will not receive any compensation for illness or injury. You should discuss this thoroughly with the principal investigator or his designee before you enroll in this study.If you have any questions about any research-related injury, you may contact <<Enter Site info>>

15. NEW FINDINGS

During the course of the study, you will be informed of any important new findings, such as changes in the risks or benefits or new alternatives to participation. If new information is provided to you, your consent to take part in the study will be re-obtained at your next follow-up visit.

16. ALTERNATIVE TO PARTICIPATION

You can choose not to take part in this study or you may choose to participate in other research studies, if available.

17. VOLUNTEER PARTICIPATION

Taking part in this study is completely voluntary. There is no cost for the clinic visits, examinations or laboratory tests that are part of this study. You may choose not to take part in this study or leave this study at any time. You will be treated the same no matter what you decide.

Some volunteers will only participate for two visits (Visits A and B) while others will participate for all the visits (C-J).

The investigator may withdraw you from this research study, (even if you would like to continue), if circumstances arise that call for such action. You may be removed from the study if you experience any of the following:

1. The sponsor ends the study for unforeseen reasons;

2. If you do not comply with study requirements;

3. If you are unwilling to continue to give blood in order for the researchers to perform the various study tests, you will be removed from this study, even if you are willing to continue to participate in other study procedures;

4. You develop health conditions which would make your continued participation in this study dangerous to you;

5. Other conditions which might occur that make your participation hazardous to your own health;

The investigator will make the decision at his/her discretion and let you know if it is not possible for you to continue. The decision may be made either to protect your health and safety, or because it is part of the research plan that people who develop certain conditions may not continue to participate.

18. PROBLEMS OR QUESTIONS

If you have questions about the study, your responsibilities as a volunteer in this study, or problems or concerns about how you are being treated in this study, contact the principal investigator <<Enter Site info>> ,

If you want to leave this study, contact the PI or any research staff at <<Enter Site info>>. You are not waiving any legal claims, rights or remedies because of your participation in this research study.

If you have any questions at any time regarding your rights as a volunteer in this research study and want to talk with someone not involved in the study, then you may contact:<<Enter IRB info>>.

19. VOLUNTEER STATEMENT

I have been asked to take part in Phase I of the study RV 217: "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand."

The principal investigator or his designee has explained the significance of the testing, the duration of the study, the testing that I will undergo, the methods to be used, and the risks and danger that I am taking.

I have been given a chance to ask any questions that I have about the purpose and nature of this research study and what is expected from my participation. All of my questions were answered to my satisfaction. I was offered a signed copy of this consent form.

_____ _____ _____
SIGNATURE OR MARK OF VOLUNTEER DATE TIME

_____
PRINT NAME OF VOLUNTEER

_____ _____ _____
SIGNATURE OF PERSON ADMINISTERING CONSENT DATE TIME

_____ _____ _____
SIGNATURE OF WITNESS (if volunteer unable to sign) DATE TIME

_____
PRINT NAME OF WITNESS (if volunteer unable to sign)

☐ The RV 217 study team **may** contact me regarding future research that I may be interested in.

☐ The RV 217 study team **may not** contact me regarding future research studies.

Volunteer Schedule (Phase I)

	Visit A	Visit B	Visit C	Visit D	Visit E	Visit F	Visit G	Visit H	Visit I	Visit J	Exit Vis
Study Day	Day 0	7 to 21 days	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months	Only if you the study
Type of Visit **(long visit is about 2 hours and a short visit is about 1 hour)**	long *may be completed over 2 days (within 1 week)	long	short	long	short	long	short	long	short	long	long
Location	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic
Does this visit require me to return to the clinic 1-3 weeks later for test results only?	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No

Starting at Visit B, and continuing for 1 year (through Visit G), you will be asked to come to SBV visits twice a week at the clinic or a location that is as convenient to you as possible.

<<Enter Site Info>>
In Collaboration with
U.S. Military HIV Research Program
And the
U.S. National Institutes of Health (NIH)

CONSENT TO BE A RESEARCH VOLUNTEER
PHASE II CONSENT FORM

1. TITLE

Phase II of RV 217: "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand."

Short Title: Early Capture HIV Cohort (ECHO)

2. <<ENTER SITE INVESTIGATOR>>

3. INFORMED CONSENT

Thank you for your interest in this study. This is phase II of the main study that you previously consented to participating in. This phase of the study uses a different schedule of visits, lasts for a longer period of time and has a stronger focus upon your body's response to HIV. The purpose of this consent is to inform you specifically about phase II. The U.S. Miltary HIV Research Program (MHRP) and <<site info>> are conducting a research study to learn more about the people that become infected with HIV. This study willl take place at this site, at two other participating sites in East Africa and at a site in Thailand. You are being asked to take part in this study because you have become HIV infected. Before you decide whether or not to take part in phase II, we would like to explain the purpose of the study, how it may help you or others, any risk to you, and what is expected of you. This process is called informed consent.

It is important that you know the following:

a. Taking part in this study is of your own free will (entirely voluntary).
b. You may choose not to participate or to withdraw from this study without prejudice.

YOUR PARTICIPATION (TAKING PART) IS VOLUNTARY.

Please ask questions about anything you do not understand. The clinic staff will talk with you about the information in this form. The study investigators encourage you to ask questions about this study at any time. You will be given the time necessary to review this form and discuss your participation in this study with your family, friends, and religious community as you feel comfortable and appropriate in order to decide whether or not you would like to participate. A copy of this informed consent will be provided to you.

4. EXPECTED LENGTH OF TIME IN THE STUDY

This study will last at least 50 months, if you so choose. The study will require a clinic visit at one week after your last visit in phase IB. Subsequent visits will occur after one week, two weeks, four weeks and every 12 weeks thereafter for at least 50 months. However, it is your

right to stop participating in this study at any time for whatever reason even if you agree to take part in the study now.

5. SAMPLE SIZE

This study is being conducted in Kenya, Uganda, Tanzania and Thailand with a total of about 37 volunteers at each site.

6. ELIGIBLE VOLUNTEERS

In order to participate in this study you must have tested HIV positive at or after Visit B of Phase I of the RV 217 study.

7. PURPOSE OF THE STUDY

The purpose of this research study is to determine the type of HIV virus you are infected with and your body's response to it and how these relate to the amount of HIV in your body.

8. PROCEDURES

Study Visits

If you are willing to take part in this study, you must sign the consent form before any tests or procedures are done. Upon completion of the informed consent process**,** you will be asked some questions, have a medical history and physical examination and have some blood drawn from a vein in your arm. Visits will last about 2 hours.

Interviews: You will answer some questions about yourself and some questions about your sex life and other possible risk factors for HIV infection. The answers to these questions will be recorded on a behavioral and knowledge questionnaire. If you are uncomfortable with some of these questions, you may choose not to answer, but the value of your participation is worth more if you answer all of the questions as truthfully as possible.Your answers will be kept confidential.

Medical History and Physical Exam: You will be asked questions about diseases or illnesses you have had in the previous 3 months. You will have your height, weight, temperature, pulse and blood pressure taken. You will also have a physical examination.

Blood Specimen Collection and Tests: Your blood will be tested for your overall health, the amount of HIV virus and type of virus and your body's response to the virus. You will be given the results of these tests once they are available. We will take about 32.5 to 107.2 ml (about 2 to 7 Tablespoons) from a vein in your arm at every study visit.

A participant who successfully completes the entire 50 months of Phase II will have donated total of 986.5 mls (about 67 Tablespoons).

We will do some additional specialized testing to try and understand why HIV disease progresses differently in some people. Most of the tests will be done at the laboratory on-site; but some of the specialized test will be done at the MHRP Laboratory in the United States of America (USA) and by their research partners.

STI (Sexually Transmitted Infection)-Test Results: Your blood will be tested for syphilis, a sexually transmitted disease**.**You will learn of your syphilis test result at your next study visit and, if you are positive you will be referred to the appropriate health facility for standard

treatment and care using your rights under the national health insurance scheme if you are positive. If during your visit, the investigator determines that you have a STI such as, gonorrhea or chlamydia, you will be treated according to <<enter site info>>.

Other: A blood smear will be done on volunteers with suspected malaria. Treatment will be given according to the current Ministry of Health guidelines. Those volunteers that may have tuberculosis (TB) will be referred to <<site info>> for additional testing and treatment.

Your saliva will also be collected in a cup after chewing on dental wax.

Exit Visit: The exit visit will occur if you choose to withdraw from this study. The exit visit will include the following activities: the questionnaire interview, medical history and physical exam, and the collection of blood and mucosal specimens.

9. RISKS

Blood Drawing Risks: Drawing blood may cause pain and bruising. On rare occasions, it may cause bacterial infection at the part of your body where the blood is taken. Sometimes, drawing blood causes people to feel dizzy or to faint. Some people, especially women, may become anemic (have a low red blood cell count).

Social Risks*:* Others might judge you for the reasons you are in this study (being HIV positive). Social harms may include physical assault, relationship issues, suicidal attempts and may or may not be related to the study, The study staff will take appropriate action to assist you with any discrimination you may experience by being in this study. However, the study staff will take great care to minimize this risk occurring. To ensure this, your personal identifying information like your name and age, will be collected at the time of enrollment and stored in a locakable cabinet to which only key study team members will have access. You will then be allocated a study number, which will be used on all subsequent visits, and not your name. Only the study team members, and no one outside of the study team will know this study number. These steps will ensure confidentiality of your personal information and minimize the chances of your HIV status, and your other information becoming known to others.

Risks from Data and Stored Samples

The greatest risk is the unplanned release of information from your study records. The chance that this information will be given to a person outside the study team without your permission is very small. Possible problems with the unplanned release of information include problems with family, friends, and your community or refusals when applying for insurance and employment. Similar problems may occur if you disclose information yourself or agree to have your medical records released.

You will not be personally identified in any publications of the results of these other research studies.

If in the course of the study you do not want your blood stored, you can inform the study team member who will provide you with a "withdraw of consent for sample storage" form, which you will have to sign to document your withdrawal.

10. BLOOD SPECIMENS

Some of the blood drawn from you as part of this study will be used for genetic tests. You will have the opportunity to review, ask questions and provide consent on having genetic tests performed on your blood samples in the "Informed Consent for Genetic Testing Form."

For specialized tests that are not available at this site, some of the blood samples will be shipped to and stored in the United States. There is a chance that the blood samples that you are donating under this study may be used in other research studies with prior approval from all related institutional regulatory boards. You will have the opportunity to review, ask questions and provide consent on the storage and use of your blood samples in the "Future Use Blood Sample Consent Form". You will not be personally identified in any publications of the results of these or other research studies that use your samples.

Although you may withdraw from the study at any time, the samples and data collected up to that time will be used in accordance with the protocol. You may choose to restrict your samples or data from future uses which are not currently in the protocol. These future uses are not anticipated or known at this time and must be reviewed and approved by the ethical and scientific review boards before use.

11. BENEFITS

Taking part in this study may provide no direct benefits to you. This phase of the study will assess your HIV disease stage and if you approve, the study team can share this information with your health care provider which may help guide your HIV treatment. You and others may benefit in the future from information that will be learned from this study.

12. COMPENSATION

You will be compensated (given) <<Enter site info>> for time and transport for the study visits and <<Enter site info>> for unscheduled visits. Visits for other illnesses will not be compensated.

13. ASSURANCE OF CONFIDENTIALITY OF VOLUNTEER'S IDENTITY:

The Principal Investigator at this clinic Dr. Hannah Kibuuka, will maintain research records of your taking part in this study.

All study volunteers will receive study numbers that are known only to the study team at the clinic and used to insure the confidentiality of research information. All blood tests will contain the date, your study visit number. Every effort will be made to keep these records as confidential as possible within the limits of the law.

Clinical and research records may be reviewed by representatives of the U.S. Military HIV Research Program, U.S. Army Medical Research and Materiel Command (USAMRMC), the U.S. Department of Defense (DoD), Local Institutional Review Boards/Ethic Committees, the National Institute of Allergy and Infectious Diseases (NIAID), the U.S. Office for Human Research Protections (OHRP), the local Ministry of Health, and people who work for these organizations, and other regulatory agencies as part of their responsibilities for insuring the protection of research volunteers. Complete confidentiality cannot be promised but every effort will be made to keep the records as confidential as possible within the limits of the law. All data and medical information obtained about you as an individual will be considered

important and held in confidence. All the above representatives are bound by rules of confidentiality not to reveal your identity to others.

Research and clinical information relating to you will be shared with other investigators and the scientific community through presentation or publication; however, you will not be identified by name or other personal information which could be used to identify you.

14. MEDICAL CARE FOR INJURY

Should you be injured as a direct result of taking part in this research study, you will be provided emergency medical care only, at no cost to you, for that injury. The US Federal Government will not provide long term care (over 6 months) for any injuries resulting from study participation. You will be given information on where to get further treatment if needed for non-emergency care and you will receive short-term care for study-related injuries. You will be responsible for any future medical treatments. You will not receive any compensation for illness or injury. You should discuss this thoroughly with the principal investigator or his designee before you enroll in this study.

If you have any questions about any research-related injury, you may contact <<Enter site info>>.

You will be referred to a HIV provider institution for your usual HIV care and treatment.

15. NEW FINDINGS

During the course of the study, you will be informed of any important new findings, such as changes in the risks or benefits or new alternatives to participation. If new information is provided to you, your consent to take part in the study will be re-obtained at your next follow-up visit.

16. ALTERNATIVE TO PARTICIPATION

You can choose not to take part in this study or you may choose to take part in any other research study, if available. If you choose not to participate in this part of the study, you will continue with follow up in the main research study.

17. VOLUNTEER PARTICIPATION

Taking part in this study is completely voluntary. There is no cost for the clinic visits, examinations or laboratory tests that are part of this study. You may choose not to take part in this study or leave this study at any time. You will be treated the same no matter what you decide. You are not waving any legal claims, rights or remedies because of your participation in this research study.

The investigator may withdraw you from this research study, even if you would like to continue, if circumstances arise that call for such action. You may be removed from the study for any of the following reasons:

1. The sponsor terminates the study for unforeseen reasons.
2. If you do not comply with study requirements;

3. If you are unwilling to continue to give blood in order for the researchers to perform the various study tests, you will be removed from this study, even if you are willing to continue to participate in other study procedures;
4. You develop health conditions which would make your continued participation in this study dangerous to you;
5. Other conditions which might occur that make your participation hazardous to your own health;

The investigator will make the decision at his/her discretion and let you know if it is not possible for you to continue. The decision may be made either to protect your health and safety, or because it is part of the research plan that people who develop certain conditions may not continue to participate.

18. PROBLEMS OR QUESTIONS

If you have questions about the study, your responsibilities as a volunteer in phase II, or problems or concerns about how you are being treated in this study, you may contact <<Enter site info>>.

If you want to leave this study, contact the PI or any research staff at <<Enter site info>>. You are not waiving any legal claims, rights or remedies because of your participation in this research study.

If you have any questions at any time regarding your rights as a volunteer in this research study and want to talk with someone not involved in the study, then you may contact: <<Enter IRB info>>.

19. PARTNER PARTICIPATION

Your partner is eligible to participate in one visit of Phase II. It is up to you to decide whether or not to tell your partner about their eligibility to participate. Please be aware that in doing so, your partner will be informed of your HIV status. You will not be penalized for refusing to notify your partner of their eligibility to participate.

VOLUNTEER STATEMENT

I have been asked to take part in phase II of the study **RV 217: "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand."**

The principal investigator or his designee has explained the significance of the testing, the duration of the study, the testing that I will undergo, the methods to be used, and the risks and danger that I am taking.

I have been given a chance to ask any questions that I have about the purpose and nature of this research study and what is expected from my participation. All of my questions were answered to my satisfaction. I was offered a signed copy of this consent form.

_____ _____ _____

SIGNATURE OR MARK OF VOLUNTEER DATE TIME

_____

PRINT NAME OF VOLUNTEER

_____

SIGNATURE OF PERSON ADMINISTERING CONSENT DATE TIME

_____

WITNESS (if volunteer unable to sign) DATE TIME

_____

PRINT NAME OF WITNESS (if volunteer unable to sign)

<<Enter Site Info>>
In Collaboration with
U.S. Military HIV Research Program
And the
U.S. National Institutes of Health (NIH)

CONSENT TO BE A RESEARCH VOLUNTEER
PHASE II PARTNER CONSENT FORM

1. TITLE

Phase II of RV 217: "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand."

Short Title: Early Capture HIV Cohort (ECHO)

2. <<ENTER SITE INVESTIGATOR>>

3. INFORMED CONSENT

Thank you for your interest in this study. You are being asked to consent to participate in one visit of phase II of RV 217. The U.S. Miltary HIV Research Program (MHRP) and <<site info>> are conducting a research study to learn more about the people that become infected with HIV. This study willl take place at this site, at two other participating sites in East Africa and at a site in Thailand. You are being asked to take part in this visit because you may be infected with HIV. Before you decide whether or not to take part in this phase II visit, we would like to explain the purpose of the study, how it may help you or others, any risk to you, and what is expected of you. This process is called informed consent.

It is important that you know the following:

a. Taking part in this study is of your own free will (entirely voluntary).
b. You may choose not to participate or to withdraw from this study without prejudice.

YOUR PARTICIPATION (TAKING PART) IS VOLUNTARY.

Please ask questions about anything you do not understand. The clinic staff will talk with you about the information in this form. The study investigators encourage you to ask questions about this study at any time. You will be given the time necessary to review this form and discuss your participation in this study with your family, friends, and religious community as you feel comfortable and appropriate in order to decide whether or not you would like to participate. A copy of this informed consent will be provided to you.

4. EXPECTED LENGTH OF TIME IN THE STUDY

The length of time for you in this study is one visit. However, it is your right to stop participating in this study at any time for whatever reason even if you agree to take part in the study now.

5. SAMPLE SIZE

This study is being conducted in Kenya, Uganda, Tanzania and Thailand with a total of about 37 volunteers at each site.

6. ELIGIBLE VOLUNTEERS

In order to participate in this visit you must have tested positive for HIV or suspect that you might have HIV.

7. PURPOSE OF THE STUDY

The purpose of this research study is to determine the type of HIV virus you are infected with and your body's response to it and how these relate to the amount of HIV in your body.

8. PROCEDURES

Study Visits

If you are willing to take part in this study, you must sign the consent form before any tests or procedures are done. Upon completion of the informed consent process**,** you will be asked some questions, have a medical history and physical examination and have some blood drawn from a vein in your arm. This visit will last about 2 hours.

Interviews: You will answer some questions about yourself and some questions about your sex life and other possible risk factors for HIV infection. The answers to these questions will be recorded on a behavioral and knowledge questionnaire. If you are uncomfortable with some of these questions, you may choose not to answer, but the value of your participation is worth more if you answer all of the questions as truthfully as possible.Your answers will be kept confidential.

Medical History and Physical Exam: You will be asked questions about diseases or illnesses you have had in the previous 3 months. You will have your height, weight, temperature, pulse and blood pressure taken. You will also have a physical examination.

Blood Specimen Collection and Tests: Your blood will be tested for your overall health, the amount of HIV virus and type of virus and your body's response to the virus. You will be given the results of these tests once they are available. We will take about 81.5 ml (about 5.5 Tablespoons) from a vein in your arm at this study visit.

We will do some additional specialized testing to try and understand why HIV disease progresses differently in some people. Most of the tests will be done at the laboratory on-site; but some of the specialized test will be done at the MHRP Laboratory in the United States of America (USA) and by their research partners.

STI (Sexually Transmitted Infection)-Test Results: Your blood will be tested for syphilis, a sexually transmitted disease**.**You will learn of your syphilis test result and, if you are positive you will be referred to the appropriate health facility for standard treatment and care using your rights under the national health insurance scheme if you are positive. If during your visit, the investigator determines that you have a STI such as, gonorrhea or chlamydia, you will be treated according to <<enter site info>>.

Other: A blood smear will be done on volunteers with suspected malaria. Treatment will be given according to the current Ministry of Health guidelines. Those volunteers that may have tuberculosis (TB) will be referred to <<site info>> for additional testing and treatment.

9. RISKS

Blood Drawing Risks: Drawing blood may cause pain and bruising. On rare occasions, it may cause bacterial infection at the part of your body where the blood is taken. Sometimes, drawing blood causes people to feel dizzy or to faint. Some people, especially women, may become anemic (have a low red blood cell count).

Social Risks*:* Others might judge you for the reasons you are in this study (being HIV positive). Social harms may include physical assault, relationship issues, suicidal attempts and may or may not be related to the study, The study staff will take appropriate action to assist you with any discrimination you may experience by being in this study. However, the study staff will take great care to minimize this risk occurring. To ensure this, your personal identifying information like your name and age, will be collected at the time of enrollment and stored in a locakable cabinet to which only key study team members will have access. You will then be allocated a study number, which will be used on all subsequent visits, and not your name. Only the study team members, and no one outside of the study team will know this study number. These steps will ensure confidentiality of your personal information and minimize the chances of your HIV status, and your other information becoming known to others.

Risks from Data and Stored Samples

The greatest risk is the unplanned release of information from your study records. The chance that this information will be given to a person outside the study team without your permission is very small. Possible problems with the unplanned release of information include problems with family, friends, and your community or refusals when applying for insurance and employment. Similar problems may occur if you disclose information yourself or agree to have your medical records released.

You will not be personally identified in any publications of the results of these other research studies.

If in the course of the study you do not want your blood stored, you can inform the study team member who will provide you with a "withdraw of consent for sample storage" form, which you will have to sign to document your withdrawal.

10. BLOOD SPECIMENS

Some of the blood drawn from you as part of this study will be used for genetic tests. You will have the opportunity to review, ask questions and provide consent on having genetic tests performed on your blood samples in the "Informed Consent for Genetic Testing Form."

For specialized tests that are not available at this site, some of the blood samples will be shipped to and stored in the United States. There is a chance that the blood samples that you are donating under this study may be used in other research studies with prior approval from all related institutional regulatory boards. You will have the opportunity to review, ask questions and provide consent on the storage and use of your blood samples in the "Future Use Blood Sample Consent Form". You will not be personally identified in any publications of the results of these or other research studies that use your samples.

Although you may withdraw from the study at any time, the samples and data collected up to that time will be used in accordance with the protocol. You may choose to restrict your samples or data from future uses which are not currently in the protocol. These future uses are not anticipated or known at this time and must be reviewed and approved by the ethical and scientific review boards before use.

11. BENEFITS

Taking part in this study may provide no direct benefits to you. This phase of the study will assess your HIV disease stage and if you approve, the study team can share this information with your health care provider which may help guide your HIV treatment. You and others may benefit in the future from information that will be learned from this study.

12. COMPENSATION

You will be compensated (given) <<Enter site info>> for time and transport for your visit.

13. ASSURANCE OF CONFIDENTIALITY OF VOLUNTEER'S IDENTITY:

The Principal Investigator at this clinic Dr. Hannah Kibuuka, will maintain research records of your taking part in this study.

All study volunteers will receive study numbers that are known only to the study team at the clinic and used to insure the confidentiality of research information. All blood tests will contain the date, your study visit number. Every effort will be made to keep these records as confidential as possible within the limits of the law.

Clinical and research records may be reviewed by representatives of the U.S. Military HIV Research Program, U.S. Army Medical Research and Materiel Command (USAMRMC), the U.S. Department of Defense (DoD), Local Institutional Review Boards/Ethic Committees, the National Institute of Allergy and Infectious Diseases (NIAID), the U.S. Office for Human Research Protections (OHRP), the local Ministry of Health, and people who work for these organizations, and other regulatory agencies as part of their responsibilities for insuring the protection of research volunteers. Complete confidentiality cannot be promised but every effort will be made to keep the records as confidential as possible within the limits of the law. All data and medical information obtained about you as an individual will be considered important and held in confidence. All the above representatives are bound by rules of confidentiality not to reveal your identity to others.

Research and clinical information relating to you will be shared with other investigators and the scientific community through presentation or publication; however, you will not be identified by name or other personal information which could be used to identify you.

14. NEW FINDINGS

During the course of the study, you will be informed of any important new findings, such as changes in the risks or benefits or new alternatives to participation. If new information is provided to you, your consent to take part in the study will be re-obtained at your next follow-up visit.

15. ALTERNATIVE TO PARTICIPATION

You can choose not to take part in this study or you may choose to take part in any other research study, if available. If you choose not to participate in this part of the study, you will continue with follow up in the main research study.

16. PROBLEMS OR QUESTIONS

If you have questions about the study, your responsibilities as a volunteer in phase II, or problems or concerns about how you are being treated in this study, you may contact <<Enter site info>>.

If you want to leave this study, contact the PI or any research staff at <<Enter site info>>. You are not waiving any legal claims, rights or remedies because of your participation in this research study.

If you have any questions at any time regarding your rights as a volunteer in this research study and want to talk with someone not involved in the study, then you may contact: <<Enter IRB info>>.

VOLUNTEER STATEMENT

I have been asked to take part in phase II of the study **RV 217: "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand."**

The principal investigator or his designee has explained the significance of the testing, the duration of the study, the testing that I will undergo, the methods to be used, and the risks and danger that I am taking.

I have been given a chance to ask any questions that I have about the purpose and nature of this research study and what is expected from my participation. All of my questions were answered to my satisfaction. I was offered a signed copy of this consent form.

_____ _____ _____

SIGNATURE OR MARK OF VOLUNTEER DATE TIME

_____

PRINT NAME OF VOLUNTEER

_____

SIGNATURE OF PERSON ADMINISTERING CONSENT DATE TIME

_____

WITNESS (if volunteer unable to sign) DATE TIME

_____

PRINT NAME OF WITNESS (if volunteer unable to sign)

<<Enter Site Here>>

In Collaboration with
U.S. Military HIV Research Program (USMHRP)
And the
U.S. National Institutes of Health (NIH)

INFORMED CONSENT FOR FUTURE USE OF STORED SPECIMENS

Study Title: RV217 "HIV-1 Prevalence, Incidence, Cohort, Retention and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand"

Short Title: Early Capture HIV Cohort (ECHO)

As we told you in the main informed consent form for this study, we will do some testing of your blood (and mucosal specimens if you decide to participate in the mucosal collection). When we do that testing, there will be some blood (and mucosal specimen) left over. We will store these extra samples in case we need to repeat any tests. When the study is over we would like to keep these samples for more tests in the future that can tell us about HIV and HIV vaccines. This consent form tells you everything we know now about possible future research using your samples.

You can decide whether or not to let us use your samples for future tests. Your decision does not affect your participation in the study or any care you receive at this clinic. If you decide to allow us to use your samples for future studies, we will ask you to sign this form. You will get a copy to keep.

1. Future research on your blood samples will be related to HIV or vaccines.

Researchers are able to measure how the immune system responds by looking at blood and mucosal samples. We will try to understand why HIV disease progresses differently in some people. As new methods (or ways) of measuring the body's immune response to HIV are made in the laboratory, we would like to test these methods on the blood samples we have already collected from you. We know that sometimes genes, passed down from your parents, can be important to a person's immune response to HIV. Because of this, we may do genetic testing on your stored blood samples. We may use methods that have not been developed yet, so we cannot describe them to you now. We will only use your stored samples to learn more about how the immune system responds to HIV and how vaccines can prevent HIV infection.

2. Your samples used for future research may be shipped to the USA or to regional laboratories.

Samples of your blood (and mucosa) will be stored in a secure central storage lab (not in the clinic). The samples will not be labeled with your name. Some of your samples will be stored and used for research in your country and some will be shipped out of the country to laboratories chosen to carry out research tests. There is no time limit on how long your samples will be stored.

3. Your privacy will be protected.

We will protect your privacy with any future research testing of your samples, just like we do with all research information from you during the main study. The blood samples will not be labeled with

your name. Instead, they will have your study ID number. After this study ends, when the samples are requested for future research, the participant ID number stays with them, or in some cases, it is removed before the samples are sent to be used, if this information is not necessary for the study.

4. An Institutional Review Board/Independent Ethics Committee will review any future research on your blood samples.

An Institutional Review Board/Independent Ethics Committee, which watches over the safety and rights of research participants, must review any research studies using your samples in future studies.

5. There may be no benefit to you if you allow us to store your samples for future research.

The researchers do not plan, in general, to contact you or your health care provider with results from future studies using your blood. This is because the use of the samples is for research not for evaluation of your health. However, if the researchers decide that a test result would provide important information for your health, we will try to contact you. If you want this information, tell the clinic staff. Always let the study clinic staffs know if you change your address and/or phone number.

Your samples may contribute to a new invention or discovery. There is no plan for you to share in any money or other benefits resulting from this invention or discovery.

6. There are few risks related to storing your samples.

When tests are done on the stored samples there is a small but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (such as information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems getting a job or insurance. The risk of this happening is extremely low, because your results are not a part of your medical record and are not given to the clinic. Also, it is possible that your participant ID could be removed from the samples. If your participant ID number is removed from any samples, we will not be able to link that sample to you.

7. You can agree now to let us use your samples for future testing and still change your mind later.

If you agree now and decide later that you do not want us to use your samples for future research, please tell us. We will ask the storage facility to destroy any remaining samples that still have your participant ID on them so that they cannot be used for future research.

8. For more information:

If you have questions about the use of your samples for future research, contact <<Enter site PI contact information>>

If you have problem that you think may be related to the use of your samples for future research, or if you want to withdraw your consent, contact the study coordinator, <<Enter site information>>

If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, you may contact <<Enter local IRB contact information>>.

If you have read this form (or have had it explained to you), and had all of your questions answered, please sign or place your thumbprint in the space provided below to declare that you consent to have your blood or mucosal sample stored for future use.

- ❑ I allow you to store samples for future testing which may include genetic testing.
- ❑ I do not allow you to store samples for future testing.

Participant's name (print)	Participant's signature or mark	Date	Time (if signed on date of enrollment)

Study staff conducting consent discussion (print)	Study staff signature	Date	Time (if signed on date of enrollment)

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

Witness's name (print)	Witness's signature	Date	Time (if signed on date of enrollment)

<<Enter Site>>

In Collaboration with
U.S. Military HIV Research Program
and the
U.S. National Institutes of Health (NIH)

MALE VOLUNTEER CONSENT FORM
-MUCOSAL SPECIMEN COLLECTION

VOLUNTEER AGREEMENT TO PROVIDE MUCOSAL SPECIMENS IN THE RV 217 STUDY "HIV-1 PREVALENCE, INCIDENCE, COHORT RETENTION, AND HOST GENETICS AND VIRAL DIVERSITY IN COHORTS IN EAST AFRICA AND THAILAND"

SHORT TITLE: EARLY CAPTURE HIV COHORT (ECHO)

<<ENTER SITE PRINCIPAL INVESTIGATOR(S)>>

PART A: NATURE OF THE RESEARCH

Background:

The male and female reproductive tracts are capable of making antibodies and cells (an immune response) to help fight some infections. Because many HIV infections occur during sexual intercourse, it is important to understand the immune responses within the reproductive tracts of both people who have HIV infection and people who do not. This information may help us see if they could fight off the virus and might also help us to make vaccines to prevent HIV in the future.

What will happen to you if you agree to provide mucosal specimens?

You will receive compensation as described below for each of these visits. You may discontinue participation at any time without penalty.

How long will the project last?
There will be a total of up to 9 visits over an 18-month period. The first two collections will be three weeks apart; the next visit will occur four weeks later and the remaining visits will occur every three months. Each visit will last approximately one and a half hours. If you are being asked to be in this study at Visit B of phase I, you will have one additional collection during Visit B.

We may perform the following things at each visit:

Semen Collection:
You will be required to refrain from ejaculation for two days.

You will be asked to ejaculate into a sterile container. This will be done in the privacy of assigned rooms in the clinic. The ejaculate is returned to the study lab immediately for processing.

Mucosal Collection from Anoscopy:
You will be required to refrain from any kind of sexual activity, douching, and inserting anything into you rectum for at least 72 hours prior to collection. The nurse will insert a short hollow tube into your rectum, which will allow access to your colon. An anascope will then be inserted into your rectum and let it rest inside you for 1 to 2 minutes. The nurse will then remove the sponge and scope and send the sponge to the lab.

All of these specimens will then be taken to our research lab for testing.

Other Collections:
You may also be asked to provide a urine sample or have your mouth swabbed.

All of these fluids will then be taken to our research lab for testing.

What benefits will you get from donating mucosal specimens?
You will not benefit directly from this activity, but the information we gain may help us to better understand the immune system (the system in your body that fights infection) and HIV infection and develop HIV vaccines in the future.

Are there any risks to donating mucosal specimens?
There are minimal risks associated with this activity. When masturbation is conducted frequently and without the use of lubricant (as is required for this project) there is a risk of abrasion.

Risks to anoscopy that are commonly listed include discomfort during the examination and for a short while afterwards, or abrasion or tearing of hemorrhoids that could cause bleeding.

Are there other risks?
No.

Will you be paid for providing mucosal specimens?
You will receive compensation <<Enter Site Info>> in the amount of for transportation, lost wages and lunch.

If you are injured during the mucosal collection:
The United States Department of Defense is funding this research project. Should you be injured as a direct result of participating in this research project, you will be provided medical care (for up to 6 months), at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release from your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this project. If you experience any symptoms which you feel may be related to participating, be sure to immediately report them to:

<<Enter Site Investigator Contact Information>>

If you don't want to provide mucosal specimens:

You may decide not to participate in this project activity or leave this project at any time. If you decide not to participate in this activity of the project, or you choose to leave this project, it will not affect your participation in the main study or the care you receive from this or any other health care facility.

PART B: VOLUNTEER STATEMENT:

I, _____, understand that I am being asked to provide mucosal specimens for the study of "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand"

I am _____ years old. I agree to provide these mucosal specimens for this project.

I have had an opportunity to ask questions concerning this evaluation and mucosal specimen collection. All of my questions have been answered satisfactorily. If I have any other questions about the research and my rights, I may contact:

<<Enter IRB Contact>>

I have read, or have had read to me, the information and I understand what is involved in participation. I am signing my name below to indicate that I wish to participate in this activity. I further understand that I can stop participating at any time without penalty or loss of benefits. I have been offered a copy of the consent form.

Signature of Volunteer	Printed Name of Volunteer	Date
Signature of Person Administering Consent Form	Printed Name of Person Administering Consent Form	Date
Signature of Witness	Printed Name of Witness	Date
Signature of Witness	Printed Name of Witness	Date

<<Enter Site Here>>

In Collaboration with
U.S. Military HIV Research Program
And the
U.S. National Institutes of Health (NIH)

FEMALE VOLUNTEER CONSENT FORM
-MUCOSAL SPECIMEN COLLECTION

VOLUNTEER AGREEMENT TO PROVIDE MUCOSAL SPECIMENS IN THE RV 217 STUDY "HIV-1 PREVALENCE, INCIDENCE, COHORT RETENTION, AND HOST GENETICS AND VIRAL DIVERSITY IN COHORTS IN EAST AFRICA AND THAILAND"

SHORT TITLE: EARLY CAPTURE HIV COHORT (ECHO)

PART A: NATURE OF THE RESEARCH

Background:

The male and female reproductive tracts are capable of making antibodies and cells (an immune response) to help fight some infections. Because many HIV infections occur during sexual intercourse, it is important to understand the immune responses within the reproductive tracts of both people who have HIV infection and people who do not. This information may help us see if they could fight off the virus and might also help us to make vaccines to prevent HIV in the future.

What will happen to you if you agree to provide mucosal specimens?

You will be required to refrain from vaginal intercourse for three days. You may not use a vaginal douche for three days prior to the collection of fluids. We will insure that you are not pregnant before collecting fluids. You should not be menstruating at the time of specimen collection.

You will receive compensation as described below for each of these visits. You may discontinue participation at any time without penalty.

We will perform the following at each of the 8 visits:

Mucosal Collection from Cervical Swab
After making sure that you are not pregnant by using a urine pregnancy test, a pelvic (gynecological) exam will be performed under the supervision of a staff physician and will consist only of the insertion of a vaginal speculum and collection of cervical and vaginal fluids. These fluids are normally present in the cervix and vagina and will not require the use of needles for collection. Instead, the cervical fluid will be obtained by squirting a small amount of sterile salt water into the vagina and then using a small plastic bulb pipette to soak

up the fluid. In addition to this, one tube of less than a teaspoon of blood (3 milliliters) total will be collected using a needle in an arm vein. All of these fluids will then be taken to our research lab for testing. This will be used to test female hormones, such as estradiol.

Mucosal Collection from Anoscopy:
You may also be asked to provide a rectal sponge sample. If so, you will be required to refrain from any kind of sexual activity, douching, and inserting anything into you rectum for at least 72 hours prior to collection. The nurse will insert a short hollow tube (anoscope) into your rectum, which will allow access to your colon. The nurse will let the anoscope rest inside you for 1 to 2 minutes. The nurse will then remove the sponge and scope and send the sponge to the lab.

Other Collections:
You may also be asked to provide a urine sample, a breast milk sample or have your mouth swabbed.

What will happen to my samples?
All of these fluids will then be taken to our research lab for processing, then sent to the United States and/or Thailand for testing. You will receive compensation as described below for each of these visits. You may discontinue participation at any time without penalty.

How long will the project last?
There will be a total of 8 visits over an 18-month period. The first two collections will be three weeks apart; the next visit will occur four weeks later and the remaining visits will occur every three months. Each visit will last approximately one and a half hours. If you are being asked to be in this study at Visit B of phase I, you will have one additional collection during Visit B.

What benefits will you get from donating mucosal specimens?
A PAP smear (test for cancer of the cervix) will be provided if you wish. Otherwise you will not benefit directly from this activity, but the information we gain may help us to better understand the immune system (the system in your body that fights infection) and HIV infection and develop HIV vaccines in the future.

Are there any risks to donating mucosal specimens?
There are minimal risks associated with this activity. As is the case with pelvic exams, there may be some discomfort associated with the insertion of the vaginal speculum. If you have a PAP smear, you may feel uncomfortable with the insertion of the brush into the cervix.

In addition to the mild discomfort, on rare occasions, a small amount of bleeding may occur when the PAP smear is collected but this resolves without intervention. The pelvic exam is inconvenient as it takes more time than blood collections and some women feel embarrassed or distressed by the procedure. Experienced professionals with a nurse present will help you through the collection however to minimize any discomforts.

Risks to anoscopy that are commonly listed include discomfort during the examination and for a short while afterwards, or abrasion or tearing of hemorrhoids that could cause bleeding.

Are there other risks?
No.

Will you be paid for providing mucosal specimens?
You will receive compensation in the amount of <<Enter site info here>> for transportation, lost wages and lunch.

If you don't want to provide mucosal specimens:

You may decide not to participate in this project activity or leave this project at any time. If you decide not to participate in this activity of the project, or you choose to leave this project, it will not affect your participation in the main study or the care you receive from this or any other health care facility.

PART B: VOLUNTEER STATEMENT:

I, _____, understand that I am being asked to provide mucosal specimens for the study of "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand"

I am _____ years old. I agree to provide these mucosal specimens for this project.

I have had an opportunity to ask questions concerning this evaluation and mucosal specimen collection. All of my questions have been answered satisfactorily. If I have any other questions about the research and my rights, I may contact:

<<Enter IRB contact info>>

I have read, or have had read to me, the information and I understand what is involved in participation. I am signing my name below to indicate that I wish to participate in this activity. I further understand that I can stop participating at any time without penalty or loss of benefits. I have been offered a copy of the consent form.

Signature of Volunteer	Printed Name of Volunteer	Date
Signature of Person Administering Consent Form	Printed Name of Person Administering Consent Form	Date
Signature of Witness	Printed Name of Witness	Date
Signature of Witness	Printed Name of Witness	Date

<<Enter Site Here>>

In Collaboration with
U.S. Military HIV Research Program
And the
U.S. National Institutes of Health (NIH)

FEMALE VOLUNTEER CONSENT FORM
-MUCOSAL SPECIMEN COLLECTION

VOLUNTEER AGREEMENT TO PROVIDE MUCOSAL SPECIMENS IN THE RV 217 STUDY "HIV-1 PREVALENCE, INCIDENCE, COHORT RETENTION, AND HOST GENETICS AND VIRAL DIVERSITY IN COHORTS IN EAST AFRICA AND THAILAND"

SHORT TITLE: EARLY CAPTURE HIV COHORT (ECHO)

PART A: NATURE OF THE RESEARCH

Background:

The male and female reproductive tracts are capable of making antibodies and cells (an immune response) to help fight some infections. Because many HIV infections occur during sexual intercourse, it is important to understand the immune responses within the reproductive tracts of both people who have HIV infection and people who do not. This information may help us see if they could fight off the virus and might also help us to make vaccines to prevent HIV in the future.

What will happen to you if you agree to provide mucosal specimens?

We may perform the following at each of the 8 visits:

Mucosal Collection from Softcup:
You will be given a device called a Softcup to collect secretions from your cervix and vagina. The Instead Softcup is approved by the United States Food and Drug Administration (US FDA) as a feminine hygiene alternative to pads and tampons for use by women during menstruation.

For the initial mucosal collection, you will undergo a pelvic examination with a PAP smear. An instrument called a speculum will be inserted into your vagina by the staff and cells from your cervix will be tested for signs of cancer. During this visit you will be instructed on how to use the Softcup. You may insert the cup yourself or ask the clinic staff to assist you. You will also be instructed on the removal of the cup.

The cup should remain in the vagina for at least three hours, but not more than 12 hours for us to be able to obtain enough secretions. To remove the cup you will be given a plastic container and be instructed to go alone into a private room in the clinic, remove the cup, place it in and close the container.

We will also check your female hormone levels each time we collect your mucosal secretions to determine which stage of the menstrual cycle you are in. This test will be conducted from blood already collected during the visit.

Before each visit, you will be asked a series of questions to be sure that you are eligible to provide the specimens.

If you use tampons during menstruation and have had a severe illness (called Toxic Shock Syndrome or TSS) associated with tampon use, caused by a bacterial toxin then you should not participate in this sub-study. And since this illness is usually associated with women using absorbent tampons and for more than 12 hours at a time, you must not use the Softcup for more than 12 hours.

It is a rare but serious disease. The warning signs include a sudden high fever, vomiting, diarrhea, a rash that looks like sunburn, dizziness, fainting or near fainting, and muscle aches. TSS can progress rapidly from flu-like symptoms to a serious illness that can be fatal. Should you become unwell or develop any of these signs when using a Softcup, remove it and immediately contact a doctor for medical care.

You cannot be currently using an intrauterine device (IUD), as there is a risk of dislodging displacing, or removing the IUD when removing the Softcup.

You cannot use the Softcup during the six-week period immediately following childbirth, miscarriage, or termination of pregnancy.

You cannot have vaginal intercourse nor use a vaginal douche for three days before the collection visit.

You should not be menstruating at the time of specimen collection.

You will also be asked to provide a urine sample to check for infection.

THE SOFT CUP CANNOT BE USED TO PREVENT PREGNANCY.

Mucosal Collection from Anoscopy:
You may also be asked to provide a rectal sample. If so, you will be required to refrain from any kind of sexual activity, douching, and inserting anything into you rectum for at least 72 hours prior to collection. The nurse will insert a short hollow tube into your rectum, which will allow access to your colon. A sponge will then be inserted into your rectum and will rest inside you for 1 to 2 minutes. The nurse will then remove the sponge and scope and send the sponge to the lab.

Other Collections:
You may also be asked to provide a urine sample, a breast milk sample or have your mouth swabbed.

What will happen to my samples?
All of these fluids will then be taken to our research lab for processing, then sent to the United States and/or Thailand for testing. You will receive compensation as described below for each of these visits. You may discontinue participation at any time without penalty.

How long will the project last?
There will be a total of 8 visits over an 18-month period. The first two collections will be three weeks apart; the next visit will occur four weeks later and the remaining visits will occur every three months. Each visit will last approximately one and a half hours. If you are being asked to be in this study at Visit B of phase I, you will have one additional collection during Visit B.

What benefits will you get from donating mucosal specimens?
A PAP smear (test for cancer of the cervix) will be provided if you wish. Otherwise you will not benefit directly from this activity, but the information we gain may help us to better understand the immune system (the system in your body that fights infection) and HIV infection and develop HIV vaccines in the future.

If there is evidence that you have a sexually transmitted infection, you will be referred for diagnosis and care. Men and women suspected of having a sexually transmitted infection may benefit from diagnosis and referral for care and treatment.

Are there any risks to donating mucosal specimens?
There are minimal risks associated with this activity. The collection will be done in a private room in order to decrease the uncomfortable feeling you may have. Inserting the Softcup into vagina may cause discomfort and slight irritation similar to a tampon but it should not cause any pain. You will be instructed in how to insert and remove the cup by clinic staff. There is no evidence of cup sampling contributing to risk of HIV or other sexually transmitted infection.

In addition to the mild discomfort, on rare occasions, a small amount of bleeding may occur when the PAP smear is collected but this resolves without intervention. The pelvic exam is inconvenient as it takes more time than blood collections and some women feel embarrassed or distressed by the procedure. Experienced professionals with a nurse present will help you through the collection however to minimize any discomforts.

Risks to anoscopy that are commonly listed include discomfort during the examination and for a short while afterwards, or abrasion or tearing of hemorrhoids that could cause bleeding.

Are there other risks?
No.

Will you be paid for providing mucosal specimens?
You will receive compensation in the amount of <<Enter site info here>> for transportation, lost wages and lunch.

If you don't want to provide mucosal specimens:

You may decide not to participate in this project activity or leave this project at any time. If you decide not to participate in this activity of the project, or you choose to leave this project, it will not affect your participation in the main study or the care you receive from this or any other health care facility.

PART B: VOLUNTEER STATEMENT:

I, _____, understand that I am being asked to provide mucosal specimens for the study of "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand"

I am _____ years old. I agree to provide these mucosal specimens for this project.

I have had an opportunity to ask questions concerning this evaluation and mucosal specimen collection. All of my questions have been answered satisfactorily. If I have any other questions about the research and my rights, I may contact:

<<Enter IRB Contact Info>>

I have read, or have had read to me, the information and I understand what is involved in participation. I am signing my name below to indicate that I wish to participate in this activity. I further understand that I can stop participating at any time without penalty or loss of benefits. I have been offered a copy of the consent form.

Signature of Volunteer	Printed Name of Volunteer	Date
Signature of Person Administering Consent Form	Printed Name of Person Administering Consent Form	Date
Signature of Witness	Printed Name of Witness	Date
Signature of Witness	Printed Name of Witness	Date

APPENDIX II: TEST OF UNDERSTANDING

<<SITE>>

In Collaboration with
U.S. Military HIV Research Program
and the
U.S. National Institutes of Health (NIH)

VOLUNTEER AGREEMENT TO PROVIDE VAGINAL OR RECTAL SWAB SPECIMENS IN THE RV 217 STUDY "HIV-1 PREVALENCE, INCIDENCE, COHORT RETENTION, AND HOST GENETICS AND VIRAL DIVERSITY IN COHORTS IN EAST AFRICA AND THAILAND"

SHORT TITLE: EARLY CAPTURE HIV COHORT (ECHO)

PART A: NATURE OF THE RESEARCH

Introduction:
You are being asked to collect vaginal or rectal swabs in order to help investigators identify the presence of HIV and thereby determine the frequency of "at-risk" exposure. The type of swab you will collect will be determined by the most common method of sexual intercourse in which you participate.

You may discontinue participation at any time without penalty.

The United States Department of Defense is funding this research project. If you should have any questions regarding your participation in this study, please contact:

<<enter Principal Investigator Contact Information>>

What will happen to you if you agree to the swab collection?
Using a swab provided by the clinic, you will be asked to swab either your anus or your vagina and place the swab into a collection tube. This will be done either at home or in the privacy of assigned rooms in the clinic during each Phase Ib visit.

The type of swab collected will be determined by you most recent sexual experience.

How long will the project last?
You will be asked to collect a swab during each Phase Ib visit. If the investigators determine that you are not infected with HIV, you will be returned to the Phase I schedule, and you will stop swab collections.

Some volunteers may have chosen to participate in swab collection for a period of 4 months during Phase I. If you are one of those volunteers, you will continue swab collections as regularly scheduled, once you are returned to Phase I visits.

What benefits will you get from donating your swabs?
You will not benefit directly from this activity, but the information we gain may help us to better understand the immune system (the system in your body that fights infection) and HIV infection and develop HIV vaccines in the future.

Are there any risks to swab collection?
There are minimal risks associated with this activity. There may be some discomfort associated with the insertion of the swab into the vagina or rectum.

Are there any other risks?
No.

Will you be paid for providing swab collections?
You will receive compensation in the amount of $2 USD <<enter local compensation>> for you time associated with this procedure.

If you don't want to provide mucosal specimens:
You may decide not to participate in this project activity or leave this project at any time. If you decide not to participate in this activity, it will not affect your participation in the main study or the care you receive from this or any other health care facility.

PART B: VOLUNTEER STATEMENT:

I have had an opportunity to ask questions concerning this swab collection. All of my questions have been answered satisfactorily. If I have any other questions about the research and my rights, I may contact:

<<Enter IRB contact>>

I have read, or have had read to me, the information as to what is involved in participation. I am signing my name below to indicate that I wish to participate in this activity. I further understand that I can stop participating at any time without penalty or loss of benefits. I have been offered a copy of the consent form.

Signature of Volunteer	Printed Name of Volunteer	Date
Signature of Person Administering Consent Form	Printed Name of Person Administering Consent Form	Date
Signature of Witness	Printed Name of Witness	Date

<<SITE>>

In Collaboration with
U.S. Military HIV Research Program
and the
U.S. National Institutes of Health (NIH)

VOLUNTEER AGREEMENT TO PROVIDE VAGINAL OR RECTAL SWAB SPECIMENS IN THE RV 217 STUDY "HIV-1 PREVALENCE, INCIDENCE, COHORT RETENTION, AND HOST GENETICS AND VIRAL DIVERSITY IN COHORTS IN EAST AFRICA AND THAILAND"

SHORT TITLE: EARLY CAPTURE HIV COHORT (ECHO)

PART A: NATURE OF THE RESEARCH

Introduction:
You are being asked to collect vaginal or rectal swabs twice a week for a period 4 months in order to help investigators identify the presence of HIV and thereby determine the frequency of "at-risk" exposure. The type of swab you will collect will be determined by the most common method of sexual intercourse in which you participate.

You may discontinue participation at any time without penalty.

The United States Department of Defense is funding this research project. If you should have any questions regarding your participation in this study, please contact:

<<enter Principal Investigator Contact Information>>

What will happen to you if you agree to the swab collection?
Using a swab provided by the clinic, you will be asked to swab either your anus or your vagina and place the swab into a collection tube. This will be done either at home or in the privacy of assigned rooms in the clinic during the time of your small blood volume (SBV) visit.
The type of swab collected will be determined by you most recent sexual experience.

How long will the project last?
You will be asked to collect a swab twice a week for a period of 4 months.

What benefits will you get from donating your swabs?
You will not benefit directly from this activity, but the information we gain may help us to better understand the immune system (the system in your body that fights infection) and HIV infection and develop HIV vaccines in the future.

Are there any risks to swab collection?
There are minimal risks associated with this activity. There may be some discomfort associated with the insertion of the swab into the vagina or rectum.

Are there any other risks?
No.

Will you be paid for providing swab collections?
You will receive compensation in the amount of $2 USD <<enter local compensation>> for you time associated with this procedure.

If you don't want to provide mucosal specimens:
You may decide not to participate in this project activity or leave this project at any time. If you decide not to participate in this activity, it will not affect your participation in the main study or the care you receive from this or any other health care facility.

PART B: VOLUNTEER STATEMENT:

I have had an opportunity to ask questions concerning this swab collection. All of my questions have been answered satisfactorily. If I have any other questions about the research and my rights, I may contact:

<<Enter IRB contact>>

I have read, or have had read to me, the information as to what is involved in participation. I am signing my name below to indicate that I wish to participate in this activity. I further understand that I can stop participating at any time without penalty or loss of benefits. I have been offered a copy of the consent form.

Signature of Volunteer	Printed Name of Volunteer	Date
Signature of Person Administering Consent Form	Printed Name of Person Administering Consent Form	Date
Signature of Witness	Printed Name of Witness	Date

<<Enter Site Here>>

In Collaboration with
U.S. Military HIV Research Program (USMHRP)
And the
U.S. National Institutes of Health (NIH)

WITHDRAWAL OF CONSENT FOR SAMPLE STORAGE

Study Title: RV 217 "HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand"

Short Title: Early Capture HIV Cohort (ECHO)

Volunteer Statement of withdraw of consent to have samples stored for future testing:

I _____ withdraw my consent to have my samples stored for future use. I do not want to donate blood samples for storage and future use. However, I would still like to continue taking part in this study. I understand that when I sign at the bottom of this form, my samples will be used for all the tests specified for this present study, but no blood will be stored.

I understand that withdrawing my consent to have my samples stored for future use will not make any difference to the care I am receiving now or in the future, or to any benefits that I am entitled to.

I have been given a chance to ask all the questions that I have about withdrawing my consent to have my samples stored. All of my questions were answered to my satisfaction. I was offered a signed copy of this consent.

Participant's name (print)	Participant's signature or mark	Date	Time (if signed on date of enrollment)

Study staff conducting consent discussion (print)	Study staff signature	Date	Time (if signed on date of enrollment)

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

Witness's name (print)	Witness's signature	Date	Time (if signed on date of enrollment)

APPENDIX III: TEST OF UNDERSTANDING ANSWERS (INTERNAL USE)

US MILITARY HIV RESEARCH PROGRAM

RV217 TEST OF UNDERSTANDING FOR STUDY VOLUNTEER- ENGLISH VERSION

Volunteer Study #: | | | | | | |

Date: ____/ _____/ _____
dd mon yyyy

Answer the following with "True" or "False"

(Please check √)

1.	Taking part in the study is voluntary.	**True**		**False**	
2.	Once you have consented to take part in this study, you are not free to withdraw at any time.	**True**		**False**	
3.	With your permission, a portion of your blood sample will be stored for future HIV related studies	**True**		**False**	
4.	The study will provide counseling, information and support if you are found to be HIV infected.	**True**		**False**	
5.	The expected length of participation in this study is approximately 24 months.	**True**		**False**	
6.	Your information and test results will be provided to your family and friends	**True**		**False**	
7.	You will be asked questions about your sex life and risk factors for HIV infection	**True**		**False**	
8.	You will be expected to pay for all laboratory tests in this study	**True**		**False**	
9.	Some of your specimens will be sent to a laboratory in the United States for testing	**True**		**False**	
10.	Volunteers who become HIV infected during the study will be offered enrollment in another part of the study that will better characterize the virus and infection	**True**		**False**	

Volunteer Study #:						

RESULTS

Test number	No. correct out of 10
# 1	
# 2	
# 3	

REMARKS:

_____

_____

_____

_____

_____

_____

INTERVIEWER NAME: _____

DATE: _____/ _____/ _____
dd mon yyyy

US MILITARY HIV RESEARCH PROGRAM
ANSWERS TO RV217 TEST OF UNDERSTANDING FOR STUDY VOLUNTEERS

About this study

1.	Taking part in the study is voluntary.	**True**	☒	**False**	☐
2.	Once you have consented to take part in this study, you are not free to withdraw at any time.	**True**	☐	**False**	☒
3.	With your permission, a portion of your blood sample will be stored for future HIV related studies	**True**	☒	**False**	☐
4.	The study will provide counseling, information and support if you are found to be HIV infected.	**True**	☒	**False**	☐
5.	The expected length of participation in this study is approximately 24 months.	**True**	☒	**False**	☐
6.	Your information and test results will be provided to your family and friends	**True**	☐	**False**	☒
7.	You will be asked questions about your sex life and risk factors for HIV infection	**True**	☒	**False**	☐
8.	You will be expected to pay for all laboratory tests in this study	**True**	☐	**False**	☒
9.	Some of the specimens will be sent to a laboratory in the United States for testing	**True**	☒	**False**	☐
10.	Volunteers who become HIV infected during the study will be offered enrollment in another part of the study that will better characterize the HIV virus and infection	**True**	☒	**False**	☐

APPENDIX IV: SCHEDULE OF EVALUATIONS #1, PHASE I

VISIT		A[1]	B[2]	C	D	E	F	G	H	I	J	Exit Visit[3]
Week of study		0	4	12	24	36	48	60	72	84	96	
DAY OF STUDY		0	28	86	168	252	336	420	504	588	672	
Clinical												
Informed Consent		X										
Test of Understanding		X										
Photo ID or Fingerprint ID		check	check	check	check	check	check	check	check	check	check	check
Questionnaire		X	X[4]		X		X		X		X	X
Vaginal/Rectal Washing Questionnaire		X	X		X		X		X		X	X
Receptive Risk Questionnaire			X[5]									
HIV Counseling		X	X[6]	X	X	X	X	X	X	X	X[7]	X
Capillary Blood Tube Collection (twice-weekly)[8]			X[9]									
Medical and Physical Exam[10]		X										X
Diagnostic specimen for suspected TB cases [11]			X	X	X	X	X	X	X	X	X	X
Laboratory[12]												
HIV Diagnostics[13]	Anti-Coagulant	10	10		10		10		10		10	10
CBC + differential, malaria[14]	Anti-Coagulant	2.7	2.7		2.7		2.7		2.7		2.7	NB[15]
T cell immunophentyoe (CD4/CD8/NK/B cells)			NB									
Serum Chemistry (Creatinine, ALT)	Coagulant	2.7										
RNA PCR	Anti-Coagulant	6	6		6		6		6		6	
HHV DNA quantification (in saliva)				X								
Research												
Syphilis (RPR/TPPA) /HSV	Anti-Coagulant	NB										
Hepatitis B, (CMV, EBV), HCV[20]	Anti-Coagulant	NB										
LPS	Anti-Coagulant	NB	NB		NB		NB		NB		NB	NB
Mucosal Collections[16]			X									
Hormone levels[17]	Coagulant		2.7									
Vaginal/rectal Swab Collection[18]			X									
Plasma/PBMC for Immunoassays	Anti-Coagulant	45										66
Serum for Immunoassays	Coagulant	10	10									
Host/Viral Genetics and GWAS	Anti-Coagulant	NB	NB		NB							
Viral Isolation			7.5									NB
PBMC for Gene Expression	Anti-Coagulant	NB										
Vector Antibody Testing		NB										
ICS		10									10	
On-site fresh HIV antigen specific flow cytometry[19]	Anti-Coag heparin		4				4				4	
Pre-Infection Control-Reposed	Anti-Coagulant		30		15		15		15		15	
Daily Volume (mL)		**86.4**	**72.9**	**NB**	**33.7**	**NB**	**37.7**	**NB**	**33.7**	**NB**	**47.7**	**76**
Microvette Blood Volume (mL)		0	0	9.6	14.4	14.4	14.4	14.4				
Cumulative Volume Total (mL)		86.4	159.3	168.9	217	231.4	283.5	297.9	331.6	331.6	379.3	

[1] Visit A may be conducted over two days within a 7-day window.

[2] Visit B may occur within 7 – 42 days of Visit A; all other visits have a visit window of ± 42 days. If a volunteer "misses" a large blood volume visit (LBV), the blood draw and questionnaire will be completed at the earliest opportunity. However, 2 LBVs should not occur within 6 weeks of each other and the next LBV may therefore need to be rescheduled within its window accordingly. If the volunteer returns only at the time of the next LBV, the missed LBV is recorded as missed, but blood and questionnaire will be completed for the current visit.

[3] Exit visit is for individuals who have had an LBV in the last six weeks, are not able to continue the study, but who do not want to withdraw consent. If last LBV within 2 weeks, exit visit will not include blood draw nor questionnaire

[4] Visit B only the vaccine knowledge and interest portion of the ACASI questionnaire is collected, if not collected at Visit A.

[5]Receptive Risk questionnaires will be collected at every SBV.

[6] At Visit B volunteers who may be infected receive counseling and the complete Visit B blood draw, and are asked to return for final results.

[7] HIV surveillance is halted for those volunteers who remain HIV negative at visit J (final visit).

[8] Stutter phase entry should be followed as described in Section 4.7 of the protocol. These visits will be tracked as follows: the first unscheduled visit after a new reactive Aptima test is Visit 1. Additionally, if any new reactive tests occur, the second round would follow as Visit 1a, the third round as 1b, etc

[9] Capillary blood tube collection will be collected via capillary puncture or venipuncture on a twice-weekly basis for one year starting at visit B and continuing through visit G. Thereafter, they will continue on a monthly basis.

[10] Visit A collect a full medical history and physical exam; at all other visits update medical history and directed physical exam.

11 Tanzania only: Specimen collected for tuberculosis (TB) diagnostic procedures (sputum, 25.5 mL of blood) will only be collected from subjects with suspected active TB.

[12] Blood volumes are actual volumes drawn into tubes.

[13] Sites may conduct Aptima at Visit A, and in case of an unscheduled visit in between the monthly visit

[14] Malaria tested only if participant febrile

[15] NB = no extra blood required

[16]Controls for mucosal collections will be solicited at the time new incident cases are identified. After that, mucosal collections will vary by site and incident infection number. A pregnancy test will be performed prior to mucosal collection for safety considerations. In addition, each site will be permitted to collect baseline samples on 20 women and 20 men. If the participant does not move out of phase I, their sample may be used as a control or discarded, based on the PI's discretion

[17] For females participating in mucosal component.

[18] At every SBV, for 4 months, self-administered vaginal or rectal swabs will be collected, depending on the most recent sexual practice of the individual. If the participant has not had sex since the previous swab, there will be no swab collected.

[19] Fresh whole blood for on-site flow cytometry to be done at visit B or the first LBV after visit B, visit F and visit J

[20] Hepatitis C testing may be done on archived samples from any time point

APPENDIX V: SCHEDULE OF EVALUATIONS #2, PHASE IB

VISIT [1]		1[9]	2	3	4	5	6	7	8	9
WEEK OF STUDY		0	0.5	1	1.5	2	2.5	3	3.5	4
DAY OF STUDY		0	3	7	10	14	17	21	24	28
Clinical										
Informed Consent										
Photo ID		check	check	check	check	check	check	check	check	check
Medical and Physical Exam[2]		X	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]
Laboratory										
CBC + differential, malaria [8]	Anti-Coagulant	2.7				2.7				2.7
T cell immunophentype (CD4/CD8/NK/B cells)		NB	NB	NB	NB	NB		NB		NB
Serum Chemistry (Creatinine, ALT)	Coagulant	2.7								
RNA PCR	Anti-Coagulant	10	10	10	10	10	10	10	10	6
HIV Diagnostics	Anti-Coagulant	6	6	6	6	6	NB	6	NB	NB
Research										
Hepatitis B, C [10]	Anti- Coagulant	NB [5]								
Syphilis(RPR/TPP)/HSV	Anti-Coagulant									
LPS	Anti-Coagulant	NB	NB	NB	NB	NB	NB	NB		NB
Mucosal Collection [3]		X		X						
Hormone Levels [4]	Coagulant	2.7		2.7						
Vaginal Swab Collection [7]		X	X	X	X	X	X	X		X
Saliva Collection		X	X	X	X	X	X	X	X	X
Urine Collection		X		X						
Plasma/PBMC for Immunoassays	Anti-Coagulant	15	7.5	15	15	75	7.5	7.5		15
Viral Isolation	Anti-Coagulant	7.5								
Viral Genetics	Anti-Coagulant	NB	NB	NB	NB	NB	NB	NB		NB
B cell responses	Anti-Coagulant		7.5	7.5	7.5	7.5	7.5	7.5		7.5
PBMC for Gene Expression	Anti-Coagulant	7.5				NB				NB
Smart Tube (ST)		2		2						
Humoral assays[6]	Coagulant	4		4		4	4	4		4
Daily Volume (mL)		60.1	31	47.2	38.5	105.2	29	35	10	35.2
Cumulative Vol (mL)		60.1	91.1	138.3	176.8	282	311	346	356	391.2

[1] The visit window for all stutter phase visits is -1 day to +2 days

[2] Visit 1 collect a full medical history and physical exam; at all other visits update medical history and directed physical exam.

[3] Samples may be collected twice during stutter at visit 2 or 3 and again at the first visit after EIA reactivity is identified. Other mucosal collections such as breast milk, oral secretions, urine and semen can be collected at 2 time points.

[4] Only for females participating as negative controls for mucosal component

[5] NB: no extra blood (will be included in the volume collected for the chemistries)

[6] Humoral immunomonitoring aliquoted from the 30mL drawn for Storage and additional testing. No exra blood required.

[7] vaginal swab collections may be done up to, but not exceeding every visit

[8] participant will be tested for malaria only if febrile

[9] if western blot is positive at visit 1 or 2 (Fiebig IV, V or VI), participant moves directly to SOE 3 and skips remainder of stutter phase

[10]Hepatitis C testing may be done on archived samples from any time point

APPENDIX VI: SCHEDULE OF EVALUATIONS #3, PHASE II

VISIT		10	11	12	13	14	15	16	17	18[1]	Exit Visit
WEEK OF STUDY		5	6	8	12	24	36	48	60		
DAY OF STUDY		35 ± 3 days	42 ± 3 days	56 ± 7 days	84 ± 14 days	168 ± 28 days	252 ± 28 days	336 ± 28 days	420 ± 28 days		
Clinical											
Informed Consent		X									
Photo ID		check	check	check	check	check	check	check	check	check	check
Medical and Physical Exam[2]		[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	X
AHI Questionnaire		X									
Laboratory [6]											
CBC + differential, malaria[3]	Anti-Coagulant		NB[4]		NB		NB				NB
RNA PCR	Anti-Coagulant		10		10	10	10	10	10	10	10
HIV Diagnostics	Anti-Coagulant		NB		NB	NB	NB	NB	NB	NB	NB
T cell immunophenotype (CD4/CD8/NK/B cells)			NB		NB		NB			NB	NB
Research											
Syphilis(RPR/TPPA)/ HSV-2	Anti-Coagulant		NB								
LPS	Anti-Coagulant		NB		NB	NB	NB	NB	NB	NB	NB
Mucosal Collection [7]					X	X	X	X	X		X
Hormone levels [5]	Coagulant				2.7	2.7	2.7	2.7	2.7		2.7
Saliva collection		X	X	X	X	X	X	X	X	X	X
Urine Collection					X	X	X	X	X		X
Plasma/PBMC for Immunoassays	Anti-Coagulant		60		75	15	15	75	15	15	15
Serum for Immunoassays	Coagulant		4		10			10			
Viral Isolation	Anti-Coagulant										
Viral Genetics	Anti-Coagulant		NB		NB	NB	NB	NB	NB	NB	NB
B cell responses	Anti-Coagulant		7.5		7.5	7.5	7.5	7.5	7.5	7.5	7.5
PBMC for Gene Expression	Anti-Coagulant										
Smart Tube (ST)					2	2					
Daily Volume (mL)		0	81.5	0	107.2	37.2	35.2	105.2	35.2	32.5	35.2
Cumulative Volume (mL)		391.2	472.7	472.7	579.9	617.1	652.3	757.5	792.7	825.2	860.4
Running 56 day total				470.7							

[1] After six months, the volunteer will be seen every 3 months—for at least 50 months
[2] Visit 10 and Exit Visit collect a full medical history and physical exam; at all other visits update medical history and directed physical exam.
[3] Malaria tested only if participant febrile
[4] NB = no extra blood required
[5] Only for females participating in mucosal component
[6] Hepatitis C testing may be done on archived samples from any time point
[7] A pregnancy test will be performed prior to mucosal collection for safety considerations.

APPENDIX VII: BASELINE QUESTIONNAIRE

Baseline 1.5 (English)

Q1. Study Number _ _ _ _ _

Q2. Site _

Welcome to this computer interview. Before you start, we would like to explain the interview process. As explained to you during screening, your participation in this interview and every aspect of the research is voluntary. You may skip any question that you prefer not to answer, but we would appreciate if you answered all the questions. These questions may be somewhat sensitive, but they are important to the success of this study. You may seek clarification for any question you do not understand or decide to stop the interview at any time.

Any information you provide for this study will be kept confidential and will not be shared with any individual, including your employer/boss, spouse, friends, or relatives. This information will be identified only by a number, not by your name.

If you have any questions please contact study staff.

Thank you. Let us begin the questions.

On the next several screens you will be given instructions on completing the questionnaire and practice questions to answer.

On the right hand side of this computer screen, there are three squares called buttons.

The button in the middle says, "Next Question". This button is used to move to the next screen after you complete the current question or information screen.

You should use your mouse to click the buttons during the interview.

Please click on "Next Question" now with your mouse.

On the right hand side there is also a "Previous Question" button. You can use this button to view or change the answers to previous questions.

The last button on the bottom right hand side is the "Repeat the Question" button. If you click on this button, the question will be repeated. Try this now then move on to the next screen.

This questionnaire contains different types of questions.

In the following screens, we will show examples of each type of question.

Practice Questions

PP1. Some questions will ask you to choose yes or no.

For Example:

Do you drink milk? Choose Yes or No.

1 Yes
0 No
9 Don't Know
8 Refuse to Answer

PP2. Some questions will ask you to choose **ONE** answer from a list of answers. These answers appear in boxes and each box has a different answer written in it. When a box has your answer in it, click on that box.

For Example:
Apple starts with what letter? (Choose one)

1 A
2 B
3 C
4 D
9 Don't Know
8 Refuse to Answer

If PP2 is not equal to 1 then Please correct your response. and skip to PP2.

The previous question asked you to choose one answer. However, some questions will ask you to choose all answers that apply to you. The following screen has an example of this type of question.

PP3. Please answer the following question: Which of the following fruits do you like? (MARK ALL ANSWERS THAT APPLY TO YOU)

To "un-mark" a response, click on the response again. After you have chosen your answers, click the "Next Question" button to move to the next screen. (Check all that apply)

__ APPLES
__ PEACHES
__ BANANAS
__ BLUEBERRIES
__ MANGOES
__ ORANGES
__ GRAPEFRUIT
__ Don't Know
__ Refuse to Answer

PP4. Some questions will ask you to select the 'most important' item from answers you selected on a previous question. Only select a button that is not blank.

Please select your favorite kind of fruit. (Choose one)

01	[:nw]&[AVP3A]
02	[:nw]&[AVP3B]
03	[:nw]&[AVP3C]
04	[:nw]&[AVP3D]
05	[:nw]&[AVP3E]
06	[:nw]&[AVP3F]
07	[:nw]&[AVP3G]
99	Don't Know
98	Refuse to Answer

If PP3A is equal to 0 and PP4 is equal to 1 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

If PP3B is equal to 0 and PP4 is equal to 2 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

If PP3C is equal to 0 and PP4 is equal to 3 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

If PP3D is equal to 0 and PP4 is equal to 4 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

If PP3E is equal to 0 and PP4 is equal to 5 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

If PP3F is equal to 0 and PP4 is equal to 6 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

If PP3G is equal to 0 and PP4 is equal to 7 then This is not a valid selection. Please select one of the labeled buttons. and skip to PP4.

PP5. Some questions will ask you to give a number for an answer. For example, how many days are in a week? Click the box with the number "7" below. When you are done, click "Next Question."

— —

99	Don't Know
98	Refuse to Answer

If PP5 is not equal to 7 then Please correct your response. and skip to PP5.

PP6. If you enter the wrong number and want to change it, click the "Clear" button. Try this now by entering a number, then click the "Clear" button. When the number is erased, enter another number. Then click "Next Question."

— — — —

9999	Don't Know
9998	Refuse to Answer

Last, there may be some questions which you don't know the answers to or that you do not want to answer. The next screen shows you where these buttons are.

PP7. On the right hand side of the screen near the top are the "Don't Know" and "Refuse to Answer" buttons. Try any of these buttons now by clicking on one of them.

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If PP7 is equal to 1 or PP7 is equal to 0 then Please correct your response. and skip to PP7.

This is the end of the practice section. Next, you will begin the interview. If you have any questions now or during the interview please ask the interviewer to assist you.

To move on to the next screen, click the button that says "Next Question".

Section A: Demographic and Background Information

A1. Are you male, female, or transgender? (Choose one)

1	Male
2	Female
3	Transgender

A2. How did you first get interested in this study? (Choose one)

1	FROM A PERSON
2	FROM MEDIA/PRINT
9	Don't Know
8	Refuse to Answer

If A2 is equal to 2, then skip to A2b.

A2a. Who first got you interested in this study? (Choose one)

1	Staff member
2	Community Leader
3	Health Care Worker
4	Other Volunteer
5	Friend/Family/Work Colleague
9	Don't Know
8	Refuse to Answer

Skip to A3a.

A2b. What first got you interested in this study? (Choose one)

1	INFORMATION PAMPHLET/SEMINAR
2	FLIER
3	POSTER
4	NEWSPAPER/MAGAZINE
5	INTERNET
6	RADIO
9	Don't Know
8	Refuse to Answer

A3a. What is/are the reason(s) for deciding to take part in this study? (Check all that apply)

__ I WANT TO KNOW MY HIV STATUS
__ PRE-MARITAL TESTING
__ PLANNING FOR THE FUTURE
__ I NEED A BLOOD TEST
__ I WANT TO DO SOMETHING GOOD POSITIVE FOR MY COMMUNITY OR COUNTRY
__ I WAS FORCED TO COME TO THIS STUDY
__ I WANT TO RECEIVE MEDICAL CARE AND TREATMENT
__ I WANT TO RECEIVE MONEY
__ Other
__ Don't Know
__ Refuse to Answer

If A3aF is not equal to 1, then skip to instruction before A3b.

A3aa. You answered that you were FORCED to come to this study, is this correct?

1 Yes
0 No
9 Don't Know
8 Refuse to Answer

If A3aa is equal to 1, then skip to end of questionnaire.

If A3aa is equal to 0 then skip to A3a.

A3b. Of the reason(s) you have stated, what was the most important for deciding to take part in this study? (Choose One) (Choose one)

01 [:nw]&[RSNA]
02 [:nw]&[RSNB]
03 [:nw]&[RSNC]
04 [:nw]&[RSND]
05 [:nw]&[RSNE]
06 [:nw]&[RSNF]
07 [:nw]&[RSNG]
08 [:nw]&[RSNH]
99 Don't Know
98 Refuse to Answer

If A3aA is equal to 0 and A3b is equal to 1 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aB is equal to 0 and A3b is equal to 2 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aC is equal to 0 and A3b is equal to 3 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aD is equal to 0 and A3b is equal to 4 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aE is equal to 0 and A3b is equal to 5 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aF is equal to 0 and A3b is equal to 6 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aG is equal to 0 and A3b is equal to 7 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

If A3aH is equal to 0 and A3b is equal to 8 then This is not a valid selection. Please select one of the labeled buttons. and skip to A3b.

A4a. What country have you lived in most of your life? (Choose one)

1	UGANDA
2	KENYA
3	TANZANIA
4	THAILAND
5	OTHER
9	Don't Know
8	Refuse to Answer

If A4a is not equal to 1, then skip to instruction before A4b2.

A4b1. What region of [Response to A4a] have you lived in most of your life? (Choose one)

1	WEST NILE
2	ACHOLI-LA
3	WESTERN (
4	BUGANDA
5	NORTH-EAS
6	SOUTH-EAS
9	Don't Know
8	Refuse to An

If A4a is not equal to 2, then skip to instruction before A4b3.

A4b2. What region of [Response to A4a] have you lived in most of your life? (Choose one)

01	RIFT VALLEY
02	WESTERN
03	NYANZA
04	CENTRAL
05	NAIROBI
06	COAST
07	EASTERN
08	NORTH EASTERN
99	Don't Know
98	Refuse to Answer

If A4a is not equal to 3, then skip to instruction before A4b4.

A4b3. What region of [Response to A4a] have you lived in most of your life? (Choose one)

01	Sourthern Highland Zone(Mbeya, Iringa, Ruvuma, Rukwa)
02	Central Zone(Dodoma, Singida, Tabora)
03	Southern Zone(Mtwara, Lindi)
04	Coastal Zone(DSM, Pwani, Tanga, Morogoro)
05	Lake Zone(Mwanza, Kigoma, Kagera, Shinyanga)
06	Northern Zone(Kilimanjaro, Arusha, Manyara)
07	Zanzibar(Pemba, Unguja)
99	Don't Know
98	Refuse to Answer

If A4a is not equal to 4, then skip to A5.

A4b4. What region of [Response to A4a] have you lived in most of your life? (Choose one)

1	NORTH
2	NORTHEAST
3	WEST
4	CENTRAL
5	EAST
6	SOUTH
9	Don't Know
8	Refuse to Answer

A5. How long have you lived in your current region of residence? (Choose one)

0	0-6 months
1	6 months to 1 year
2	1-2 years
3	2-5 years
4	Greater than 5 years
9	Don't Know
8	Refuse to Answer

A6. What is your religious affiliation? (Choose one)

01	CATHOLIC
02	PROTESTANT
03	MUSLIM
04	SEVENTH DAY ADVENTIST
05	BORN AGAIN CHRISTIAN
06	JEHOVAH WITNESS
07	TRADITIONAL RELIGION
08	BUDDHIST
09	NO RELIGION
10	OTHER
99	Don't Know
98	Refuse to Answer

A7. What is your highest level of education? (Choose one)

00	NONE
01	PRIMARY NOT COMPLETED
02	PRIMARY COMPLETED
03	O'LEVEL NOT COMPLETED
04	O'LEVEL COMPLETED
05	A'LEVEL NOT COMPLETED
06	A'LEVEL COMPLETED
07	POST-SECONDARY COLLEGE/ POLYTECHNIC NOT COMPLETED
08	POST-SECONDARY COLLEGE/POLYTECHNIC COMPLETED
09	UNIVERSITY NOT COMPLETED
10	UNIVERSITY GRADUATE
99	Don't Know
98	Refuse to Answer

For the following questions:

HOUSEHOLD IS DEFINED AS PERSON(S) LIVING WITHIN ONE HOMESTEAD.

HOUSEHOLD MEMBER IS SOMEONE THAT HAS BEEN LIVING IN YOUR HOUSEHOLD FOR AT LEAST A MONTH PRIOR TO THIS INTERVIEW.

A8. How many other people do you live with in your household?

__ __ __	
999	Don't Know
998	Refuse to Answer

If A8 is equal to 0, then skip to instruction before A10.

A9. What is your relationship to those you live with? (Check all that apply)

__	PARTNER/SPOUSE
__	CHILDREN
__	BROTHER/SISTER
__	MOTHER/FATHER
__	OTHER RELATIVE
__	FRIEND
__	CO-WORKERS
__	HOUSE HELPER
__	Don't Know
__	Refuse to Answer

For the following questions:

NEVER MARRIED= YOU HAVE NEVER BEEN MARRIED OR COHABITATED WITH SOMEONE

MARRIED= IT CAN BE A RELIGOUS OR GOVERNMENT MARRIAGE OR BASED ON CUSTOMARY LAW

COHABITATING=YOU HAVE BEEN LIVING WITH SOMEONE OF THE SAME OR OPPOSITE SEX AS SPOUSES FOR AT LEAST SIX MONTHS, BUT ARE NOT "LEGALLY" MARRIED THROUGH EITHER CIVIL, CUSTOMARY OR RELIGIOUS LAW

SPOUSE= YOUR HUSBAND OR WIFE, WHETHER LEGALLY MARRIED OR COHABITATING

DIVORCED/SEPARATED= ANY MARRIAGE OR COHABITATION THAT HAS ENDED

WIDOWED= SPOUSE (MARRIED OR COHABITATING) HAS DIED

A10. What is your present marital status? (Choose one)

01	MARRIED, ONE SPOUSE
02	MARRIED, MORE THAN ONE SPOUSE
03	COHABITATING, ONE SPOUSE
04	COHABITATING, MORE THAN ONE SPOUSE
05	SEPARATED
06	DIVORCED
07	WIDOWED
08	NEVER MARRIED OR COHABITED
99	Don't Know
98	Refuse to Answer

If A10 is equal to 8, then skip to B1a.

A11. How many times have you gotten married or lived/cohabitated with a sexual partner?

— —	
99	Don't Know
98	Refuse to Answer

If A4a is equal to 4, then skip to instruction before A13.

IN MANY CULTURES, WHEN A MAN DIES, HIS WIFE IS "GIVEN" TO THE NEAREST MALE RELATIVE, WHO IS EXPECTED TO CARE FOR HER AND THE DECEASED MAN'S CHILDREN. THE INHERITED WOMAN IS EXPECTED TO HAVE SEXUAL INTERCOURSE WITH THE MAN WHO INHERITS HER.

A12. Have you ever inherited a wife/been inherited as a wife?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If A10 is equal to 6 or A10 is equal to 7, then skip to B1a.

A13. If currently married or cohabitating with a sexual partner, for how long have you lived in this union? (Choose one)

0	0-6 months
1	6 months to 1 year
2	1-2 years
3	2-5 years
4	More than 5 years
9	Don't Know
8	Refuse to Answer

A14. There are some couples that live apart due to the nature of the wife's/husband's/partner's work or for some other reason. Is this the case with you?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If A14 is not equal to 1, then skip to instruction before B1a.

A15. Are you living apart temporarily or have you stopped living together permanently? (Choose one)

1	STOPPED FOR GOOD
2	AWAY FOR THE TIME BEING
9	Don't Know
8	Refuse to Answer

You have completed the first section of the questionnaire. We will now begin the second section.

Section B: Work

B1a. What is the occupation from which you earn most of your income? (Choose one)

1	NOT WORKING
2	PUPIL/STUDENT
3	ENTERTAINMENT/SERVICE
4	RESTAURANT/BAR/HOTEL/TRADE BUSINESS
5	DRIVER/LABORER
6	OFFICE/HEALTH CARE/MILITARY/POLICE
9	Don't Know
8	Refuse to Answer

If B1a is equal to 1 or B1a is equal to 2 or B1a is equal to 8 or B1a is equal to 9, then skip to instruction before B4.

If B1a is equal to 4, then skip to instruction before B1ac.

If B1a is equal to 5, then skip to instruction before B1ad.

If B1a is equal to 6, then skip to instruction before B1ae.

B1ab. You specified [Response to B1a], which response below best describes your occupation? (Choose one)

1	Bar, Karaoke Worker
2	Sex Worker
3	Entertainer/Performer
4	Massage Parlor Worker
5	Hairdresser/Manicure
6	Other
9	Don't Know
8	Refuse to Answer

If B1a is equal to 3, then skip to B1b.

B1ac. You specified [Response to B1a], which response below best describes your occupation? (Choose one)

01	Guesthouse Attendant
02	Restaurant/Hotel Worker
03	Bar Owner or Manager
04	Business/Trader
05	Local Brew Seller
06	Food Vendor
07	Home Brewing
08	Other
99	Don't Know
98	Refuse to Answer

If B1a is equal to 4, then skip to B1b.

B1ad. You specified [Response to B1a], which response below best describes your occupation? (Choose one)

01	Local Driver (within country)
02	Long Distance (transnational)
03	Domestic Employee
04	Motorcyclist/Boda-Boda/Songtaew
05	Transport
06	Fishing
07	Farming
08	Laborer
09	Factory Worker
10	Other
99	Don't Know
98	Refuse to Answer

If B1a is equal to 5, then skip to B1b.

B1ae. You specified [Response to B1a], which response below best describes your occupation? (Choose one)

1	NGO Employee
2	Health Care Provider
3	Office Worker
4	Military/Police
5	Other
9	Don't Know
8	Refuse to Answer

B1b. Do you have another occupation?

1	Yes	
0	No	***Skip to B2***
9	Don't Know	
8	Refuse to Answer	

B1c. What is your second most income-generating occupation? (Choose one)

1	PUPIL/STUDENT
2	ENTERTAINMENT/SERVICE
3	RESTAURANT/HOTEL BUSINESS
4	DRIVER/LABORER
5	OFFICE/HEALTH CARE/MILITARY/POLICE
9	Don't Know
8	Refuse to Answer

B2. How long have you been working at your occupation where you make the most money? (Choose one)

0	0-6 months
1	6 months- 1 year
2	1- 2 years
3	2-5 years
4	More than 5 years
9	Don't Know
8	Refuse to Answer

B3. What is your approximate average monthly income in local currency? (Include all sources, but do not include spouses' income)

———————	
9999999	Don't Know
9999998	Refuse to Answer

If A10 is equal to 6 or A10 is equal to 7 or A10 is equal to 8 or A10 is equal to 98 or A10 is equal to 99, then skip to instruction before C1.

B4. If you have a spouse, what is the most income-generating occupation of your spouse? (Choose one)

1	NOT WORKING
2	PUPIL/STUDENT
3	ENTERTAINMENT/SERVICE
4	RESTAURANT/HOTEL BUSINESS
5	DRIVER/LABORER
6	OFFICE/HEALTH CARE/MILITARY/POLICE
9	Don't Know
8	Refuse to Answer

You have completed the second section of the questionnaire. We will now begin the third section. This section is about pregnancy and birth control.

Baseline 1.5

Section C: Contraception and Birth Control

If A1 is equal to 3, then skip to C5.

If A1 is equal to 1, then skip to C2.

C1. Are you currently pregnant?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

C2. During the past 3 months did you or your sexual partner do anything to avoid pregnancy? (Choose one)

0	NO	***Skip to instruction before C4***
1	YES	
2	NEVER HAD SEX	
9	Don't Know	***Skip to instruction before C4***
8	Refuse to Answer	***Skip to instruction before C4***

If C2 is equal to 0 or C2 is equal to 2, then skip to instruction before C4.

C3. During the past 3 months, which methods did you or your partner use to delay/avoid pregnancy/having children? (Check all that apply)

__ HORMONAL CONTRACEPTION (Pill, Injectables,Implant,Emergency Pill)
__ INUTERINE CONTROL DEVICE
__ ME AND/OR MY PARTNER ARE STERILIZED
__ DIAPHRAGM/FOAM
__ MALE CONDOM
__ FEMALE CONDOM
__ WITHDRAWAL
__ RHYTHM/CALENDAR
__ USING HERBS
__ TYING STRINGS ON THE BODY
__ ABORTION
__ OTHER
__ Don't Know
__ Refuse to Answer

If C2 is equal to 1 or C2 is equal to 2, then skip to instruction before C5.

C4. What are the reasons for not using any method? (Check all that apply)

__ DO NOT KNOW ANY METHOD
__ CANNOT AFFORD TO PAY FOR BIRTH CONTROL SERVICES
__ BIRTH CONTROL SERVICES NOT AVAILABLE IN MY COMMUNITY
__ MY SPOUSE/SEXUAL PARTNER DOES NOT APPROVE
__ PARTNER REFUSED TO USE CONDOM(S)
__ FEAR SIDE EFFECTS
__ I WANT TO GET PREGNANT/FATHER A CHILD
__ IT IS AGAINST MY RELIGIOUS BELIEF
__ Don't Know
__ Refuse to Answer

If A4a is not equal to 4, then skip to instruction before D1a&C.

If A1 is not equal to 3, then skip to instruction before D1a&C.

C5. Where are you in the transition from male to female? (Check all that apply)

__ NONE
__ LIVED IN CROSS-GENDER ROLE/DRESSED AS A WOMAN
__ HORMONES
__ PSYCHOLOGICAL EVALUATION/COUNSELING
__ GENITAL (SEX REASSIGNMENT SURGERY)
__ OTHER
__ Don't Know
__ Refuse to Answer

Section D: Sexuality

We are now on Section D. We will now ask you some questions about your relationship with the person or people with whom you have had sexual relations (even if you are no longer seeing them).

When we ask you about persons you have had sex with, please respond with all partners: male, female and transgender. We understand that this is a sensitive and personal topic. However, it is important for the purpose of this study that you are as open and honest as possible. Again, we want to assure you that anything you say will be kept confidential.

For this section the following definitions apply:

For all questions, "sex", "sexual contact", "having sex" or "playing sex" refers only to vaginal or anal sexual intercourse (receptive or insertive).
"Oral sex", or "mutual masturbation" are not considered "having sex" or "playing sex".

For all questions, "unprotected sex" is sex without a a barrier such as a condom.

For this section the following definitions apply:

When asked about Steady Partners, steady partners can include:
Someone with whom you are involved in an ongoing or regular sexual relationship, such as a boyfriend/girlfriend, a spouse/cohabitating partner, or a sugar daddy/mummy.

When asked about Casual Partners, casual partners can include:
Someone who is not your steady partner with whom you are not romantically involved but with whom you have sex with once or on an occasional basis by mutual consent where no money, goods, or services are exchanged, for example, a stranger or a workmate.

When asked about Customer/Client Partners, customer/client partners include:
Sexual contact with a person in which you receive or give money, goods, or services in exchange for sex, in the absence of a romantic relationship or emotional attachment, for example a sex worker or a sex client.

When asked about Other Partners, other partners can include:
Someone who is not your steady, casual, or customer/client partner but with whom you have had sex with by mutual consent or force, such as a boss, teacher, rapist, relative other than spouse, work subordinate, or student.

D1a. Have you ever had sex with a [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D1a&C is equal to 0 or D1a&C is equal to 8, then skip to next category.

D1b. How old were you when you first had/played sex with your [Female partner(s), Male partner(s), ...]?

— — — —

9999	Don't Know
9998	Refuse to Answer

D1c. Who was the first [Female partner(s), Male partner(s), ...] you had/played sex with? (Choose one)

1	STEADY PARTNER
2	CASUAL PARTNER
3	CUSTOMER/CLIENT PARTNER
4	OTHER PARTNER
9	Don't Know
8	Refuse to Answer

If D1c&C is not equal to 1, then skip to instruction before D1c2&C.

D1c1. You specified a steady [Female partner(s), Male partner(s), ...], which response below best describes your partner? (Choose one)

1	Boy/Girlfriend
2	Spouse/Cohabitating Partner
3	Sugar Daddy/Mummy
4	Other Steady Partner
9	Don't Know
8	Refuse to Answer

If D1c&C is not equal to 2, then skip to instruction before D1c3&C.

D1c2. You specified a casual [Female partner(s), Male partner(s), ...] , which response below best describes your partner? (Choose one)

1	Stranger
2	Workmate
3	Other Casual Sex Partner
9	Don't Know
8	Refuse to Answer

If D1c&C is not equal to 3, then skip to instruction before D1c4&C.

D1c3. You specified a customer/client [Female partner(s), Male partner(s), ...] , which response below best describes your partner? (Choose one)

1	Sex Worker
2	Sex Client
9	Don't Know
8	Refuse to Answer

If D1c&C is not equal to 4, then skip to D1d.

Baseline 1.5

D1c4. You specified any other [Female partner(s), Male partner(s), ...] , which response below best describes your partner? (Choose one)

01	Boss/Work Supervisor
02	Teacher
03	Relative Other than Spouse
04	Student
05	Other Authority Figure
06	Employee/Work Subordinate
07	Rapist
99	Don't Know
98	Refuse to Answer

D1d. In the past 3 months, how many different [Female partner(s), Male partner(s), ...] have you had unprotected vaginal and/or anal intercourse with? (Choose one)

0	1-2 partners
1	3-10 partners
2	More than 10 partners
9	Don't Know
8	Refuse to Answer

D2a. In the past 3 months, have you had sex with a steady [Female partner(s), Male partner(s), ...]? (A steady partner includes a boyfriend/girlfriend, spouse/cohabitating partner, sugar daddy/mummy)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D2a&C is equal to 0 or D2a&C is equal to 8, then skip to D3a.

D2b. In the past 3 months, how many <u>times</u> did you play/have sex with your steady [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D2c. In the past 3 months, how <u>many different steady [Female partner(s), Male partner(s), ...]</u> have you had/played sex with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D2d. In the past 3 months, how many of your steady [Female partner(s), Male partner(s), ...] did you have sex with for the first time? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D2e. During the last time you had/played sex with your steady [Female partner(s), Male partner(s), ...], did you use a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D2f. In the past 3 months, how often did you use a condom when you had/played sex with your steady [Female partner(s), Male partner(s), ...]? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D3a. In the past 3 months, have you had sex with a casual [Female partner(s), Male partner(s), ...]? (Casual includes a stranger, workmate, or other casual partner)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D3a&C is equal to 0 or D3a&C is equal to 8, then skip to D4a.

D3b. In the past 3 months, how many <u>times</u> did you play/have sex with your casual [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D3c. In the past 3 months, how <u>many different</u> casual [Female partner(s), Male partner(s), ...] have you had/played sex with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D3d. In the past 3 months, how many of your casual [Female partner(s), Male partner(s), ...] did you have sex with for the first time? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D3e. During the last time you had/played sex with your casual [Female partner(s), Male partner(s), ...], did you use a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D3f. In the past 3 months, how often did you use a condom when you had/played sex with your casual [Female partner(s), Male partner(s), ...]? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D4a. In the past 3 months, have you had sex with a customer/client [Female partner(s), Male partner(s), ...]?
(Customer/client includes a sex worker or a sex client)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D4a&C is equal to 0 or D4a&C is equal to 8, then skip to D5a.

D4b. In the past 3 months, how many times did you play/have sex with your customer/client [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D4c. In the past 3 months, how many different customer/client [Female partner(s), Male partner(s), ...] have you had/played sex with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D4d. In the past 3 months, how many of your customer/client [Female partner(s), Male partner(s), ...] did you have sex with for the first time? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D4e. During the last time you had/played sex with your customer/client [Female partner(s), Male partner(s), ...], did you use a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D4f. In the past 3 months, how often did you use a condom when you had/played sex with your customer/client [Female partner(s), Male partner(s), ...]? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D5a. In the past 3 months, have you had sex with other [Female partner(s), Male partner(s), ...]? (Other includes boss/work supervisor, teacher, other authority figure and relative other than spouse, student, employee/work subordinate, or rapist)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D5a&C is equal to 0 or D5a&C is equal to 8, then skip to D6.

D5b. In the past 3 months, how many times did you play/have sex with your other [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D5c. In the past 3 months, how many other different [Female partner(s), Male partner(s), ...] have you had/played sex with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D5d. In the past 3 months, how many of your other [Female partner(s), Male partner(s), ...] did you have sex with for the first time? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D5e. During the last time you had/played sex with your other [Female partner(s), Male partner(s), ...], did you use a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D5f. In the past 3 months, how often did you use a condom when you had/played sex with your other [Female partner(s), Male partner(s), ...]? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D6. In the past 3 months, how often did you have sex with your [Female partner(s), Male partner(s), ...] after you had been drinking beer or any other alcoholic drink(s)? (Choose one) 1 ALW

2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D7. In the past 3 months, did you receive money or other benefits in exchange for vaginal or anal sex with a [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D7&C is equal to 0 or D7&C is equal to 8, then skip to D8.

D7a. In the past 3 months, how many times have you received money or other goods in exchange for sex with your [Female partner(s), Male partner(s), ...]? (Choose one) 1 1-5 TIMES

2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D8. In the past 3 months, did you pay/give money or other benefits in exchange for sex with a [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D8&C is equal to 0 or D8&C is equal to 8, then skip to D9.

D8a. In the past 3 months, how many times have you given or paid money or other benefits in exchange for sex with a [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 times
2	6-10 times
3	More than 10 times
9	Don't Know
8	Refuse to Answer

D9. In the past 3 months, have you had sex with a non-spouse/non-cohabiting [Female partner(s), Male partner(s), ...] older than you by approximately 10 years or more?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D9&C is equal to 0 or D9&C is equal to 8 or D9&C is equal to 9, then skip to D10.

D9a. In the past 3 months, how many times have you had sex with a non-spouse/cohabitating [Female partner(s), Male partner(s), ...] older than you by approximately 10 years or more? (Choose one)

1	1-5 T
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D10. In the past 3 months, have you had vaginal or anal sex with any [Female partner(s), Male partner(s), ...] whom you know was infected with HIV?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D10&C is equal to 0 or D10&C is equal to 8 or D10&C is equal to 9, then skip to D11.

D10a. How often did you use a condom when you had/played sex with the [Female partner(s), Male partner(s), ...] you knew or suspected was infected with HIV? (Choose one)

1	ALW
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D11. In the past 3 months, have you had sex with any of the following [Female partner(s), Male partner(s), ...], Bar/Pub/Karaoke Worker, Someone diagnosed with an STI, Boda-Boda driver, Long distance truck driver, sex worker?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D11&C is equal to 0, then skip to D12.

D11a. Did you use a condom the last time you had sex with the [Female partner(s), Male partner(s), ...] mentioned in the previous question?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D12. In the past 3 months, have you been forced to have sex with any [Female partner(s), Male partner(s), ...] without your agreement or consent?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

	Female	Male	Transgender
D1a	|__|	|__|	|__|
D1b	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|
D1c	|__|	|__|	|__|
D1c1	|__|	|__|	|__|
D1c2	|__|	|__|	|__|
D1c3	|__|	|__|	|__|
D1c4	|__|__|	|__|__|	|__|__|
D1d	|__|	|__|	|__|
D2a	|__|	|__|	|__|
D2b	|__|	|__|	|__|
D2c	|__|	|__|	|__|
D2d	|__|	|__|	|__|
D2e	|__|	|__|	|__|
D2f	|__|	|__|	|__|
D3a	|__|	|__|	|__|
D3b	|__|	|__|	|__|
D3c	|__|	|__|	|__|
D3d	|__|	|__|	|__|
D3e	|__|	|__|	|__|
D3f	|__|	|__|	|__|
D4a	|__|	|__|	|__|
D4b	|__|	|__|	|__|
D4c	|__|	|__|	|__|
D4d	|__|	|__|	|__|
D4e	|__|	|__|	|__|
D4f	|__|	|__|	|__|
D5a	|__|	|__|	|__|
D5b	|__|	|__|	|__|
D5c	|__|	|__|	|__|
D5d	|__|	|__|	|__|
D5e	|__|	|__|	|__|
D5f	|__|	|__|	|__|
D6	|__|	|__|	|__|
D7	|__|	|__|	|__|
D7a	|__|	|__|	|__|
D8	|__|	|__|	|__|
D8a	|__|	|__|	|__|
D9	|__|	|__|	|__|

D9a	|__|	|__|	|__|
D10	|__|	|__|	|__|
D10a	|__|	|__|	|__|
D11	|__|	|__|	|__|
D11a	|__|	|__|	|__|
D12	|__|	|__|	|__|

D13. In the past 3 months, have you had anal intercourse with a [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D13&C is equal to 0 or D13&C is equal to 8, then skip to next category.

D13a. In the past 3 months, have you had anal intercourse with a steady [Female partner(s), Male partner(s), ...]?
(A steady partner includes a boyfriend/girlfriend, spouse/cohabitating partner, sugar daddy/mummy)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D13a&C is equal to 0 or D13a&C is equal to 8, then skip to D14a.

D13b. In the past 3 months, how many different steady [Female partner(s), Male partner(s), ...] have you had/played anal intercourse with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D13c. The last time you had receptive anal sex with a steady [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD RECEPTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D13d. The last time you had insertive anal sex with a steady [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD INSERTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D14a. In the past 3 months, have you had anal sex with a casual [Female partner(s), Male partner(s), ...]? (Casual includes a stranger, workmate, or other casual partner)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D14a&C is equal to 0 or D14a&C is equal to 8, then skip to D15a.

D14b. In the past 3 months, how many different casual [Female partner(s), Male partner(s), ...] have you had/played anal intercourse with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D14c. The last time you had receptive anal sex with a casual [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD RECEPTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D14d. The last time you had insertive anal sex with a casual [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD INSERTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D15a. In the past 3 months, have you had anal sex with a customer/client [Female partner(s), Male partner(s), ...]?
(Customer/client includes a sex worker or a sex client)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D15a&C is equal to 0 or D15a&C is equal to 8, then skip to D16a.

D15b. In the past 3 months, how many different customer/client [Female partner(s), Male partner(s), ...] have you had/played anal intercourse with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D15c. The last time you had receptive anal sex with a customer/client [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD RECEPTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D15d. The last time you had insertive anal sex with a casual [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD INSERTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D16a. In the past 3 months, have you had anal sex with any other [Female partner(s), Male partner(s), ...]?
(Other includes boss/work supervisor, teacher, other authority figure and relative other than spouse, student, employee/work subordinate, or rapist)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D16a&C is equal to 0 or D16a&C is equal to 8, then skip to instruction before D17.

D16b. In the past 3 months, how many different other [Female partner(s), Male partner(s), ...] have you had/played anal intercourse with? (Choose one)

0	1-2 partners
1	3-10 partners
2	more than 10 partners
9	Don't Know
8	Refuse to Answer

D16c. The last time you had receptive anal sex with any other [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD RECEPTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

D16d. The last time you had insertive anal sex with any other [Female partner(s), Male partner(s), ...] did you use a condom? (Choose one)

0	NO
1	YES
2	NEVER HAD INSERTIVE ANAL SEX
9	Don't Know
8	Refuse to Answer

	Female	Male	Transgender
D13	|__|	|__|	|__|
D13a	|__|	|__|	|__|
D13b	|__|	|__|	|__|
D13c	|__|	|__|	|__|
D13d	|__|	|__|	|__|
D14a	|__|	|__|	|__|
D14b	|__|	|__|	|__|
D14c	|__|	|__|	|__|
D14d	|__|	|__|	|__|
D15a	|__|	|__|	|__|
D15b	|__|	|__|	|__|
D15c	|__|	|__|	|__|
D15d	|__|	|__|	|__|
D16a	|__|	|__|	|__|
D16b	|__|	|__|	|__|
D16c	|__|	|__|	|__|
D16d	|__|	|__|	|__|

If A4a is not equal to 4, then skip to instruction before E1.

If D13F is not equal to 1 and D13M is not equal to 1 and D13T is not equal to 1, then skip to D19.

D17. Have you ever used water based lubricants (such as KY or other sexual gel products) during anal sex? (Choose one)

0	NO, HAVE NEVER USED
1	YES, HAVE USED
9	Don't Know
8	Refuse to Answer

If D17 is not equal to 1, then skip to instruction before E1.

D18. How often do you use water based lubricants (such as KY or other sexual gel products) during anal sex? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D19. In the last 3 months, have you or your partner used a female condom? (Choose one)

0	NO
1	YES
2	I HAVE NEVER HEARD OF FEMALE CONDOMS
9	Don't Know
8	Refuse to Answer

If D19 is not equal to 1, then skip to instruction before E1.

D20. You answered that you or your partner have used a female condom, was it for vaginal or anal sex? (Check all that apply)

__	VAGINAL
__	ANAL
__	Don't Know
__	Refuse to Answer

You have finished the fourth section of the questionnaire. We will now begin the last section of the questionnaire.

Section E

E1. Have you been circumcised?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If E1 is equal to 8 or E1 is equal to 9, then skip to E5.

If E1 is equal to 0, then skip to instruction before E4.

E2. How old were you when you got circumcised?

__ __	
99	Don't Know
98	Refuse to Answer

E3. Who circumcised you? (Choose one)

1	HEALTH CARE WORKER
2	TRADITIONAL HEALER OR CULTURAL CIRCUMCISER
3	RELIGIOUS CIRCUMCISER
4	OTHER
9	Don't Know
8	Refuse to Answer

If E1 is equal to 1, then skip to E5.

If A1 is equal to 2, then skip to E5.

E4. If you are not circumcised and circumcision was offered, would you be interested? (Choose one)

0	NO
1	YES
2	NOT SURE
9	Don't Know
8	Refuse to Answer

E5. Have you ever had any of the following skin cutting/piercing for beauty, cultural or treatment purposes? (CHECK ALL ANSWERS THAT APPLY TO YOU) (Check all that apply)

__ FACIAL CUTTINGS
__ EAR PIERCING
__ NOSE PIERCING
__ TEETH REMOVED
__ TATTOING ON THE BODY
__ OTHER BODILY CUTTINGS OR PIERCING
__ CLITORIS CUT/MUTILATED
__ PENILE BEADS
__ NONE
__ Don't Know
__ Refuse to Answer

E6. In your lifetime, do you recall having received a blood transfusion where you got someone else's blood during a procedure or a surgery?

1 Yes
0 No
9 Don't Know
8 Refuse to Answer

E7. If in the past 3 months you got ill, where did you seek medical care/treatment? (Check all that apply)

__ DID NOT GET ILL
__ GOT ILL BUT DID NOT SEEK MEDICAL CARE
__ TRADITIONAL HERBALIST
__ DISTRICT/PUBLIC HOSPITAL/COMMUNITY HEALTH CENTER
__ PRIVATE HOSPITAL/HEALTH CENTER
__ PRIVATE CLINIC
__ COMMUNITY HEALTH CARE WORKER
__ DRUG SHOP/PHARMACY
__ Don't Know
__ Refuse to Answer

E8. Do you use injectable recreational drugs? (Choose one)

1 NEVER
2 YES, MORE THAN 1 WEEK AGO
3 YES, LESS THAN ONE WEEK AGO
4 IN THE PAST BUT NO LONGER
9 Don't Know
8 Refuse to Answer

If E8 is equal to 1 or E8 is equal to 8, then skip to instruction before E10.

Baseline 1.5

E9. Do you share needles with another person for the injection of recreational drugs, male hormones, or female hormones? (Choose one)

1	NEVER
3	YES, MORE THAN ONE WEEK AGO
4	YES, LESS THAN ONE WEEK AGO
5	IN THE PAST BUT NO LONGER
9	Don't Know
8	Refuse to Answer

If A1 is equal to 2 or C5E is equal to 1, then skip to instruction before E11.

E10. In the past 3 months, have you experienced any of the following?
(CHECK ALL ANSWERS THAT APPLY TO YOU) (Check all that apply)

__	PAIN OR BURNING ON URINATION
__	DISCHARGE FROM PENIS OR ANUS
__	LUMPS, TENDERNESS, OR PAIN IN GENITAL AREA
__	ULCER ON PENIS OR ANUS OR GENITAL AREA
__	PAINFUL SEX
__	NONE
__	Don't Know
__	Refuse to Answer

If A1 is equal to 1 or C5E is equal to 0, then skip to end of questionnaire.

E11. In the past 3 months, have you experienced any of the following?
(CHECK ALL ANSWERS THAT APPLY TO YOU) (Check all that apply)

__	PAIN OR BURNING ON URINATION
__	ITCHING AND DISCHARGE FROM VAGINA OR ANUS
__	LUMPS, TENDERNESS, OR PAIN IN GENITAL AREA
__	ULCER ON ANUS OR GENITAL AREA
__	PAINFUL SEX
__	NONE
__	Don't Know
__	Refuse to Answer

Now you will be happy to know that we have finished all the questions. I would like to thank you for your patience, co-operation, and honesty in answering the questions. We know it was a long and exhausting process for you, with some sensitive and personal questions. We are grateful for your co-operation.

Let us repeat that the questionnaire is confidential and none of the information you have given us will be shared with anybody.

Thank you very much

Baseline 1.5

If A3aF is equal to 1 then THANK YOU FOR PARTICIPATING IN THIS STUDY. PLEASE CONTACT STUDY STAFF..

APPENDIX VIII: FOLLOW-UP QUESTIONNAIRE

fupquestD-J_1.0 (English)

Q1. Study Number _ _ _ _ _

Q2. Site _

Q3. FOLLOW-UP STUDY VISIT (Choose one)

3	STUDY VISIT D
4	STUDY VISIT F
5	STUDY VISIT H
6	STUDY VISIT J
9	Don't Know
8	Refuse to Answer

Before you start, we would like to remind you about the interview process. Your participation in this interview and every aspect of the research study are completely voluntary. You may skip any question that you prefer not to answer, but we would appreciate if you answered all the questions. These questions may be somewhat sensitive but they are important for the success of the study. You can also ask study staff to clarify questions that you do not understand, or you can decide to stop the interview at any time. The majority of the questions we will asking you cover the 3 month period since your last visit.

Any information you provide for this study will be kept confidential and cannot be shared with any individual, including your employer/boss, spouse, friends, or relatives. This information you provide to us is identified only by a number, not by your name.

This questionnaire has five sections.

If you have any questions please contact study staff.

Thank you. Let us begin the questions.

Section A: Demographic and Background Information

A1. Are you male, female, or transgender? (Choose one)

1	Male
2	Female
3	Transgender
9	Don't Know
8	Refuse to Answer

A2. Over the past 3 months, have you changed you residence?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If A2 is equal to 0 or A2 is equal to 8 or A2 is equal to 9, then skip to A4.

For the following questions:

HOUSEHOLD IS DEFINED AS PERSON(S) LIVING WITHIN ONE HOMESTEAD.

HOUSEHOLD MEMBER IS SOMEONE THAT HAS BEEN LIVING IN YOUR HOUSEHOLD FOR AT LEAST ONE MONTH PRIOR TO THIS INTERVIEW.

A3. How many other people do you live with in your household?

___	
999	Don't Know
998	Refuse to Answer

If A3 is equal to 0 or A3 is equal to 999 or A3 is equal to 998, then skip to A4.

A4. What is your relationship to those you live with? (Check all that apply)

__ PARTNER/SPOUSE

__ BIOLOGICAL CHILDREN

__ BIOLOGICAL BROTHER/SISTER

__ BIOLOGICAL MOTHER/FATHER

__ OTHER RELATIVE

__ FRIEND

__ CO-WORKERS

__ HOUSE HELPER

__ Don't Know

__ Refuse to Answer

For the following questions:

NEVER MARRIED= YOU HAVE NEVER BEEN MARRIED OR COHABITATED WITH SOMEONE

MARRIED= IT CAN BE A RELIGiOUS OR GOVERNMENT MARRIAGE OR BASED ON CUSTOMARY LAW

COHABITATING=YOU HAVE BEEN LIVING WITH SOMEONE OF THE SAME OR OPPOSITE SEX AS SPOUSES FOR AT LEAST SIX MONTHS, BUT ARE NOT "LEGALLY" MARRIED THROUGH EITHER CIVIL, CUSTOMARY OR RELIGIOUS LAW

SPOUSE= YOUR HUSBAND OR WIFE, WHETHER LEGALLY MARRIED OR COHABITATING

DIVORCED/SEPARATED= ANY MARRIAGE OR COHABITATION THAT HAS ENDED

WIDOWED= SPOUSE (MARRIED OR COHABITATING) HAS DIED

A5. In the past 3 months has your marital status changed?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If A5 is equal to 0 or A5 is equal to 8 or A5 is equal to 9, then skip to instruction before A7.

A6. How has your marital status changed? (Choose one)

1	I am now married to one spouse
2	I got married to more spouses
3	I am now cohabitating with one spouse
4	I am now cohabitating with more than one spouse
5	I got separated
6	I got divorced
7	I became widowed
9	Don't Know
8	Refuse to Answer

If Q2 is equal to 400, then skip to B1.

IN MANY CULTURES, WHEN A MAN DIES, HIS WIFE IS "GIVEN" TO THE NEAREST MALE RELATIVE, WHO IS EXPECTED TO CARE FOR HER AND THE DECEASED MAN'S CHILDREN. THE INHERITED WOMAN IS EXPECTED TO HAVE SEXUAL INTERCOURSE WITH THE MAN WHO INHERITS HER.

A7. In the past 3 months, have you ever inherited a wife/been inherited as a wife?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If A7 is equal to 0 or A7 is equal to 8 or A7 is equal to 9, then skip to B1.

A8. How old is the wife or husband that you inherited during the past 3 months?

— —	
99	Don't Know
98	Refuse to Answer

Section B: Work

B1. In the past 3 months, has your employment status changed?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If B1 is equal to 0 or B1 is equal to 8 or B1 is equal to 9, then skip to instruction before C1.

B2. In the past 3 months, how has your employment status changed? (Choose one)

1	Now not working/unemployed
2	Now working/employed
3	Changed jobs/employer
9	Don't Know
8	Refuse to Answer

If B2 is equal to 1, then skip to instruction before C1.

B3a. What is the occupation from which you earn most of your income? (Choose one)

1	NOT WORKING
2	PUPIL/STUDENT
3	ENTERTAINMENT/SERVICE
4	RESTAURANT/HOTEL BUSINESS
5	DRIVER/LABORER
6	OFFICE/HEALTH CARE/MILITARY/POLICE
9	Don't Know
8	Refuse to Answer

If B3a is equal to 1 or B3a is equal to 2 or B3a is equal to 8 or B3a is equal to 9, then skip to instruction before C1.

If B3a is equal to 4, then skip to instruction before B3ac.

If B3a is equal to 5, then skip to instruction before B3ad.

If B3a is equal to 6, then skip to instruction before B3ae.

B3ab. You specified [Response to B3a], which response below best describes your occupation? (Choose one)

1	Bar, Karaoke Worker
2	Sex Worker
3	Entertainer/Performer
4	Massage Parlor Worker
5	Hairdresser/Manicure
6	Other
9	Don't Know
8	Refuse to Answer

If B3a is equal to 3, then skip to B3b.

B3ac. You specified [Response to B3a], which response below best describes your occupation? (Choose one)

01	Guesthouse Attendant
02	Restaurant/Hotel Worker
03	Bar Owner or Manager
04	Business/Trader
05	Local Brew Seller
06	Food Vendor
07	Home Brewing
08	Other
99	Don't Know
98	Refuse to Answer

If B3a is equal to 4, then skip to B3b.

B3ad. You specified [Response to B3a], which response below best describes your occupation? (Choose one)

01	Local Driver (within country)
02	Long Distance (transnational)
03	Domestic Employee
04	Motorcyclist/Boda-Boda/Songtaew
05	Transport
06	Fishing
07	Farming
08	Laborer
09	Factory Worker
10	Other
99	Don't Know
98	Refuse to Answer

If B3a is equal to 5, then skip to B3b.

B3ae. You specified [Response to B3a], which response below best describes your occupation? (Choose one)

1	NGO Employee
2	Health Care Provider
3	Office Worker
4	Military/Police
5	Other
9	Don't Know
8	Refuse to Answer

B3b. Do you have another occupation?

1	Yes	
0	No	***Skip to B3c***
9	Don't Know	
8	Refuse to Answer	

If B3b is equal to 0, then skip to B4.

B3c. What is your second most income-generating occupation? (Choose one)

1	PUPIL/STUDENT
2	ENTERTAINMENT/SERVICE
3	RESTAURANT/BAR/HOTEL/TRADE BUSINESS
4	DRIVER/LABORER
5	OFFICE/HEALTH CARE/MILITARY/POLICE
9	Don't Know
8	Refuse to Answer

B4. What is your approximate average monthly income in local currency? (Include all sources, but do not include spouses' income)

_ _ _ _ _ _ _

9999999	Don't Know
9999998	Refuse to Answer

Section C: Contraception and Birth Control

If A1 is equal to 3, then skip to instruction before D1a&C.

If A1 is equal to 1, then skip to C2.

C1. Are you currently pregnant?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

C2. During the past 3 months did you or your sexual partner do anything to avoid pregnancy? (Choose one)

0	NO
1	YES
2	NEVER HAD SEX
3	NOT APPLICABLE/MSM
9	Don't Know
8	Refuse to Answer

If C2 is not equal to 1, then skip to instruction before D1a&C.

C3. During the past 3 months, which methods did you or your partner use to delay/avoid pregnancy/having children?
(CHECK ALL ANSWERS THAT APPLY TO YOU) (Check all that apply)

__ HORMONAL CONTRACEPTION (Pill, Injectables,Implant,Emergency Pill)

__ IUCD

__ I AND/OR MY PARTNER ARE STERILIZED

__ DIAPHRAGM/FOAM

__ MALE CONDOM

__ FEMALE CONDOM

__ WITHDRAWAL

__ RHYTHM/CALENDAR

__ USING HERBS

__ TYING STRINGS ON THE BODY

__ ABORTION

__ OTHER

__ Don't Know

__ Refuse to Answer

You have completed the third section of the questionnaire. We will now begin the fourth section.

Section D: Sexuality

We are now on Section D. We will now ask you some questions about your relationship with the person or people with whom you have had sexual relations (even if you are no longer seeing them).

When we ask you about persons you have had sex with, please respond with all partners: male, female and transgender. We understand that this is a sensitive and personal topic. However, it is important for the purpose of this study that you are as open and honest as possible. Again, we want to assure you that anything you say will be kept confidential.

For this section the following definition apply:

For all questions, "sex", "sexual contact", "having sex" or "playing sex" refers only to vaginal or anal sexual intercourse (receptive or insertive).
"Oral sex" or "mutual masturbation" are not considered "having sex" or "playing sex".

For all questions, "unprotected sex" is sex without a barrier such as a condom.

For this section the following definitions apply:

When asked about Steady Partners, steady partners can include:
Someone with whom you are involved in an ongoing or regular sexual relationship, such as a boyfriend/girlfriend, a spouse/cohabitating partner, or a sugar daddy/mummy.

When asked about Casual Partners, casual partners can include:
Someone who is not your steady partner with whom you are not romantically involved but with whom you have sex with once or on an occasional basis by mutual consent where no money, goods, or services are exchanged, for example, a stranger or a workmate.

When asked about Customer/Client Partners, customer/client partners include:
Sexual contact with a person in which you receive or give money, goods, or services in exchange for sex, in the absence of a romantic relationship or emotional attachment, for example a sex worker or a sex client.

When asked about Other Partners, other partners can include:
Someone who is not your steady, casual, or customer/client partner but with whom you have had sex with by mutual consent or force, such as a boss, teacher, rapist, relative other than spouse, work subordinate, or student.

D1a. In the last 3 months, have you ever had sex with a [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D1a&C is equal to 0 or D1a&C is equal to 8 or D1a&C is equal to 9, then skip to next category.

D1b. In the past 3 months, how many different [Female partner(s), Male partner(s), ...] have you had unprotected vaginal and/or anal intercourse with? (Choose one)

0	1-2 partners
1	3-10 partners
2	More than 10 partners
9	Don't Know
8	Refuse to Answer

D2a. In the past 3 months, have you had sex with a steady [Female partner(s), Male partner(s), ...]? (A steady partner includes a boyfriend/girlfriend, spouse/cohabitating partner, sugar daddy/mummy)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D2a&C is equal to 0 or D2a&C is equal to 8 or D2a&C is equal to 9, then skip to D4a.

D2b. In the past 3 months, how many times did you play/have sex with your steady [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D3a. In the past 3 months, have you had sex with a casual [Female partner(s), Male partner(s), ...]? (Casual includes a stranger, workmate, or other casual partner)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D3a&C is equal to 0 or D3a&C is equal to 8 or D3a&C is equal to 9, then skip to D5a.

D3b. In the past 3 months, how many times did you play/have sex with your casual [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D4a. In the past 3 months, have you had sex with a customer/client [Female partner(s), Male partner(s), ...]?
(Customer/client includes a sex worker or a sex client)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D4a&C is equal to 0 or D4a&C is equal to 8 or D4a&C is equal to 9, then skip to D6a.

D4b. In the past 3 months, how many times did you play/have sex with your customer/client [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D5a. In the past 3 months, have you had sex with other [Female partner(s), Male partner(s), ...]?
(Other includes boss/work supervisor, teacher, other authority figure and relative other than spouse, student, employee/work subordinate, or rapist)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D5a&C is equal to 0 or D5a&C is equal to 8 or D5a&C is equal to 8, then skip to D6c.

D5b. In the past 3 months, how many times did you play/have sex with your other [Female partner(s), Male partner(s), ...]? (Choose one)

1	1-5 TIMES
2	6-10 TIMES
3	MORE THAN 10 TIMES
9	Don't Know
8	Refuse to Answer

D6a. In the past 3 months, how many different non-spouse/non-cohabitating [Female partner(s), Male partner(s), ...] have you had/played sex with? (Choose one)

0	1-2 partners
1	3-10 partners
2	More than 10 partners
9	Don't Know
8	Refuse to Answer

If D6a&C is equal to 0, then skip to D7a.

D6b. In the past 3 months, how many of your non-spouse/non-cohabitating [Female partner(s), Male partner(s), ...] did you have sex with for the first time?

_ _ _ _

9999	Don't Know
9998	Refuse to Answer

D6c. Did you use a condom, the last time you had/played sex with a non-spouse/non-cohabitating [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D6d. How often did you use a condom when you had/played sex with non-spouse/non-cohabitating [Female partner(s), Male partner(s), ...]? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

D7a. In the past 3 months, did you receive money or other benefits in exchange for vaginal or anal sex with a [Female partner(s), Male partner(s), ...]?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D7a&C is equal to 0 or D7a&C is equal to 8 or D7a&C is equal to 9, then skip to D7c.

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D7b. In the past 3 months, how many times have you received money or other goods in exchange for sex with your [Female partner(s), Male partner(s), ...]? (Choose one)

1 LESS THAN
2 5-10 TIMES
3 MORE THAN 10 TIMES
9 Don't Know
8 Refuse to Answer

D7c. In the past 3 months, did you pay/give money or other benefits in exchange for sex with a [Female partner(s), Male partner(s), ...]?

1 Yes
0 No
9 Don't Know
8 Refuse to Answer

If D7c&C is equal to 0 or D7c&C is equal to 8 or D7c&C is equal to 9, then skip to D8a.

D7d. In the past 3 months, how many times have you given or paid money or other benefits in exchange for sex with a [Female partner(s), Male partner(s), ...]? __ __

99 Don't Know
98 Refuse to Answer

D8a. In the past 3 months, have you had sex with a non-spouse/non-cohabiting [Female partner(s), Male partner(s), ...] older than you by approximately 10 years or more?

1 Yes
0 No
9 Don't Know
8 Refuse to Answer

If D8a&C is equal to 0 or D8a&C is equal to 8 or D8a&C is equal to 9, then skip to D9a.

D8b. In the past 3 months, how many times have you had sex with a non-spouse/cohabitating [Female partner(s), Male partner(s), ...] older than you by approximately 10 years or more? (Choose one)

1	LES	
2	5-10 TIMES	
3	MORE THAN 10 TIMES	
9	Don't Know	
8	Refuse to Answer	

D9a. In the past 3 months, have you had vaginal or anal sex with any [Female partner(s), Male partner(s), ...] whom you know or strongly suspect was infected with HIV?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D9a&C is equal to 0 or D9a&C is equal to 8 or D9a&C is equal to 9, then skip to D9c.

D9b. How often did you use a condom when you had/played sex with the [Female partner(s), Male partner(s), ...] you knew or suspected was infected with HIV? (Choose one)

1	ALV	
2	NEARLY ALWAYS	
3	SOMETIMES	
4	NEARLY NEVER	
5	NEVER	
9	Don't Know	
8	Refuse to Answer	

D9c. In the past 3 months, have you had sex with any of the following [Female partner(s), Male partner(s), ...], Bar/Pub/Karaoke Worker, Someone diagnosed with an STI, Food vendor, Long distance truck driver, sex worker?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D9c&C is equal to 0 or D9c&C is equal to 8 or D9c&C is equal to 9, then skip to D10.

D9d. Did you use a condom the last time you had sex with the [Female partner(s), Male partner(s), ...] mentioned in the previous question?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D10. In the past 3 months, have you been forced to have sex with any [Female partner(s), Male partner(s), ...] without your agreement or consent?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

	Female	Male	Transgender
D1a	|__|	|__|	|__|
D1b	|__|	|__|	|__|
D2a	|__|	|__|	|__|
D2b	|__|	|__|	|__|
D3a	|__|	|__|	|__|
D3b	|__|	|__|	|__|
D4a	|__|	|__|	|__|
D4b	|__|	|__|	|__|
D5a	|__|	|__|	|__|
D5b	|__|	|__|	|__|
D6a	|__|	|__|	|__|
D6b	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|
D6c	|__|	|__|	|__|
D6d	|__|	|__|	|__|
D7a	|__|	|__|	|__|
D7b	|__|	|__|	|__|
D7c	|__|	|__|	|__|
D7d	|__|__|	|__|__|	|__|__|
D8a	|__|	|__|	|__|
D8b	|__|	|__|	|__|
D9a	|__|	|__|	|__|

D9b	|__|	|__|	|__|
D9c	|__|	|__|	|__|
D9d	|__|	|__|	|__|
D10	|__|	|__|	|__|

D11. In the past 3 months, have you had anal intercourse with a [Female partner(s), Male partner(s), ...]?

1 Yes

0 No

9 Don't Know

8 Refuse to Answer

If D11&C is equal to 0 or D11&C is equal to 8 or D11&C is equal to 9, then skip to next category.

D12. In the past 3 months, have you had anal intercourse with a steady [Female partner(s), Male partner(s), ...]?
(A steady partner includes a boyfriend/girlfriend, spouse/cohabitating partner, sugar daddy/mummy)

1 Yes

0 No

9 Don't Know

8 Refuse to Answer

If D12&C is equal to 0 or D12&C is equal to 8 or D12&C is equal to 9, then skip to D13.

D12a. In the past 3 months, how many times did you play/have anal sex with your steady [Female partner(s), Male partner(s), ...]?

— —

99 Don't Know

98 Refuse to Answer

D13. In the past 3 months, have you had anal sex with a casual [Female partner(s), Male partner(s), ...]?
(Casual includes a stranger, workmate, or other casual partner)

1 Yes

0 No

9 Don't Know

8 Refuse to Answer

If D13&C is equal to 0 or D13&C is equal to 8 or D13&C is equal to 9, then skip to D14.

D14. In the past 3 months, have you had anal sex with a customer/client [Female partner(s), Male partner(s), ...]?
(Customer/client includes a sex worker or a sex client)

1 Yes

0 No

9 Don't Know

8 Refuse to Answer

If D14&C is equal to 0 or D14&C is equal to 8, then skip to D15.

D15. In the past 3 months, have you had anal sex with any other [Female partner(s), Male partner(s), ...]?
(Other includes boss/work supervisor, teacher, other authority figure and relative other than spouse, student, employee/work subordinate, or rapist)

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If D15&C is equal to 0 or D15&C is equal to 8, then skip to D16a.

D15a. In the past 3 months, how many times did you play/have anal sex with your other [Female partner(s), Male partner(s), ...]?

— —

99	Don't Know
98	Refuse to Answer

D16a. The last time you had anal sex with a [Female partner(s), Male partner(s), ...] did you have receptive anal sex with a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D16b. The last time you had anal sex with a [Female partner(s), Male partner(s), ...] did you have receptive anal sex without a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D16c. The last time you had anal sex with a [Female partner(s), Male partner(s), ...] did you have insertive anal sex with a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

D16d. The last time you had anal sex with a [Female partner(s), Male partner(s), ...] did you have insertive anal sex without a condom?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

	Female	Male	Transgender
D11	|__|	|__|	|__|
D12	|__|	|__|	|__|
D12a	|__|__|	|__|__|	|__|__|
D13	|__|	|__|	|__|
D14	|__|	|__|	|__|
D15	|__|	|__|	|__|
D15a	|__|__|	|__|__|	|__|__|
D16a	|__|	|__|	|__|
D16b	|__|	|__|	|__|
D16c	|__|	|__|	|__|
D16d	|__|	|__|	|__|

You have finished the fourth section of the questionnaire. We will now begin the last section of the questionnaire.

We are now going to ask some questions about your health. We understand this is a sensitive and personal topic. However, it is important for the purpose of this study that you are as open and honest as possible. Again, We want to assure you that anything you say will be kept confidential from your employer, co-workers, spouse/partner, or any other person.

Section E

E1. Have you been circumcised?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If E1 is equal to 0 or E1 is equal to 8 or E1 is equal to 9, then skip to E3.

E2. Who circumcised you? (Choose one)

1	HEALTH CARE WORKER
2	TRADITIONAL HEALER OR CULTURAL CIRCUMCISER
3	RELIGIOUS CIRCUMCISER
4	OTHER
9	Don't Know
8	Refuse to Answer

E3. Have you ever had any of the following skin cutting/piercing for beauty, cultural or treatment purposes? (CHECK ALL ANSWERS THAT APPLY TO YOU) (Check all that apply)

__ FACIAL CUTTINGS

__ EAR PIERCING

__ NOSE PIERCING

__ TEETH REMOVED

__ TATTOOING ON THE BODY

__ OTHER BODILY CUTTINGS OR PIERCING

__ CLITORIS CUT/MUTILATED

__ PENILE BEADS

__ NONE

__ Don't Know

__ Refuse to Answer

E4. In your lifetime, do you recall having received a blood transfusion where you got someone else's blood during a procedure or a surgery?

1 Yes

0 No

9 Don't Know

8 Refuse to Answer

E5. If in the past 3 months you got ill, where did you seek medical care/treatment? (Check all that apply)

__ DID NOT GET ILL

__ GOT ILL BUT DID NOT SEEK MEDICAL CARE

__ TRADITIONAL HERBALIST

__ DISTRICT/PUBLIC HOSPITAL/COMMUNITY HEALTH CENTER

__ PRIVATE HOSPITAL/HEALTH CENTER

__ PRIVATE CLINIC

__ COMMUNITY HEALTH CARE WORKER

__ DRUG SHOP/PHARMACY

__ Don't Know

__ Refuse to Answer

E6. In the past 3 months, have you drunk beer, spirits, or other alcoholic drinks?

1	Yes
0	No
9	Don't Know
8	Refuse to Answer

If E6 is equal to 0 or E6 is equal to 8 or E6 is equal to 9, then skip to E8.

E7. In the past 3 months, how often did you drink beer, spirits, or any other alcoholic drinks? (Choose one)

1	ALWAYS
2	NEARLY ALWAYS
3	SOMETIMES
4	NEARLY NEVER
5	NEVER
9	Don't Know
8	Refuse to Answer

E8. Do you use injectable recreational drugs? (Choose one)

1	NEVER
2	YES, MORE THAN 1 WEEK AGO
3	YES, LESS THAN ONE WEEK AGO
4	IN THE PAST BUT NO LONGER
9	Don't Know
8	Refuse to Answer

If E8 is equal to 1 or E8 is equal to 8 or E8 is equal to 9, then skip to end of questionnaire.

E9. Do you share needles with another person for the injection of recreational drugs, male hormones, or female hormones? (Choose one)

1	NEVER
3	YES, MORE THAN ONE WEEK AGO
4	YES, LESS THAN ONE WEEK AGO
5	IN THE PAST BUT NO LONGER
9	Don't Know
8	Refuse to Answer

Now you will be happy to know that we have finished all the questions. I would like to thank you for your patience, co-operation, and honesty in answering the questions. We know it was a long and exhausting process for you, with some sensitive and personal questions. We are grateful for your co-operation.

Let us repeat that the questionnaire is confidential and none of the information you have given us will be shared with anybody.

Thank you very much

APPENDIX IX: WASHING QUESTIONNAIRE

Site	RV217	Echo Study	Visit: _____
☐ 1 MUWRP Uganda ☐ 2 WRP Kenya ☐ 3 MMRP Tanzania ☐ 4 AFRIMS Thailand	Study Number ___ ___ ___ ___ ___		Date DD/MON/YYYY __ __/__ __ __/__ __ __ __

INTRAVAGINAL PRACTICES QUESTIONNAIRE

We would like to ask you some questions about internal vaginal cleaning that you may do before or after the sex act. We would like to find out if any of these practices may put you at an increased risk of getting HIV.

1a. Do you practice internal vaginal washing/cleaning?

☐ 0 No, go to Item1b.
☐ 1 Yes

2a. How frequently do you do this?

Before sex

☐ 1 Yes
☐ 0 No

If Yes, when?
☐ 1 Nearly never
☐ 2 Sometimes
☐ 3 Always or nearly always
☐ 9 Other, specify_____

After sex

☐ 1 Yes
☐ 0 No

If Yes, when?
☐ 1 After each act
☐ 2 After 2 – 3 acts
☐ 3 Once a day
☐ 4 Depends on partner
☐ 9 Other, specify _____

3a. With which type of partners do you do this?

Regular partners (i.e. husband, boyfriend)

☐ 1 Yes
☐ 0 No

If Yes, why? *(Check all that apply)*
☐ Known HIV
☐ Suspected HIV
☐ Partner has poor hygiene
☐ Condom not used
☐ Forced sex
☐ Other, specify _____

Clients (those that pay for sex)

☐ 1 Yes
☐ 0 No

If Yes, why? *(Check all that apply)*
☐ Known HIV
☐ Suspected HIV
☐ Partner has poor hygiene
☐ Condom not used
☐ Forced sex
☐ Other, specify _____

→Go to Item 4a

4a. Which products do you use? *(Check all that apply)*

Liquids	Before	After
Water only	☐	☐
Water & soap	☐	☐
Coke-Cola	☐	☐
Soda water	☐	☐
Other soda, specify _____	☐	☐
Diluted vinegar	☐	☐
Toothpaste	☐	☐
Lemon juice	☐	☐
Antiseptic (Dettol)	☐	☐
Commercial feminine hygiene product	☐	☐
Bleach (such as diluted JIK)	☐	☐
Cloth		
Fresh cloth	☐	☐
Used previously	☐	☐
Toilet paper	☐	☐
Other, specify _____	☐	☐

5a. Why do you wash or clean the vagina? *(Check all that apply)*

I believe it avoids HIV	☐	☐
I believe it avoids STIs	☐	☐
I believe it avoids pregnancy	☐	☐
Partner request	☐	☐
Cleanliness/personal hygiene/ feel fresh	☐	☐
Remove condom smell	☐	☐
Dry sex	☐	☐
Other, specify _____	☐	☐

6a. Are there any comments you would like to make? *If Yes, please include here.*

_____

_____

Interviewer (Print Name): _____

Site	RV217	Echo Study	Visit: ____
☐ 1 MUWRP Uganda ☐ 2 WRP Kenya ☐ 3 MMRP Tanzania ☐ 4 AFRIMS Thailand	Study Number ___ ___ ___ ___ ___		Date DD/MON/YYYY __ __/__ __ __/__ __ __ __

INTRAANAL PRACTICES QUESTIONNAIRE

We would like to ask you some questions about internal anal cleaning that you may do before or after the sex act. We would like to find out if any of these practices may put you at an increased risk of getting HIV.

1b. Do you practice internal anal washing/cleaning?

☐ 0 No, stop questionnaire
☐ 1 Yes

2b. How frequently do you do this?

Before sex

☐ 1 Yes
☐ 0 No

If Yes, when?
☐ 1 Nearly never
☐ 2 Sometimes
☐ 3 Always or nearly always
☐ 9 Other, specify_____

After sex

☐ 1 Yes
☐ 0 No

If Yes, when?
☐ 1 After each act
☐ 2 After 2 – 3 acts
☐ 3 Once a day
☐ 4 Depends on partner
☐ 9 Other, specify _____

3b. With which type of partners do you do this?

Regular partners (i.e. husband, boyfriend)

☐ 1 Yes
☐ 0 No

If Yes, why? *(Check all that apply)*
☐ Known HIV
☐ Suspected HIV
☐ Partner has poor hygiene
☐ Condom not used
☐ Forced sex
☐ Other, specify _____

Clients (those that pay for sex)

☐ 1 Yes
☐ 0 No

If Yes, why? *(Check all that apply)*
☐ Known HIV
☐ Suspected HIV
☐ Partner has poor hygiene
☐ Condom not used
☐ Forced sex
☐ Other, specify _____

→Go to Item 4b

4b. Which products do you use? *(Check all that apply)*

Liquids	Before	After
Water only	☐	☐
Water & soap	☐	☐
Coke-Cola	☐	☐
Soda water	☐	☐
Other soda, specify _____	☐	☐
Diluted vinegar	☐	☐
Toothpaste	☐	☐
Lemon juice	☐	☐
Antiseptic (Dettol)	☐	☐
Commercial feminine hygiene product	☐	☐
Bleach (such as diluted JIK)	☐	☐
Cloth		
Fresh cloth	☐	☐
Used previously	☐	☐
Toilet paper	☐	☐
Other, specify _____	☐	☐

5b. Why do you wash or clean the anus? *(Check all that apply)*

I believe it avoids HIV	☐	☐
I believe it avoids STIs	☐	☐
I believe it avoids pregnancy	☐	☐
Partner request	☐	☐
Cleanliness/personal hygiene/ feel fresh	☐	☐
Remove condom smell	☐	☐
Dry sex	☐	☐
Other, specify _____	☐	☐

6b. Are there any comments you would like to make? *If Yes, please include here.*

_____

_____

Interviewer (Print Name): _____

APPENDIX X: RECEPTIVE RISK QUESTIONNAIRE

U.S. MILITARY HIV RESEARCH PROGRAM

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		**Date** *DD/MON/YYYY* ___ ___/___ ___ ___/___ ___ ___ ___

RECEPTIVE RISK QUESTIONNAIRE

for participants not participating in swab collection

Date of Reference: ___ ___/___ ___ ___ **Day of the week** _____

DD / MON

Note to staff: subtract 3 from today's date.

1. Are you participating in swab collection?

☐ 1 Yes ☐ 0 No ***If 1, then STOP – go to appropriate RR questionnaire***

2. What type of receptive sex did you most recently engage in? (choose one)

☐ 1 Receptive vaginal sex ☐ 2 Receptive anal sex ☐ 3 Neither ☐ 8 Don't know

If 3 or 8, then STOP

3. Since the reference date, with how many different sexual partners have you had receptive sex?

Total number of partners: _____

If 0, then STOP

4. Of the receptive intercourse you mentioned, how many times did someone ejaculate inside you (that is, sex without a condom or a condom broke)? ___ ___ ___

5. Of the sexual partners you mentioned, was there anyone who is HIV infected?

☐ 1 Yes ☐ 0 No ☐ 8 Don't know

6. Was a condom used every time, without breakage, from the beginning to end?

☐ 1 Yes ☐ 0 No ☐ 8 Don't know

STOP

Interviewer *(print name, if interview administered)*: _____

U.S. MILITARY HIV RESEARCH PROGRAM

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		**Date** *DD/MON/YYYY* ___ ___/___ ___ ___/___ ___ ___ ___

RECEPTIVE RISK QUESTIONNAIRE

for participants collecting swab at home

Date of Reference: ___ ___/___ ___ ___ **Day of the week** _____ ***Note to staff: subtract 3 from today's date.***

DD / MON

1. Are you participating in swab collection?

☐ 1 Yes ☐ 0 No ***If 0, then STOP – administer RR questionnaire for those not participating in swab collection***

2. Was the swab collected at home or will the swab be collected in the clinic?

☐ 1 I collected the swab at home ☐ 2 I will collect the swab at the clinic today ***If 2, then STOP – administer clinic RR questionnaire***

3. What type of receptive sex did you most recently engage in? (choose one)

☐ 1 Receptive vaginal sex ☐ 2 Receptive anal sex ☐ 3 Neither ☐ 8 Don't know

If 3 or 8, then go to question 10

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4. Since the reference date, with how many different sexual partners have you had receptive sex?

Total number of partners: _____

If 0, then go to question 10

5. Of the receptive intercourse you mentioned, how many times did someone ejaculate inside you (that is, sex without a condom or a condom broke)? ___ ___ ___

6. Of the sexual partners you mentioned, was there anyone who is HIV infected?

☐ 1 Yes ☐ 0 No ☐ 8 Don't know ***If 0 or 8, then go to question 8***

7. Was a condom used every time, without breakage, from the beginning to end?

☐ 1 Yes ☐ 0 No ☐ 8 Don't know

U.S. MILITARY HIV RESEARCH PROGRAM

Site	RV217	Echo Study
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___	**Date** *DD/MON/YYYY* __ __/__ __ __/__ __ __ __

RECEPTIVE RISK QUESTIONNAIRE PAGE 2

for participants collecting swab at home

Swab Collected at the home

8. How long after you had receptive sex did you take the swab?

☐ 1 < 1 hour after sex ☐ 2 1-6 hours after sex ☐ 3 6 hours-24 hours after sex ☐ 4 Greater than 24 hours after sex ☐ 8 Don't Know

9. Did you douche between sex and swabbing?

☐ 1 Yes ☐ 0 No

10. Swab site?

☐ 1 Vagina ☐ 2 Anus

11. Swab collected, time:___ ___ ___ ___ (24 hour)

Interviewer *(print name, if interview administered)*: _____

U.S. MILITARY HIV RESEARCH PROGRAM

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		**Date** *DD/MON/YYYY* ___ ___/___ ___ ___/___ ___ ___ ___

RECEPTIVE RISK QUESTIONNAIRE *for participants collecting swab at clinic*

Date of Reference: ___ ___/___ ___ ___ **Day of the week** _____ ***Note to staff: subtract 3 from today's date.***
DD / MON

1. Are you participating in swab collection?

☐ 1 Yes ☐ 0 No ***If 0, then STOP – administer RR questionnaire for those not participating in swab collection***

2. Was the swab collected at home or will the swab be collected in the clinic?

☐ 1 I collected the swab at home ☐ 2 I will collect the swab at the clinic today ***If 1, then STOP – administer home RR questionnaire***

3. What type of receptive sex did you most recently engage in? (choose one)

☐ 1 Receptive vaginal sex ☐ 2 Receptive anal sex ☐ 3 Neither ☐ 8 Don't know

If 3 or 8, then go to question 12

4. Since the reference date, with how many different sexual partners have you had receptive sex?

Total number of partners: _____

If 0, then go to question 12

5. Of the receptive intercourse you mentioned, how many times did someone ejaculate inside you (that is, sex without a condom or a condom broke)? ___ ___ ___

6. Of the sexual partners you mentioned, was there anyone who is HIV infected?

☐ 1 Yes ☐ 0 No ☐ 8 Don't know ***If 0 or 8, then go to question 8***

7. Was a condom used every time, without breakage, from the beginning to end?

☐ 1 Yes ☐ 0 No ☐ 8 Don't know

U.S. MILITARY HIV RESEARCH PROGRAM

Site	RV217	Echo Study
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___	**Date** *DD/MON/YYYY* __ __/__ __ __/__ __ __ __

RECEPTIVE RISK QUESTIONNAIRE PAGE 2

for participants collecting swab at clinic

Swab Collected at the clinic

8. When was the last time someone ejaculated in you?

☐ 1 Today ☐ 2 Yesterday ☐ 3 2 days ago ☐ 4 3 days ago ☐ 5 More than 3 days ago ☐ 8 Don't Know

9. What time of day did they ejaculate in you?

☐ 1 Morning ☐ 2 Afternoon ☐ 3 Evening ☐ 8 Don't Know

10. Did you douche after?

☐ 1 Yes ☐ 0 No ***If 0, then go to Question 12***

11. How long after sex did you douche?

☐ 1 < 1 hour after sex ☐ 2 1-6 hours after sex ☐ 3 6 hours-1 day after sex ☐ 4 Greater than 1 day after sex ☐ 8 Don't Know

12. Swab site?

☐ 1 Vagina ☐ 2 Anus

13. Swab collected, time: ___ ___ : ___ ___ (24 hour)

Interviewer *(print name, if interview administered)*: _____

APPENDIX XI: PHASE II ENTRY QUESTIONNAIRE

Site	RV217	Echo Study	Visit: 10
☐ 1 MUWRP Uganda ☐ 2 WRP Kenya ☐ 3 MMRP Tanzania ☐ 4 AFRIMS Thailand	Study Number ___ ___ ___ ___ ___		Date DD/MON/YYYY __ __/__ __ __/__ __ __ __

1. Have you experienced any of the following symptoms over the past 8 weeks?

Symptoms / Signs	Yes	No	Start Date	End Date	Treatment
Fever	☐ 1	☐ 0			
Fatigue	☐ 1	☐ 0			
Sore throat	☐ 1	☐ 0			
Skin rash or lesions	☐ 1	☐ 0			
Muscle aches	☐ 1	☐ 0			
Joint aches	☐ 1	☐ 0			
Headache or pain behind your eyes	☐ 1	☐ 0			
Feeling of the light hurting your eyes	☐ 1	☐ 0			
Stiff or painful neck	☐ 1	☐ 0			
Swollen and/or tender glands (lymph nodes)	☐ 1	☐ 0			
Nausea and/or vomiting	☐ 1	☐ 0			
Abdominal pain	☐ 1	☐ 0			
Diarrhea	☐ 1	☐ 0			
Loss of appetite	☐ 1	☐ 0			
Difficulty or pain on swallowing	☐ 1	☐ 0			
Confusion/difficulty thinking	☐ 1	☐ 0			
Memory loss	☐ 1	☐ 0			
Numbness, tingling, pain in extremities	☐ 1	☐ 0			
Weakness (generalized or in a specific area?	☐ 1	☐ 0			
Specify location if not general _____					
Hair loss	☐ 1	☐ 0			
Night sweats	☐ 1	☐ 0			
Skin or mucous membrane ulcers	☐ 1	☐ 0			
Depression and/or anxiety	☐ 1	☐ 0			
Cough	☐ 1	☐ 0			
Feeling of being ill or sick	☐ 1	☐ 0			
Thrush (white plaques) in your mouth	☐ 1	☐ 0			
Weight loss amount _____ kg	☐ 1	☐ 0			

2. Any other symptoms (describe): _____

3. Please describe the circumstances which you feel may account for acquiring HIV. This may include the partners and types of sexual or other risk interaction. _____

_____

_____

Interviewer: *(Print Name)* _____

ATTACHMENT I: CASE REPORT FORMS

ELIGIBILITY & RISK ASSESSMENT

ERA

Site	RV217	Echo Study	Visit: A Phase 1
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number**	___ ___ ___ ___ ___	

Date of Visit
DD/MON/YYYY __ __/__ __ __/__ __ __ __

Age: ☐☐ years Gender: ☐ 0 Male ☐ 1 Female ☐ 3 Transgender

ELIGIBILITY

			Yes	No*
1.	Has volunteer successfully completed the Test of Understanding? Score #1 _____ Score #2 _____ Score #3 _____		☐ 1	☐ 0
2.	Has volunteer signed the study informed consent form?		☐ 1	☐ 0
3.	Is the volunteer's age between 18 and 50 years?		☐ 1	☐ 0
4.	Will the volunteer be available for follow-up for a total duration of 24 months?		☐ 1	☐ 0
5.	Is the volunteer willing to receive HIV counseling and testing?		☐ 1	☐ 0
6.	Is the volunteer willing to receive HIV test results?		☐ 1	☐ 0
7.	Is the volunteer willing to provide his/her location and contact information?		☐ 1	☐ 0
8.	Is the volunteer willing to have his/her picture taken for the study ID, or provide a photo ID and/or fingerprint?		☐ 1	☐ 0
9.	Does the volunteer understand English or local language as approved by IRB?		☐ 1	☐ 0
10.	Does the volunteer meet the risk category for eligibility in the study?	☐ 8 NA	☐ 1	☐ 0
11.	Is the volunteer free of any significant condition (including medical and psychological/psychiatric disorder) that in the opinion of the study investigator might interfere with the conduct of the study?	☐ 8 NA	☐ 1	☐ 0

If the check box is marked "No", the volunteer is not eligible for enrollment into the study.

Is the volunteer eligible for inclusion into the study? **Yes** ☐ 1 **No** ☐ 0

Form Completed by:

Print Name: _____

Signature: _____

Date Signed
DD/MON/YYYY
__ __/__ __ __/__ __ __ __

Site	RV217	Echo Study	Visit: A Phase 1
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		

Date of History
DD/MON/YYYY __ __/__ __ __/__ __ __ __

PREVIOUS ILLNESSES

☐ Check If **All** answers are **"No"**

ILLNESS	Ever Had?		Have it now?	
	No	Yes	No	Yes
Heart Disease	☐ 0	☐ 1	☐ 0	☐ 1
Renal Disease	☐ 0	☐ 1	☐ 0	☐ 1
Diabetes	☐ 0	☐ 1	☐ 0	☐ 1
Thyroid or other hormonal disorder	☐ 0	☐ 1	☐ 0	☐ 1
Psychiatric disease or depression	☐ 0	☐ 1	☐ 0	☐ 1
Cancer	☐ 0	☐ 1	☐ 0	☐ 1
Liver problem	☐ 0	☐ 1	☐ 0	☐ 1
Tuberculosis (treated: ☐ yes ☐ no)	☐ 0	☐ 1	☐ 0	☐ 1
Sexually Transmitted Infections	☐ 0	☐ 1	☐ 0	☐ 1
Allergies to Drug/Food	☐ 0	☐ 1	☐ 0	☐ 1
Allergic Reaction to Vaccination	☐ 0	☐ 1	☐ 0	☐ 1
Migraine or Seizures	☐ 0	☐ 1	☐ 0	☐ 1
Perineal/Anal Conditions	☐ 0	☐ 1	☐ 0	☐ 1
Other (1), specify _____	☐ 0	☐ 1	☐ 0	☐ 1
Other (2), specify _____	☐ 0	☐ 1	☐ 0	☐ 1
Other (3), specify _____	☐ 0	☐ 1	☐ 0	☐ 1

Interviewer:
(Print Name) _____

Site	*RV217*	*Echo Study*	**Visit: A Phase 1**
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		

DD/MON/YYYY __ __/__ __ __/__ __ __ __

RECENT MEDICAL HISTORY

☐ Check If **All** answers are **"No"**

Genitourinary Symptom	Past 3 months?		Have it now?	
	No	**Yes**	**No**	**Yes**
Burning during urination	☐ 0	☐ 1	☐ 0	☐ 1
Urethral discharge	☐ 0	☐ 1	☐ 0	☐ 1
Difficulties with defecation and / or rectal discharge	☐ 0	☐ 1	☐ 0	☐ 1
Scrotal swelling	☐ 0	☐ 1	☐ 0	☐ 1
Lower abdominal pain and painful sex	☐ 0	☐ 1	☐ 0	☐ 1
Vaginal discharge with itching and / or foul smell	☐ 0	☐ 1	☐ 0	☐ 1
Itching and/or discomfort in the perineum and / or perianal and pubic region	☐ 0	☐ 1	☐ 0	☐ 1
Anogenital ulcer	☐ 0	☐ 1	☐ 0	☐ 1
Genital Warts	☐ 0	☐ 1	☐ 0	☐ 1
Genital rashes, lumps and sores	☐ 0	☐ 1	☐ 0	☐ 1
Abnormal neovaginal or rectal bleeding	☐ 0	☐ 1	☐ 0	☐ 1
Other Medical (1), specify _____	☐ 0	☐ 1	☐ 0	☐ 1
Other Medical (2), specify _____	☐ 0	☐ 1	☐ 0	☐ 1
Other Medical (3), specify _____	☐ 0	☐ 1	☐ 0	☐ 1

Interviewer:
(Print Name) _____

Site		
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	***RV217*** **Study Number** ___ ___ ___ ___ ___	***Echo Study***	**Visit: A** **Phase 1**

Date of History
DD/MON/YYYY __ __/__ __ __/__ __ __ __

RECENT MEDICAL HISTORY	☐ Check If **All** answers are **"No"**			
General Symptoms	**Past 3 months?**		**Have it now?**	
	No	**Yes**	**No**	**Yes**
Fever	☐ 0	☐ 1	☐ 0	☐ 1
Skin Rash	☐ 0	☐ 1	☐ 0	☐ 1
Unintentional weight loss	☐ 0	☐ 1	☐ 0	☐ 1
Diarrhea	☐ 0	☐ 1	☐ 0	☐ 1
Sores in the mouth	☐ 0	☐ 1	☐ 0	☐ 1
Headache	☐ 0	☐ 1	☐ 0	☐ 1
Feeling of the light hurting your eyes	☐ 0	☐ 1	☐ 0	☐ 1
Stiff or painful neck	☐ 0	☐ 1	☐ 0	☐ 1
Nausea	☐ 0	☐ 1	☐ 0	☐ 1
Abdominal pain	☐ 0	☐ 1	☐ 0	☐ 1
Loss of appetite	☐ 0	☐ 1	☐ 0	☐ 1
Confusion / difficulty thinking	☐ 0	☐ 1	☐ 0	☐ 1
Night sweats	☐ 0	☐ 1	☐ 0	☐ 1
Fatigue	☐ 0	☐ 1	☐ 0	☐ 1
Muscle aches	☐ 0	☐ 1	☐ 0	☐ 1
Joint aches	☐ 0	☐ 1	☐ 0	☐ 1
Feeling of being ill or sick	☐ 0	☐ 1	☐ 0	☐ 1
Feeling of loss or absence of energy	☐ 0	☐ 1	☐ 0	☐ 1
Sore throat	☐ 0	☐ 1	☐ 0	☐ 1
Pain behind your eyes	☐ 0	☐ 1	☐ 0	☐ 1
Vomiting	☐ 0	☐ 1	☐ 0	☐ 1
Cough	☐ 0	☐ 1	☐ 0	☐ 1
Shortness of breath	☐ 0	☐ 1	☐ 0	☐ 1

Interviewer:
(Print Name) _____

Recent Medical History RMH3

Site	RV217	Echo Study	Visit:____
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** __ __ __ __ __		

Date of History
DD/MON/YYYY __ __/__ __ __/__ __ __ __

RECENT MEDICAL HISTORY

Other Medical Conditions	Past 3 months?		Have it now?	
	No	Yes	No	Yes
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1
	☐ 0	☐ 1	☐ 0	☐ 1

Interviewer:
(Print Name) ______________________________

Site	RV217	Echo Study	Visit: A Phase 1
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		

Date of Exam
DD/MON/YYYY __ __/__ __ __/__ __ __ __

VITAL SIGNS — Obtained by: *(Print Name)* _____

Height *(cm)*	Weight *(kg)*	Temperature *(˚C)* ☐ 1 *Oral* ☐ 2 *Axillary*	SITTING MEASUREMENTS: Pulse *(beats/min)*	Respirations *(breaths/min)*	Systolic BP *(mmHg)*	Diastolic BP *(mmHg)*

COMPLETE PHYSICAL EXAMINATION

Examine the following and check appropriate box for each body system

BODY SYSTEM	Normal	Abnormal	Not Done	ONLY COMMENT ON ABNORMALITIES
General Appearance	☐ 0	☐ 1	☐ 9	
Skin	☐ 0	☐ 1	☐ 9	
HEENT	☐ 0	☐ 1	☐ 9	
Lymphatic	☐ 0	☐ 1	☐ 9	
Pulmonary	☐ 0	☐ 1	☐ 9	
Cardiovascular	☐ 0	☐ 1	☐ 9	
Abdomen	☐ 0	☐ 1	☐ 9	
Genitourinary	☐ 0	☐ 1	☐ 9	
Musculo-skeletal	☐ 0	☐ 1	☐ 9	
Spine & Extremities	☐ 0	☐ 1	☐ 9	
Neurologic	☐ 0	☐ 1	☐ 9	
Other (1), *specify* _____	☐ 0	☐ 1	☐ 9	
Other (2), *specify* _____	☐ 0	☐ 1	☐ 9	
Other (3), *specify* _____	☐ 0	☐ 1	☐ 9	

Obtained by: *(Print Name)* _____

Site	RV217	Echo Study	Visit: B Phase 1
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		

Date of Exam
DD/MON/YYYY __ __/__ __ __/__ __ __ __

VITAL SIGNS

Obtained by:
(Print Name) _____

Height *(cm)*	Weight *(kg)*	Temperature *(˚C)* ☐ 1 *Oral* ☐ 2 *Axillary*	SITTING MEASUREMENTS			
			Pulse *(beats/min)*	**Respirations** *(breaths/min)*	**Systolic BP** *(mmHg)*	**Diastolic BP** *(mmHg)*

DIRECTED PHYSICAL EXAMINATION

Examine the following and check appropriate box for each body system

☐ Check If Not Indicated

BODY SYSTEM	Normal	Abnormal	Not Done	ONLY COMMENT ON ABNORMALITIES
General Appearance	☐ 0	☐ 1	☐ 9	
Skin	☐ 0	☐ 1	☐ 9	
HEENT	☐ 0	☐ 1	☐ 9	
Lymphatic	☐ 0	☐ 1	☐ 9	
Pulmonary	☐ 0	☐ 1	☐ 9	
Cardiovascular	☐ 0	☐ 1	☐ 9	
Abdomen	☐ 0	☐ 1	☐ 9	
Genitourinary	☐ 0	☐ 1	☐ 9	
Musculo-skeletal	☐ 0	☐ 1	☐ 9	
Spine & Extremities	☐ 0	☐ 1	☐ 9	
Neurologic	☐ 0	☐ 1	☐ 9	
Other (1), *specify* _____	☐ 0	☐ 1	☐ 9	
Other (2), *specify* _____	☐ 0	☐ 1	☐ 9	
Other (3), *specify* _____	☐ 0	☐ 1	☐ 9	

Obtained by:
(Print Name) _____

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		**Visit:** _____

RV217 STATUS CHANGE

Date of Visit
DD/MON/YYYY __ __/__ __ __/__ __ __ __

Date of last lettered or numbered visit completed before the change occurred:

__ __/__ __ __/__ __ __ __
DD/MON/YYYY

Type of Status Change?

☐ 0 Discontinued early from study, complete a TOS Form

☐ 1 Basic Surveillance to Stutter Phase (PI to PIB)

☐ 2 Stutter Phase to Acute HIV Infection (PIB to PII)

☐ 3 Stutter Phase to Basic Surveillance Phase (PIB to PI)

Other Status Change *(check if applicable):*

☐ 4 Retained for masking

☐ 5 Became incarcerated

☐ 6 Re-consent in study and resume continuation in the study

☐ 9 Other status change, specify _____

Biweekly Visit Summary

BVS

Site	RV217	Echo Study	Visit: _____
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		

BVS Summary

Date of Visit
DD/MON/YYYY __ __/__ __ __/__ __ __ __

Number of scheduled BVS visits in the interval: ____ ____

Number of attended visits with a blood draw: ____ ____

Participant Completed:

☐ 0 <25% of visits
☐ 1 25%-50% of visits
☐ 2 51%-75% of visits
☐ 3 >75% of visits

Continuing to participate in the study?

☐ 1 No
☐ 2 Yes

If participant completed less than 50% of visits and is continuing in the study specify reason:

_____

_____

_____

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	Study Number __ __ __ __ __		**Visit:** _____

TERMINATION FROM STUDY

Date of Termination from Study
DD/MON/YYYY __ __/__ __ __/__ __ __ __

What was the last lettered or numbered visit completed by the participant? _____

Reason for Termination

☐ 1 Per Protocol (Completed Phase I or Phase II)

☐ 2 Withdrawal of consent (participant has indicated that participation is terminated permanently and no further contact is permitted)

Does participant permit use of specimens collected? ☐ 0 No ☐ 1 Yes

☐ 3 Death, complete additional information below

Date of Death __ __/__ __ __/__ __ __ __
D D M O N Y Y Y Y

Primary Cause of Death ☐ 8 Unknown ☐ 1 Other, specify _____

☐ 4 Non-compliance with visit schedule

☐ 5 Protocol Violation, specify _____

☐ 6 Concurrent Illness, specify _____

☐ 7 Reaction to blood collection, specify _____

☐ 8 Lost To Follow-up

☐ 9 Family or job conflict, specify_____

☐ 10 Refused or unable to continue, specify if known_____

☐ 11 Other Reason, specify _____

PI Signature	Date Signed *DD/MON/YYYY* __ __/__ __ __/__ __ __ __

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	**Study Number** ___ ___ ___ ___ ___		**Visit:** _____

CRF REVIEW STATEMENT

I have reviewed all data contained in this case report form binder and verified that the contents are consistent with observations and source records. They accurately reflect the condition of the participant before, during and at the completion of the study.

Principal Investigator's Signature: **Date Signed:**

_____ __ __/__ __ __/__ __ __ __
D D M O N Y Y Y Y

PREGNANCY REPORT - Page _____ of _____ **PREG REP**

Site	RV217	Echo Study	
☐ 1 *MUWRP Uganda* ☐ 2 *WRP Kenya* ☐ 3 *MMRP Tanzania* ☐ 4 *AFRIMS Thailand*	Subject Number ____ ____ ____ ____ ____		**Visit:** _____

Date of Last Menstrual Period __ __/__ __ __/__ __ __ __ (D D M O N Y Y Y Y)

Estimated Date of Confinement __ __/__ __ __/__ __ __ __ (D D M O N Y Y Y Y)

PART A: Past OB history of volunteer

Number of previous pregnancies: _____ Number of live births: _____ Number of still births: _____

Number of children still alive: _____ Number of abortions : _____

Does the subject have a history of children born with congenital abnormalities? ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

If yes, description of congenital abnormalities: _____

Does the subject have a history of spontaneous abortions ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

PART B: Current pregnancy and Outcomes

1. Outcome of Pregnancy ☐ 1 Live Birth ☐ 2 Still Born ☐ 3 Terminated

Date of Delivery: __ __/__ __ __/__ __ __ __ (D D M O N Y Y Y Y) Gestational Age at Delivery: ____ weeks ☐ Unknown

Place of Delivery: ☐ 1 Home ☐ 2 Hospital

Method of Delivery: ☐ 1 Vaginal delivery ☐ 2 C-Section

Delivery by Skilled Birth Attendant: ☐ 1 Yes ☐ 0 No

Date of termination/abortion: __ __/__ __ __/__ __ __ __ (D D M O N Y Y Y Y) ☐ Unknown

Weeks of Gestation at termination/abortion: _____ ☐ Unknown

2. Complications during Pregnancy ☐ 1 Yes ☐ 0 No If Yes: *(check all that apply)*

☐ Illness ☐ Maternal Age (AMA) ☐ Maternal Drug Use ☐ Alcohol Use

☐ Hospitalization due to complications during pregnancy please explain _____

☐ Other infections during current pregnancy: ☐ Syphilis ☐ GC ☐ Chlamydia ☐ Herpes

☐ Other _____ ☐ Unknown

3. Mother's HIV status at delivery/termination: ☐ 0 Negative ☐ 1 Positive ☐ 8 Unknown

4. Gestational Age at HIV Diagnosis _____weeks ☐ N/A ☐ Unknown

5. Was volunteer offered enrollment into PMTCT program or received prenatal care during pregnancy ? 1 ☐ Yes ☐ 0 No

6. Was ARV therapy initiated ☐ 1 Yes ☐ 0 No ☐ 3 N/A **a.** If Yes, trimester/ weeks of gestation when ARV was initiated? _____

b. Duration of ARV therapy? _____ **c.** Was ARV therapy taken as prescribed ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

d. Type of ARV prescribed *(check all that apply)* ☐ Antepartum AZT ☐ Antepartum triple ARV prophylaxis

☐ Antepartum HAART ☐ Intrapartum SD NVP ☐ Intrapartum AZT ☐ Intrapartum 3TC

☐ Postpartum AZT ☐ Postpartum 3TC ☐ Postpartum triple ARV ☐ Postpartum HAART

☐ Other _____ ☐ Unknown Antepartum therapy ☐ Unknown Intrapartum therapy

BIRTH REPORT - Page _____ of _____ **BIRTH**

Site	RV217	Echo Study	Visit: _____
☐1 *MUWRP Uganda* ☐2 *WRP Kenya* ☐3 *MMRP Tanzania* ☐4 *AFRIMS Thailand*	**Subject Number** ____ ____ ____ ____ ____		

Infant Identifier: _____ ☐ 0 Male ☐ 1 Female Birth Weight: ___ ___ ___ ___ gm

Estimated Gestational Age at Delivery: ___ ___ weeks

APGAR Score (at 5mins): __ __ or ☐ Unknown Date of delivery __ __/__ __ __/__ __ __ __ (*D D M O N Y Y Y Y*)

Was the infant born with congenital anomalies ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

If Yes, please describe: _____

☐ **Check if Mother was HIV Negative at time of delivery**

1. Was infant prescribed ARV? ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

2. What type of ARV/prophylaxis prescribed: (*check all that apply)* ☐ Co-trimoxazole (Bactrim/Septra/Septrim)

☐ Continuous NVP ☐ 3TC ☐ AZT ☐ Triple ARV ☐ SD NVP

☐ Other (*please specify*)_____ ☐ Unknown

3. Duration of ARV prescribed to child post-partum : _____ days _____ weeks _____ months ☐ Unknown

4. Was ARV given to infant as prescribed: ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

5. Was mother counseled on Infant Feeding: ☐ 1 Yes ☐ 0 No ☐ 8 Unknown

If Yes, which method was use: ☐ 1 Exclusive breastfeeding ☐ 2 Exclusive Replacement Feeding ☐ 3 Mixed Feeding

6. Duration of Breastfeeding: _____ days _____ weeks _____ months ☐ Not Applicable ☐ 8 Unknown

7. Current Health Status of Infant: ☐ 1 Alive and well ☐ 2 Alive and ill ☐ 8 Unknown

☐ 3 Deceased: date: __ __/__ __ __/__ __ __ __ (*D D M O N Y Y Y Y*) ☐ Unknown Date Age: _____ days _____ months

8. Infant HIV testing:

0-6 weeks: DNA PCR ☐ 0 Negative ☐ 1 Positive ☐ 2 Indeterminate ☐ 8 Unknown

VL (RNA PCR): _____ EIA/WB _____ RDT _____ ☐ Unknown

☐ **No additional infant information to report for this pregnancy.**

Site ☐1 *MUWRP Uganda* ☐2 *WRP Kenya* ☐3 *MMRP Tanzania* ☐4 *AFRIMS Thailand*	***RV217***	***Echo Study***	**Visit: _____**
	Subject Number ___ ___ ___ ___ ___		

Infant Identifier: _____ ☐ Male ☐ Female | Date of Delivery: __ __/__ __ __/__ __ __ __ (*D D M O N Y Y Y Y*)

☐ **Check if Mother was HIV Negative at time of delivery**

HIV Status of Infant

1. 6wks-9months: ☐ 0 Negative ☐ 1 Positive ☐ 2 Indeterminate ☐ 8 Unknown

2. 9-18 months: ☐ 0 Negative ☐ 1 Positive ☐ 2 Indeterminate ☐ 8 Unknown

3. > 18 months: ☐ 0 Negative ☐ 1 Positive ☐ 2 Indeterminate ☐ 8 Unknown

4. Definitive HIV status of infant: ☐ 0 Negative ☐ 1 Positive ☐ 8 Unknown

. **a.** If Positive, was infant started on ART: ☐ 1 Yes ☐ 0 No

b. If Yes, duration of ART: _____ days _____ weeks _____ months ☐ On-going ART therapy

☐ **No additional infant information to report for this pregnancy.**

Site	RV217	Echo Study	Visit: _____
☐ 1 MUWRP Uganda ☐ 2 WRP Kenya ☐ 3 MMRP Tanzania ☐ 4 AFRIMS Thailand	**Study Number** ___ ___ ___ ___ ___	**Visit Date:** |__|__|-|__|__|__|-|__|__|__|__|	**Phase 2**

MEDICATION RECORD

☐ Check if **None** *(Never taken ARVs)*

Baseline Visit: *Record medications subject is currently taking, and also prior antiretroviral regimens.*

Subsequent Visits*: Refer to medications listed on the this form from the last visit, record medications the subject is still taking and medications the subject has stopped and why (discontinuation code), and add any medications since the last visit.*

***PEP** = post exposure prophylaxis* ***PMTCT** = prevention of mother to child transmission*

Medication	Indication*	Start Date	Stop Date	Stop Codes
☐ 1. Abacavir (ABC)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 2. Didanosine (ddl)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 3. Didanosine (ddl ec)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 4. Lamivudine (3TC)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 5. Stavudine (d4T)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 6. Tenofovir (TDF)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 7. Zidovudine (AZT)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 8. Efavirenz (EFV)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 9. Nevirapine (NVP)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 10. Atazanavir (ATZ)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 11. Lopinavir/Ritonavir (Kaletra or Alluvia)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 12. Ritonavir Boost	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 13. Saquinavir	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____
☐ 14. Emtricitabine (FTC)	☐ a. PEP ☐ b. PMTCT ☐ c. HIV Treatment	|__|__|__|-|__|__|__|__|	|__|__|__|-|__|__|__|__| ☐ Ongoing	_____

Does the pill count confirm adherence to ARVs? ☐ 1. Yes ☐ 0. No ☐ 7. Not Performed

ATTACHMENT II: COLLABORATING INSTITUTIONS

RV217 External Collaborator List 21 MAY 2012

Collaborator Name	Institution	Work to be done	Sample types used	PI Contact
George Shaw	UPenn	Sequencing	ACD plasma	George M. Shaw, M.D., Ph.D. Perelman School Of Medicine University of Pennsylvania 409 Johnson Pavilion 3610 Hamilton Walk Philadelphia, PA 19104 215-746-8514 Fax 215-573-8976 gshaw@uab.edu
Andrew McMichael Nilu Goonetilleke	Oxford	T cell immunology	Cells	Nilu Goonetilleke nilu.goonetilleke@ndm.ox.ac.uk CHAVI Group WIMM. John Radcliffe Hospital Oxford 0x39sj +44 (0) 1865 222 145
David Montefiori	Duke	Infectious titer and neutralzing antibody	ACD Plasma	David C. Montefiori, Ph.D. Department of Surgery SORF Bldg., LaSalle St. Ext., Rm 130 Duke University Medical Center Durham, NC 27710 Tel 919-684-5278 Fax 919-684-4288
Georgia Tomaras	Duke (DHVI)	Humoral Immunology	ACD Plasma	Georgia D. Tomaras, Ph.D., Assistant Professor 919) 681-5598, gdt@duke.edu
Tony Moody Bart Haynes	Duke	B cell tetramer	Cells	M. Anthony Moody, MD, Assistant Professor of Pediatrics (919) 668-2551, moody007@mc.duke.edu Barton F. Haynes, MD, Professor and Director DHVI ((919) 684-5279, hayne002@mc.duke.edu

RV217 External Collaborator List 21 MAY 2012

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Rafick Sekaly	VGTI	HIV quantification, microarrays, systems biology, T cell immunology	Cells/Plasma	pierre.sekaly@umontreal.ca, sekaly@vgti-fl.org
Bette Korber	LANL	Sequencing/analysis	Sequences and plasma/PBMC	MS K710, T-10 Los Alamos National Laboratory Los Alamos, NM 87545 Tel: 505-665-4453 Fax: 505-665-3493 E-mail: btk@lanl.go
Danny Douek	VRC / NIH	TCR clonotypes	PBMC	Daniel Douek, M.D., MRCP, Ph.D. **Phone:** 301-594-8484 **Fax:** 301-480-2565 Vaccine Research Center Bldg 40 Room 3504 MSC 3022 Bethesda, MD 20892-3005
John Mascola	VRC/NIH	Humoral Immunology	Plasma	Dr. John Mascola NIH/Vaccine Research Center 40 Convent Drive Bldg. 40, Room 4500 Bethesda, MD 20892-3005
Doug Richman Davey Smith Susan Little	University of California, San Francisco	Sequencing, neutralizing antibody	Plasma	Douglas Richman, M.D. Professor of Pathology and Medicine University of California, San Diego Stein Clinical Research Building, Room 329 La Jolla, CA 92093 Telephone: (858) 552-8585 x 7439 Email: drichman@ucsd.edu
Richard A Jenkins	National Inst. On Drug Abuse (NIDA)	Questionnaire Analysis	Behavioral Data	Richard A. Jenkins, Ph.D. Health Scientist Administrator (301) 443-1923

RV217 External Collaborator List 21 MAY 2012

	Prevention Research Branch (PRB)			jenkinsri@mail.nih.gov

LIST OF CHANGES FOR RV 217 AMENDMENT

A. Site-Specific Addenda:

1. African sites may permit enrollment for female workers in bars, shops, and stores in high transmission locations. Added footnote to Section 5.2: [1] Any modification to the three African sites enrollment criteria will be described in the site-specific addenda.
2. Removal of Boda-Boda workers and STIAs as a target population for Uganda in Part B.
3. Revision of Tanzania SSA substudy: "The influence of pre-existing infections on HIV disease progression"

B. Investigator Changes:

1. Main Protocol and RV 217a: Associate PI: Delete David Guwatudde, PhD
2. Main Protocol: Removed Oliver Hoffmann, MD; Jeff Currier, PhD and Francine McCutchan, PhD
3. Main Protocol: Added Leigh Anne Eller, MSc., Mark de Souza, PhD., and Amy Weintrob, MD as AIs.
4. Main Protocol: Address changes for MHRP investigators.
5. RV 217c: PI: Add Erica Sanga and Christof Geldmacher as AIs
6. RV 217b: Removed George Nyale
7. Deleted Medical Monitors
8. Addition of collaborating institutions that will participate in analysis, to include Rich Jenkins

C. Protocol

1. Blood volumes have been adjusted to accommodate intended analytic plans in view of experience in the lab since submission of the original protocol and the addition of gene expression analysis at a lab outside MHRP, which requires its own dedicated sample.
2. Summary, revised the following sentence (where bold) to reflect the change of SBVs from twice-weekly to monthly after visit G:

   *These "small blood volume" (SBV) visits will occur twice weekly **(until the participant completes visit G, and then will occur once per month)** and afford the opportunity to diagnose HIV infection prior to the advent of detectable antibody by the most sensitive techniques available.*
3. Schema, under "Primary Objectives" item #3—removed 3.7% incidence criterion and modified to note that study team will *observe an **increased** incidence with at least 30% of the incident cases identified prior to the advent of detectable antibody.*

   This change has been made throughout the protocol.
4. Schema, under "Participants", 2nd paragraph, last sentence added: ***Some shifting of enrollment numbers per site might occur in order to complete the study at the same time at all sites.***
5. Schema, under "Study Duration", 2nd sentence: Removed the bolded language:

   *Approximately nine months will be required for enrollment into Part A at all sites **and assessment of protocol operations based upon retention and compliance characteristics will require an additional 12 months.***
6. Schema, under "Study Duration" and throughout protocol: Added the following as the penultimate statement:

   Part B will commence when a site achieves 3 Fiebig 1/ 2 AHI and documents an increased incidence.
7. Section 1.1 State of MHRP Vaccine Research: added results of the Thai Phase III trial starting in paragraph 5:

   The results of the Phase III HIV vaccine clinical trial involving more than 16,000 adult volunteers in Thailand demonstrated that an investigational HIV vaccine regimen was safe and

modestly effective in preventing HIV infection in the modified intent-to-treat analysis. According to final results released by the trial sponsor, the U.S. Army Surgeon General, the prime boost combination of ALVAC® HIV and AIDSVAX® B/E lowered the rate of HIV infection by 31.2% compared with placebo.

In the final analysis, 74 placebo recipients became infected with HIV compared to 51 in the vaccine regimen arm. The efficacy result is statistically significant as the lower bound of the 95% confidence interval is above zero. The vaccine regimen had no effect on the amount of virus in the blood of volunteers who became HIV-infected during the study.

*This study, RV144, tested a prime-boost vaccine strategy that combined two vaccines based on strains (subtypes) of HIV that circulate in Thailand. The prime canary pox vector called ALVAC-HIV vCP1521, was developed by Sanofi Pasteur; **t**he booster vaccine, AIDSVAX B/E, that was previously tested in two efficacy studies and developed by VaxGen but now licensed to Global Solutions for Infectious Diseases. The results of RV 144 demonstrate that an efficacious HIV vaccine for the prevention of HIV vaccine is now possible, and we plan to utilize the cohorts developed through this proposal for further efficacy testing.*

8. Section 1.5.1 HIV Diagnostics: Added the following subsection:

   Smart Maximal Antibody Response Tube, SMARTube, for *in vitro* stimulation of anti-HIV antibody from *in vivo* primed B lymphocytes

   The Smart Maximal Antibody Response Tube, SMARTube HIV & HCV (ST), is a tissue culture tube containing 2 ml of culture medium with a proprietary cocktail of stimuli which promote proliferation and differentiation of HIV or HCV antigen primed B lymphocytes from fresh whole blood specimens. Two published reports outlined below and discussions with the product developer suggest that this pretreatment device accelerates production of anti-HIV antibody to levels detectable by commercially available serological diagnostic assays and thus enables detection and diagnosis of window period seronegative acute/primary HIV infected individuals weeks to months prior to seroconversion (Novikov I 2009; Mumo J 2008). In corporation of this assay into ECHO screening may provide a powerful means to resolve the infection status of ECHO participants who periodically test Aptima reactive, but seemingly do not progress; thus, providing a means to discriminate between Aptima false reactive ("spurious blips") and the actual detection of RNA signal and resolution of HIV infection status.

   Novikov and Jehuda-Cohen report the development of this "Stimmulogy" assay and its use to identify seronegative HIV infected individuals among two Ethiopian émigré populations immigrating to Israel in 1992 and 1998. Parallel serological testing of paired de-identified pre and ST stimulated herapinized samples was conducted. Pre ST plasma samples were tested directly by standard EIA. For ST samples, 1 ml of heparinized whole blood was added to the culture tube and incubated in a 5% CO_2 humidified incubator at 37^0C for five days. Post incubation ST plasma was tested by conventional HIV enzyme immunoassays (EIA) following adjustment of EIA diluents to compensate for dilution into the smart media. Studies with Smart media and post incubation stimulated ST plasma demonstrated that neither ST plasma, nor Smart media impacted the signal to cut-off for EIAs in conventional HIV EIAs. Of 285 samples from the 1992 population, 7/285 were HIV infected by both standard and ST EIA. An additional 8/285 (2.7%) were identified as HIV EIA reactive, HIV Western Blot positive post ST stimulation. Of these, 5/8 pre stimulation and 2/8 post stimulation samples were PCR positive. Of the 1998 population samples, 26/537 (4.84%) pre stimulated samples were HIV positive. Post ST stimulation an additional 2/537 window period seronegative HIV infected individuals were identified (Novikov I 2009). Mumo et al 2009 using the same approach examining adult and student Kenyan blood donors demonstrated similar findings.

   In addition to the aforementioned studies, Mumo et al, 2009 demonstrated that this assay identified HIV infected individuals weeks to months prior to seroconversion and detection by standard serological screening assays. Of 20 pregnant women visiting an antenatal clinic and tested for HIV antibody, 7/20 were HIV infected by both standard and ST EIA. Among the 13 seronegative women, 5/20 were HIV antibody reactive post ST stimulation. Serconversion

estimates based on taking the midpoint between the time of last seronegative and first seroreactive sample by standard EIA of serial samples indicated that ST stimulation detected seronegative individuals 3-10 months earlier than standard EIA (Mumo J 2008). This phenomena, earlier detection weeks to months prior to seroconversion, has also reportedly been demonstrated in current clinical trials underway with this device (personal communication, Dr. Jehuda-Cohen).

Finally, application of this technology to recent infection detection has been proposed. While not yet fully mature as a concept, a Stimulation Index for estimating incidence based on a semi-quantitative comparison of antibody levels in plasma to ST stimulated plasma has been proposed and is under consideration and development.

These provocative findings strongly suggest, albeit they have not been independently corroborated, that ST stimulation can accelerate the production of anti-HIV antibody from HIV primed lymphocyte populations leading to early detection and diagnosis in seronegative HIV infected individuals. If true, these findings also suggest that the immune system may be capable of regulating viral exposure and subsequent progress of infection for prolonged periods in some individuals; that the eclipse phase extends well beyond current consideration

9. Section 1.5.3: Added following subsection:

Analysis of HIV Antigen Specific Immune Responses in Uninfected Volunteers

Transient elevation of type I interferons and other cytokines and chemokines occurs prior to peak viremia in acute HIV infection, followed by TNF-α and IFN-γ, suggesting that systemic activation of plasmacytoid dendritic cells leads to a cascade of to activation and recruitment of other immune cells including NK cells and eventually T cells. Recently, Tiemessen, CT. et al demonstrated that HIV-1 specific responses in NK cells could be detected in the CD3-/CD8+ compartment only in the presence of plasma from fresh heparinized whole blood in infants born to HIV+ mothers who remained uninfected (Tiemessen 2009). The ability to reproduce and build upon this finding in other cohorts, especially those with other types of repetitive high risk HIV exposure, is critical to understanding innate HIV specific immune protection.

And

Regulatory T cells

Regulatory T-cells have been proposed to down regulate the immune reponse within the CD4 and CD8 T-cell compartments and loss of their function due to HIV-1 infection may be associated with hyperimmune activation and subsequent CD4 depletion observed in natural infection resulting in disease progression (Eggena, 2005). Some of the commonly distinguishable markers of regulatory T cells include CD25 (IL-2 receptor) bright expression, CD62L (L-selectin) high, and Foxp3 (forkhead box protein 3 transcription factor). Most studies have addressed immune activation and the role of regulatory T-cells in the context of cross sectional studies or longitudinal analysis of HIV positive volunteers on antiretroviral therapy, therefore not much is characterized about early HIV infection. Additionally, it would be useful to describe the effects of infecting HIV-1 subtype on the immune system as measured through activation and regulatory cell surface markers.

And

Functional Genomics and HIV Surrogate Marker Exploration

Functional genomics is a powerful tool to investigate host responses to infection and therapy. The success of gene chips is due, in part, to their ability to interrogate the entire genome, offering

the broadest and most comprehensive coverage of the transcriptome. Many genetic polymorphisms affect disease trajectory, drug response or propensity to develop drug toxicities. Known host determinants of HIV-1 disease progression include genes for chemokine receptors (eg, CCR5 delta 32) and HLA molecules (eg, B57 and B27), natural killer cell killer immunoglobulin-like receptor (KIR3DS1), and APOBEC3F and APOBEC3G proteins. There are likely more unknown host genetic determinants yet to be discovered. While the search for elusive correlates of protection from HIV disease progression continues, we propose to use global gene expression profiling because it offers the most comprehensive coverage of the human genome - especially those genes involved in immunity or response to viral infection. Global gene expression profiling of the earliest emergent adaptive HIV-specific T cells in acute infection provides the most likely approach to discovery of novel correlate(s) of protection. In combination with other novel and exploration assay systems it would from a central component of a systematic multi-factorial approach to identify critical facets of the adaptive immune response associated with control or delay of initial peak viremia.

And

The Proteome Analysis of Activated CD4+ T-Cells Infected with Different Clades of HIV-1
MHC class I molecules are expressed on nucleated cells whereas MHC class II molecules are predominantly expressed on professional antigen presenting cells. For the clearance of intracellular pathogens, epitopes bound to either MHC class I or MHC class II molecules are presented at the cell surface for interaction with CD8+ T-cells and CD4+ T-cells, respectively (Cresswell 2005; Ramachandra 2009). Antigen processing and presentation is a complicated process and there are significant differences between MHC class I and class II pathways, although antigens that enter the MHC class II pathway are capable of being presented by MHC class I molecules and vice versa (Giodini 2010).

MHC class I processing involves many proteins such as ubiquitination proteins, chaperone proteins, loading and transporter proteins, and proteases. These proteins need to work in a defined order for the antigens in the cytosol to be proteolytically cleaved and presented on the cell surface as an 8-10 amino acid epitope bound to an MHC class I molecule (Rock 2004). In MHC class II processing, antigens are taken up by the cell through endocytosis / phagocytosis. The antigens are processed using a different series of proteases and chaperone molecules and presented on the cell surface as a 12-15 amino acid epitope bound to an MHC class II molecule (Ramachandra 2009). Disruption of any protein(s) involved in the MHC class I or class II pathways can either negate the presentation of antigenic epitopes on these molecules or lead to an altered epitope repertoire.

Intracellular pathogens have devised numerous methods for evading the host's immune response. One principal mechanism is by interfering with antigen processing and presentation thus preventing the appearance of foreign peptides on the cell surface (Loureiro 2006). The predominant protease is the proteasome complex that is required for the creation of a vast majority of MHC class I precursor epitopes. The proteasome consists of two forms, the constitutive proteasome found in all cell types and the immunoproteasome found in cells activated with interferongamma. The proteasome is a barrel shaped complex of 4 rings consisting of 28 subunits. The alpha subunits (a1-a7) make up the outer rings and the beta subunits (b1-b7) make up the inner rings. The three active enzymatic subunits of the constitutive proteasome, b1, b2, and b5 are replaced with inducible subunits (b1i, b2i, and b5i) for the formation of the immunoproteasomes, leading to changes in the enzymatic activity of the proteasome complex and an altered epitope repertoire (Klotzel 2004; Steers 2008).

HIV-1 interferes with antigen processing and presentation by disrupting the composition of the

immunoproteasome and by down regulating the MHC class I molecule. HIV-1 Tat, an early stage antigen prevents the b1i subunit incorporation into the immunoproteasome (Gavioli 2004) and HIV-1 Gag-p24, a late stage antigen alters the immunoproteasome composition by interfering with the PA28b and b2i subunits (Steers 2009). The alteration of the immunoproteasome composition at different stages of the viral replication cycle could potentially alter the repertoire of MHC class I precursor epitopes. HIV-1 Nef protein decreases the expression of MHC class I molecules at the cell surface (Stove 2006) thus preventing the HIV-1-peptide-MHC complex from interacting with cytotoxic CD8+ T-cell lymphocytes. Presently, the impact of HIV-1 infection on other proteins intricately involved in MHC class I processing and presentation is unknown.

10. Section 1.5.4: Added to the end of the section:

    In addition to studies mentioned above, it is important to examine the antibodies that recognize lipids in serum of HIV-1 infected patients during the course of acute HIV-1 infection. Naturally occurring antibodies to many types of phospholipids are present in normal human sera (Alving1984; Cabiedes 1998), and antibodies to phospholipids are also commonly transiently induced as an epiphenomenon during many infectious diseases (Vaarala 1986), including HIV-1 (Silvestris 1996). The possibility exists that some or many of the antibodies in the plasma pools have the ability to recognize pure lipids. Such antibodies would be analogous to, or similar to, the broadly neutralizing human 4E10 and 2F5 monoclonal antibodies that bind to pure phospholipids (Haynes 2005). The existence of these antibodies would also be analogous to the observation that monoclonal antibodies to phosphatidylinositol-4-phosphate (PIP) neutralize primary isolates of HIV-1, as shown by our demonstration of such activities (Brown 2007). As an illustration of the relevance of such antibodies, it has been demonstrated that the 4E10 monoclonal antibody also binds to PIP (Brown 2007; Beck 2007; Matyas 2009).

    Naturally occurring antibodies to cholesterol, both IgM and IgG, are present in sera from essentially 100% of humans (Alving 1989; Avila 1996; Alving 1999; Horvath 2001; Fust et al., 2005). Interestingly, the titers of antibodies to cholesterol were markedly higher in the sera of HIV infected patients when compared to HIV seronegative controls (Horvath 2001; Fust 2005). Introduction of highly active antiretroviral therapy (HAART) resulted in a significant and gradual drop in the anti-cholesterol antibody titers in parallel with a decrease in viral load and increase in CD4+ cell counts (Horvath 2001). Recent studies have demonstrated that a murine monoclonal IgG antibody to cholesterol can neutralize HIV-1 infection of macrophages (Beck 2010). However, it is not clear at present whether the increased titers of antibodies to cholesterol associated with HIV-1 infection were an epiphenomenon, or whether the antibodies actually had some beneficial effects against acute HIV-1 infection.

    The Alving laboratory has a long history and considerable experience in the practical measurement of human and murine antibodies to cholesterol, including murine multispecific antibodies that recognize both cholesterol and gp41 from HIV-1 (Swartz 1988; Alving 1989; Alving 1991; Alving 1999; Alving 2006; Karasavvas 2008; Beck 2008).

11. Section 2.1: Correct to read that Uganda is targeting **two** populations for Part B—Sex workers and bar workers.

12. Section 4.2.2 Modified Transition to Part B—Added this new section to the protocol.

    In view of the success of the pilot phase of the study (Part A) we propose to transition to Part B permitting full enrollment to 500 participants per site. However, in discussion with DAIDS, the primary funding agency, the transition to part B must be made on a site-by-site basis based upon an increased incidence and at least 3 acute infections identified in Fiebig stage 1 or 2. To manage work flow, logistics, and costs each site will only enroll new volunteers in part B as existing participants enrolled under part A complete the intensive phase of surveillance at one year. For the purpose of protocol management we will define the visit at 60 weeks, visit G, as the end of a full year of intensive surveillance. All participants completing visit G remain in the protocol but the frequency of SBV visits change from twice weekly to once per month, but they

will continue to come in for all other visits as scheduled until study termination. As volunteers complete visit G, more volunteers can be enrolled under the expansion of enrollment in Part B. This will permit a gradual expansion of the overall enrollment yet maintain a population of approximately 200 participants in the intensive, twice-weekly surveillance phase.

13. Section 4.2.3 Part B (Full Study Implementation): Corrected visit window for follow-up of Visit A to within approximately 6 weeks. The protocol previously stated 30 days.
14. Section 4.3.1 HIV Diagnostics, 3rd paragraph, modified where bold:

    HIV testing for cohort enrollment will be by anti-HIV antibody using a 3rd generation EIA, Genetic Systems HIV-1/HIV-2 Plus O EIA (BioRad Laboratories, Redmond, WA), or equivalent, or ***4th generation methodology****. Repeatedly reactive EIA samples will be tested using Genetic Systems HIV-1 Western Blot (WB) (BioRad Laboratories, Redmond, WA****). Samples may be subjected to*** *Aptima HIV-1 RNA Qualitative Assay (Aptima: Gen-Probe) (see Figure 6 below****) to detect acute HIV infection. Diagnosis will be made based on detection of serological markers of infection. Diagnostically challenging cases may be resolved by supplemental confirmatory testing for HIV proviral DNA****. The enrolled* ***HIV*** *prevalent participants will also be concurrently screened using the capillary microvette sampling method described below as concordant detection of HIV-1 prevalent cases by microvette sampling.* ***Standard EIA/WB testing will be employed as*** *a critical internal control for the small blood volume sampling methodology proposed.*
15. Section 4.3.1 HIV Diagnostics, paragraph following Figure 6: Corrected language "one serum and one EDTA microvette sample will be acquired.." to read "600 ul of blood will be collected at each visit and then tested for the presence of HIV-1 RNA"
16. Section 4.3.1 HIV Diagnostics, 3rd paragraph following Figure 6: Modified last two sentences as follows (where bold):

    Staging will be performed on large blood volume samples (LBVs) using 1) ultrasensitive HIV-1 RNA qualification (Aptima HIV-1 Qualitative Assay, Gen-Probe) and quantification (Abbott HIV-1 Real Time, Abbott Diagnostics) nucleic acid amplification technologies (NAAT), 2) HIV-1 DNA polymerase chain reaction (PCR), and 3) serological assays (p24 antigen EIA [Perkin Elmer or equivalent] 3rd generation IgM sensitive EIA (Genetic Systems HIV-1/HIV-2 Plus O [BioRad Laboratories], 4th generation p24 antigen, anti-HIV antibody EIA, HIV-1 WB [BioRad Laboratories; ***Abbott Laboratories], 4th generation Rapid Diagnostic Tests [RDTs; Determine HIV-1/2 Ag/Ab Combo; Alere, Inc and equivalent], HIV WB [BioRad Laboratories] and, SMART plasma/cells, SMARTube HIV/HCV blood enhance device; [SMART Biotech, Ltd].*** *Estimations of the emergence of HIV viremia, p24 antigenemia, anti-HIV antibody, and WB reactivity, and* ***proviral DNA*** *in these subtype diverse cohorts will be generated.*

    Alternative NAAT or serological assays may be employed if data suggests that subtype diversity impacts detection sensitivity/specificity or if technology advances ***occur during the course*** *of this study.*
17. Section 4.3.1 HIV Diagnostics: Description of SMART Tube process added as 4th paragraph after Figure 6:

    A 2ml blood sample will be collected as a heparin specimen or directly into a SMART tube A 2ml blood sample will be collected as a heparin specimen or directly into a SMART tube containing 2ml of anti-coagulant and reagent. Specimens will be acquired on Visits 1 and 3 within the stutter phase and in conjunction with the proposed ACD blood draw post a reactive Aptima result and prior to transition to stutter phase. Additional specimens will be collected at visits 13 and 14 for evaluation of this product as a recent infection detection device. Samples will be incubated in a 5% CO_2 humidified incubator (or candle jar if feasible) at 37^0C for 3-5 days. Post ST stimulation, the diluted plasma from the SMART tube will be screened using standard EIA and subsequently reflexed to HIV Western Blot if EIA reactive. The approximate remaining 700ul diluted plasma from the SMART tube will be reposed for future studies. While ST stimulated diluted plasma has been examined by standard serological methods an analysis of the ~ 1 million stimulated PBMC for integration of proviral DNA has not been conducted (personal

communication, Dr. Jehuda-Cohen) nor have there been attempts to sequence infecting virus. The PBMCs will be preserved as dry cell pellets or isolated by ficoll hypaque methods for proviral integration studies and, if possible, sequence analysis. Results will be compared to nucleic acid testing results and standard serological HIV diagnostic testing. Sample numbers will be small and the intent is to provide descriptive analysis of the comparisons.

18. Section 4.3.1 HIV Diagnostics, 6th paragraph, revised as follows (where bold):

    As qualitative and quantitative NAATs for detection of HIV RNA and HIV Ag/Ab tests are continuously advancing, panels constructed from laboratory staging studies will be used to assess the most promising. ***Several promising technologies are advancing, which include, but are not limited to****:*

    1. ***IQuum Lab-in-a-Tube (LIAT). This assay integrates plasma specimen processing (200 ul), nucleic acid extraction, reverse-transcription, and real-time PCR in a single closed-tube format. The LIAT analyzer provides an interpreted result in less than 60 minutes. A recent evaluation of product against gold standard Abbott m2000sp/Real Time HIV-1 RNA assay demonstrated that HIV-1 RNA levels were over quantified approximately 1.5-fold higher and with 1.3-fold less precision than the gold standard platform over a analytical range from 1.5 x10^3 to 1.5 x10^6 that is suitable for monitoring HIV-1 infection (Coombs et al. Abstract).***

    3. ***The Simple Amplification Based Nuclei Acid Test (SAMBA) (Diagnostics for the Real World, Inc, Sunnyvale, C) is qualitative point of care (POC) HIV viral load platform which leverages sample preparation, isothermal nucleic acid amplification (< 1 hour), and dipstick based rapid visual detection of nucleic acid (<0.5 hour). Amplification and detection steps are combined in a disposable cartridge which is processed on the POC device permitting containment of amlicons. An extraction module is under development. Detector probes labeled with multiple hapten moieties forml lattice-like structures by binding to anti-hapten antibodies conjugated with multiple colored particles. In the presence of target RNA or DNA, visible lines develop in the capture zone of the test strip. Studies with Group M subtype panels (A-K), Groups N and O, and clinical samples demonstrated sensitivity to 200 copies/ml (Lee et al. 2010).***

    4. ***Wave 80 BioSciences (San Francisco, CA) EOSCAPE HIV platform is based on continuous-flow microfluidic, microchip technology, with on board lyophilized reagents requiring no fluid exchange. The product employs thermostable branched DNA-like amplification with luminescent readout, is disposable, and has a flexible instrument design. The product is scheduled for beta testing in 2012.***

19. Section 4.3.1 HIV Diagnostics: added as 6th paragraph from end of section:

    HCV serology status will be determined using Ortho Diagnostics HCV EIA 3.0 (Ortho Diagnostics, Raitan, NJ) or following followed by supplemental confirmatory testing (Chiron RIBA HCV 3.0 SIA (Novartis Vaccine and Diagnostics, Inc, Emeryville, CA), and/or HCV viral load (COBAS AmpliScreen HCV version 2.0; Roche Diagnostics, Indianapolis, IN). HCV genotyping may also be performed.

20. Section 4.3.1 HIV Diagnostics: Added to end of section is the following text:

    Among participants who are known to be on anti-retroviral therapy or have demonstrated control of HIV viremia without a history of anti-retroviral therapy, drug levels will be ascertained to verify treatment status.

21. Section 4.3.2 Viral Evolution: Added to the 2nd-4th paragraphs:

    Full-length (FL) *viral sequences will be attained* ***by single genome amplification (SGA)*** *strategy. Five FL genomes will be generated initially and, if two or more distinct variants are observed, ten additional genomes* ***or more*** *will be generated. We expect 80% of samples to the*

homogeneous and 20% heterogeneous, based on current reports (Salazar-Gonzalez, Bailes et al. 2008) and Keele, Giorgi, et al. 2008. A consensus sequence of the viral proteome will be generated and provided to the cellular immunology group for use in the synthesis of autologous peptides for evaluation of CTL epitopes. The full genome PCR products will be used to generate an infectious molecular clone ***and chimeric/ IMC with swapped envelope. These reagents*** *will be used for biological characterization of the earliest viral strain, for studied of virus neutralization, and to generate autologous infected target cells for certain cellular immune studies.*

*Envelope sequencing will be performed at closely spaced intervals in very early infection. We will again use SGA of RNA extracted from plasma, generating approximately 20****-30*** *envelope sequences from the first RNA positive sample and at* ***3, 8, and 24*** *weeks post infection, starting with 10 acute infections initially to explore the utility of the frequent sampling approach.*

From the study of recognized CTL epitopes and their early escape variants, ***we will also perform 5-10 genomes at 3 and 8 weeks post-infection. From the sequence diversity obtained comparing to the transmitted/founder (T/F) viruses we will select approximately 5 regions across the viral genome to perform*** *in-depth longitudinal analysis for each study subject. Viral RNA from the first RNA positive sample, and* ***from 3 and 8 weeks*** *post-infection, will be subjected to pyrosequencing to interrogate the viral quasispecies in great depth.* ***Other timepoints may be added for pyrosequencing.*** *This technology provides a more complete description of the population of viral genomes present in a given sample, and can permit identification of variants present as low as* ***0.1%*** *of the quasispecies.* ***Envelope SGA will be performed to study the earliest viral quasi-species identified in mucosal compartments arising during acute HIV infection. The specimens used will be semen and vaginal/rectal swab collections and sigmoid gut biopsies (collected via approved protocols RV 254 and RV 304) at the earliest positive time point. Sharing between RV 217, RV 254 and RV 304 is planned to optimize use of controls and minimize overall risk to volunteers.***

Additionally, several sequences from the first sequencing timepoint to the viral peak, before onset of immune pressure, will be analyzed for mutation rates due to reverse transcriptase misincorporation. Sequences will also be analyzed in order to refine models currently available on the LANL website for estimate timing of infection, most recent common ancestor sequence and onset of host selection. (Giorgi 2010).

In addition, the Multi-region hybridization assay may be conducted on the plasma samples in order to determine HIV subtype. This will likely be performed on all acute cases.

22. Section 4.3.3 Cellular Immunology: The following subsections were added:

<u>Analysis of HIV Antigen Specific Immune Responses in Uninfected Volunteers:</u>

These analyses will only be conducted when feasible based on arrival time in the laboratory. Heparinized fresh whole blood from exposed uninfected HIV-1 individuals will be activated with several different HIV-1 peptide pools for 6 hr at 37°C in the presence of anti-CD28/49d, BFA and monensin. To maintain cell integrity, the assay will be performed within 8 hr after blood is drawn. After stimulation, red blood cells will be lysed by FACS lysing solution and remaining cells permeabilized with FACS Perm2 buffer. Samples will be stained with monoclonal antibodies against the relevant lymphocyte, cytokine and degranulation markers. Samples will be analyzed by FACS-Canto II or LSR-II and data analyzed by FlowJo.

AND

<u>Functional and phenotypic characterizations of immune cells:</u>

Efforts will focus on the comprehensive detailed phenotypic and functional characterization of immune cells in AHI with and without HIV specific stimulation, including but not limited to, B cells, T cells, dendritic cells and NK. Importantly, we will identify the cellular sources of some of the key cytokines involved in the acute phases of HIV infection. PBMC samples collected at the pre-stutter time point and during the acute and chronic phases of infection will be assayed for each volunteer. At a minimum, 3 samples will be included for study: those drawn within 1 week

after the detection of viremia (where early peak cytokines are expected), within 2 weeks and within 1-3 months after the detection of viremia (for late peak cytokines). Multiparameter flow cytometry will be used to identify and characterize immune cell populations isolated from whole blood. Fluorescence conjugated antibodies against the markers for lymphocytes, chemokine receptors, activation, apoptosis and other functional markers will be used. Markers may be added or substitutes as new reagents become available. All antibodies are titrated prior to use to determine the optimal concentrations for staining. Samples will be acquired on an LSR-II flow cytometer equipped with 4 lasers and 18 detectors. Flow cytometric data will be analyzed using FlowJo (Treestar). In addition, PBMCs in complete media will be stimulated with heat-inactivated influenza virus as a positive control, aldrithiol-2 treatment of HIV-1 particles (AT2-HIV) as a stimulus, or Jurkat cell-derived microvesicles (MV) as a control for 18 h at 37°C. After stimulation, supernatants will be collected for cytokine detection. Cells will be stained for various phenotypic and activation markers and analyzed by flow cytometry. Similarly, in order to detect cytokines intracellularly, PBMCs in complete media will be stimulated with heat-inactivated influenza virus as positive control, AT2-HIV, or control MV for 4 h at 37°C followed by an additional 4 h in the presence of protein transport inhibitor (Brefeldin A and/or monensin). Surface staining will be performed and cells will be fixed and permeabilized and intracellular cytokine staining will be performed. Samples will be analyzed on an LSR-II flow cytometer and data analyzed with FlowJo software. Plasma cytokine levels will also be quantified using a custom Q-PlexTM Human Cytokine–IR Array according to the manufacturer's protocol. Images are taken on an Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE) and analyzed using Quansys Q-viewTM Plus software (Quansys Biosciences). At this time the Type 1 interferons are not yet optimized in the multiplex array. As such, standard cytokine ELISAs will be utilized for the quantitation of IFN-⟨ and IFN-® (PBL Biosciences / R&D Systems). In addition, other markers of immune activation and inflammation will be studied.

AND

Regulatory T cells:

Regulatory T cells may be examined on cryopreserved PBMC samples, when cell yields allow, using multiparameter flow cytometry on the LSR-II. Potential markers that may be analyzed in combinantion with some of the core markers described in the above sections will be CD25 (IL-2 receptor) bright expression, CD62L (L-selectin) high, and Foxp3 (forkhead box protein 3 transcription factor), CD69, CD122, CD127 and CTLA-4. Tregs will be functionally interogated by screening for IL-10 and TGFbeta production or other cytokines. In addition a4b7 expressing cells will examined to look at the effects of gp120 binding to these cells and their cytokines.

23. Section 4.3.3 Cellular Immunology, subsection 'Pilot Studies of Gene Expression Profiling and Novel Assay Platforms', added the following to the end:

    Gene array profiling will be used to identify signaling and transcriptional signatures of innate immune cells as well as that of responding T cells during acute HIV-1 infection and a systems biology approach may be taken to elucidate signaling pathways and transcriptional networks.

24. Section 4.3.3 Cellular Immunology: Added the following subsection:

    Proteome Analysis of Activated CD4+ T cells Infected with Different Clades of HIV-1:

    PBMCs from HIV-1 clade A and clade C infected individuals at different time points will be used for the isolation of CD4+ T-cells. A small aliquot of the PBMCs from each of the individuals will be analyzed on an LSRII to determine the % of CD4 infected T cells by intracellular p24 staining. PMBCs will be stained for CD3, CD4, and MHC class I and sorted based on the surface expression of these markers using a FACS ARIA cell sorter. CD4+ T- cells will be sorted into two populations: (i) CD3hi, CD4hi and MHC class Ihi (uninfected), and (ii) CD3hi, CD4low and MHC class Ilow (HIV-1 infected). The proteome profile of HIV-1 infected and uninfected CD4+ T-cells will be analyzed by 2-D isoelectrophoresis followed by image analysis. Protein spots that are absent or have a greater than a twofold difference in spot intensities between HIV-1 infected and uninfected CD4+-T cells will be identified. The appropriate proteins will be excised from the gel, digested with trypsin and peptides will be subjected to mass spectrometry.

25. Section 4.3.4 Humoral Immunology, subsection 'Binding antibody assays': Added the following to the 2nd paragraph (where bold):

    Binding antibody assays

    A customized multiplex assay (using Luminex technology) for the detection of IgM, IgA1, IgA2, IgG1, IgG2, IgG3, IgG4 antibodies with specificities against HIV Env gp140 and gp41, Gag p55, Tat, Nef, ***RT, p31*** *and Env epitopes (ie. MPER) will be assessed in longitudinal samples. Autoantibody responses will be assessed including a rheumatoid factor test. Samples will be separated into IgG and non-IgG fractions for analysis. Positive and negative controls are included in every assay. Fully characterized samples in terms of antibody type and specificity will then be assessed for functional activity. The formation immune complexes (antibody-virion) will also be measured using developmental antibody capture assays to assess the possible role of antibodies complexed with virus in acute infection.* ***Capture assays will be done for both IgG and IgA by measuring the capture of infectious virions (in addition to total virions). This work will be performed in collaboration with Dr. Georgia Tomaras at Duke University****.*

26. Section 4.3.4 Humoral Immunology, added the following subsection:

    Mapping the epitopes for the early antibody responses:

    Broad epitope mapping may be done at select timepoints using a peptide microarray that covers multiple clades and CRFs (Tomaras). In addition, early immunodominant non-protective epitopes (IDNPE) may be mapped through use of binding to a series of engineered mutant proteins (Nara). These initial studies will be a follow-up to ongoing immune refocusing efforts using the CM235 CRF01 AE env. Dr. Tomaras has under development the peptide microarray technology predominantly used for mapping linear and non-conformational epitopes. This method may be applied to select samples of interest to determine the binding epitopes. This may be of interest for both IgGs and for IgAs. Dr Peter Nara at Biological Mimetics, Inc. has requested samples to identify the earliest immunodominant non-protective epitopes; these may be mapped through use of binding to a series of engineered mutant proteins that are produced in a vaccinia expression system.

27. Section 4.3.4 Humoral Immunology, subsection 'ADCC Neutralization and other functional assays': revised (where bold)

    The laboratory at AFRIMS in Bangkok has extensive experience performing ADCC using subtype B and CRF01_AE gp120, ***and an assay optimized at Duke University will also be performed by Guido Ferrari.*** *We will purchase gp120 of subtypes A, C, and D from Immune Technology, Inc, and will also test vaccinia-expressed o-gp140 from the strains used in our A, C, D and CRF01_AE vaccines.* ***An ADCVI assay may also be used to assess antibody-dependent, cell mediated virus inhibition; this assay would be performed in the Rockville labs using donor PBMC infected with IMC as targets for suppression****.*

28. Section 4.3.4 Humoral Immunology, added the following subsections:

    Determination of the TCID50 of infectious virus present in plasma throughout acute HIV infection

    Unpublished data indicate that there may be a fall-off in the infectious titer of plasma virus during acute HIV infection, suggesting that there could be an immune basis to the drop in viral load that is observed in early infection. This would indicate a potential role for HIV-antibody immune complexes, or an as yet unidentified antibody population. A standard TCID50 assay to determine infectious viral titers in plasma will be employed using PHA-stimulated donor PBMC as viral targets and a p24 readout for infection. This will be done in collaboration with David Montefiori at Duke University.

Analysis of antibodies binding to proteins and lipids

ELISA and Biacore assays will be utilized to analyze the serum samples during the course of an HIV-1 infection for the presence of antibodies to lipids. We will initially screen the sera for antibodies to four representative lipids, cholesterol, phosphatidylinositol-4-phosphate (PIP), cardiolipin, and galactosyceramide (GalCer) using a modified ELISA procedure (Swartz 1988; Matyas 2006; Matyas 2009). If antibodies to cholesterol, GalCer, cardiolipin and PIP are found, the sera may be screened for additional antibodies to other lipids described in Matyas (2009). Biacore assays (Beck 2007; Karasavvas 2008) will also be used to analyze the serum samples to HIV-1 proteins (env, p17, and p24). The subclass of the captured anti-HIV IgG antibodies will be determined by sequentially injecting through the Bioacord anti-IgG1, anti-IgG2, anti-IgG3, and anti-IgG4 to allow binding and isotype determination. Serum samples will also be analyzed for their ability to bind primary human macrophages by confocal microscopy and flow cytometry. Supernatants from macrophage cultures incubated with serum samples will be analyzed for the induction of chemokines, MIP-1 alpha, MIP-1beta, RANTES, and IFN-gamma by ELISA or by flow cytometry.

29. Section 4.3.4 Humoral Immunology, "Mucosal Specimen Collection"—added cervical cups and rectal sponges for mucosal specimen collection. The following paragraphs were added:

Mucosal Specimen Collection

*Paragraph 1: Mucosal secretions for this study are defined as CVL**, cervical cups, rectal sponges**, and semen.*

Paragraph 2: ***CVL collection will be conducted on those participants who have already previously donated this way. As longitudinal comparisons would be difficult if a participant were to switch from CVL to cup, cervical cup collection will only be used for new participants. This will allow comparisons between the two collection devices and also bridge the gap between those studies that used only CVL collection and those studies that use the cervical cup for collection.***

Paragraph 3: ***Rectal secretions will be collected by rectal sponge, and will be collected in addition to the CVL/cervical cup and semen collections. The rectal secretions will be offered to both male and female participants.***

Paragraphs 8-13:

Collection of Cervico-vaginal Secretions Using the Cervical Cup:
The collection of mucosal samples will be subject to the volunteer's acceptance and tolerability and laboratory constraints. Detailed sampling technique will be as per SOP. All specimens will be processed within 8 hours of collection.

We will evaluate the concentration and specificity of antibodies in vaginal mucus using a new collection method called Instead Softcup. The Instead Softcup is an FDA approved feminine hygiene alternative to pads and tampons for use by women during menses. The cup is a disposable single use device that can be placed by the woman in the vagina for up to 12 hours to collect blood, or in this case, vaginal secretions. An advantage for this collection method is that the woman can insert the cup intra-vaginally herself so no pelvic exam is required. It also eliminates the dilution factor as the mucus will is collected in its natural state and normalization of the samples will not be needed.

Softcups will be used according to manufacturer's instructions. Specifically, use of the Softcup is not recommended for those using an IUD, for those with a history of Toxic Shock Syndrome (TSS), and during the period immediately following childbirth, miscarriage, or termination of pregnancy. For this protocol, this period will be defined as within six weeks.

For preservation of cervical sample integrity, participants should be advised not to have vaginal intercourse or douching for the 72 hours preceding collection. Female volunteers enrolling in the mucosal secretion substudy will receive an initial pelvic examination by clinic staff with PAP smear. If the volunteer requests, clinic staff may assist and instruct the volunteer on insertion of Softcup device during this pelvic examination process. Volunteers with abnormal results on the PAP smear will be referred for further care and treatment as clinically indicated. They will be referred for further care and treatment and deferred from enrollment into the mucosal substudy.

Additionally, they will be queried for signs and symptoms for sexually transmitted infections (such as gonorrhea or Chlamydia), abnormal discharge, and date of last menses. Additionally, urinalysis will be conducted to look for the presence of white cells (leukocyte esterase). Women with signs and symptoms of an active STI will be referred for care and treatment and deferred from giving mucosal specimen. Menstruating women will have their mucosal collection deferred, up to the end of the study visit window, after which the mucosal collection time point will be missed. The stage of the menstrual cycle will be determined by checking LH and FSH levels at the time of each mucosal collection.

Collection of Mucosal Samples by Rectal Sponge:
The inclusion of rectal sponges to collect rectal secretions is a new methodology that has not been tested yet with our assays. It is being included so we can compare against other mucosal collection methodologies. The advantage of using the sponge is that the amount of mucosal secretions collected should increase dramatically. The process should only take 1 minute (retention of sponge, not including preparatory work). Mucosal secretions would be undiluted. Since samples will be undiluted, we could avoid normalization and have more confidence in the results. The product we are planning to use is the Schindler Ear Packing Ear Wick (Merocel hemoX). It has to be introduced using a disposable anoscope, and retained for 1 minute before it is retrieved. The anoscope is kept in place until the sponge is retrieved to prevent the fluid from squeezing out. Detailed sampling technique will be as per SOP.

30. Section 4.3.4 Humoral Immunology, "Mucosal Specimen Collection"—added that we intend to perform pregnancy test prior to mucosal collection for safety considerations. (this is already noted in consent).
31. Section 4.3.4 Humoral Immunology, "Mucosal Specimen Collection", paragraph 5— revised to remove the pool of controls being established at visit B; instead will match a control at the time a new HIV infection is identified (changed where bold):

    At the time an incident case arises, specimens will be collected on HIV seronegative subjects to *assess specificity of the assays and ensure that their performance is comparable across the different sites. For each HIV acute infection in the study,* ***an HIV seronegative will be matched on age and gender, and sp****ecimen collection will follow the schedule for HIV acute infection (Phase II).*
32. Section 4.3.4 Humoral Immunology, "Mucosal Specimen Collection", added paragraph 6:

    In addition, each site will be permitted to collect baseline samples (as noted on SOE#1) on 20 women and 20 men. If the participant does not move out of phase I, their sample may be used as a control or discarded, based on the PI's discretion.
33. Section 4.5 Susceptibility to HIV Infection from Co-Incident Infections Design, 1st sentence: revised as follows (where bold):

Study volunteers will be tested for malaria ***if febrile; for syphilis and HSV-2 at Visit B and also at Visit 1 of stutter;*** *and Hepatitis B (if appropriate), eosinophil****ia, and*** *LPS upon study entry, within 42 days (Visit B) and subsequently every 6 months during the course of the study. If volunteer becomes HIV(+) they will be tested at the time of phase II enrollment for malaria if febrile, syphillis, HSV-2, LPS, and* ***eosinophilia****.*

34. Section 4.6 Overview of Volunteer Activities (Phase I- Surveillance): Revised the window for follow-up after visit A to no later than six weeks after visit A (within 7 to 42 days of Visit A).
35. Section 4.6 Overview of Volunteer Activities (Phase I-Surveillance): Added the following language to the end of the section (concerning swab collections):

    All participants entering twice-weekly surveillance after visit B will be asked to consent to the twice-weekly collection of vaginal and/or rectal swabs (according to the most recent sexual practice of the individuals) in order to identify the presence of HIV and determine quasispecies evolution at the site of infection prior to the advent of and after systemic viremia. Any volunteer within the first 2 months of commencing twice-weekly surveillance is eligible for vaginal/rectal swab collection.

    Volunteers will self-administer these swabs and place them into collection tubes provided by the site. This collection may be done in the clinic in conjunction with their SBV visits or at home. The intent is to begin these mucosal swab collections early after enrollment, when transmission rates appear highest, and continue the collection for four months. The volunteers will be compensated for the vaginal/rectal swab collection.

    At the end of the 4-month swabbing period, the swabs collected during the first 2 months can be discarded. At the end of 6 months since the beginning of collections, all retained swabs on an individual can be destroyed. The timing of discarding the swabs relates to the fact that the swabs can be kept at room temperature for only 60 days. The swabs should not be discarded until the analyses stipulated below are accomplished.

    Receptive Risk Questionnaire
    In addition, at the time of SBV, a questionnaire will be completed by the volunteer if literate or administered with staff assistance to document the frequency and type of sexual exposure occurring since the last SBV visit. The purpose of this brief questionnaire is to accurately measure the frequency and type of sexual acts to link to the "at-risk" exposure data.

    The questionnaire will be independently collected from all volunteers who are undergoing SBV visits, regardless of whether they choose to consent to the swab collections.

    Swab Analysis
    The swabs will be analyzed using the APTIMA analysis platform.

    For all volunteers participating in swab collection, prior to swab discard, 10-20 samples (roughly one per week of observation) will be analyzed for HIV RNA. Any positive samples will be frozen and transferred to Rockville, MD for sequence analysis.

    If a volunteer participating in swab collections develops a reactive APTIMA plasma sample during the four months of swab surveillance or within 2 months after the last swab collection all of the swabs collected (that have not been discarded) will be analyzed. This analysis will occur even if the participant does not enter or remain in phase IB, i.e. analysis of all collected swabs will occur for an individual with a false reactive APTIMA as well as true reactive.

    All individuals with a reactive APTIMA plasma sample, regardless of their participation in the prospective collection of vaginal/rectal swabs, will have a vaginal or rectal swab collected at each phase IB visit. A volunteer with a reactive APTIMA that has not been participating in the swab collections will be consented to allow swabbing during phase IB. Such a volunteer, if at visit 1 is no longer APTIMA reactive, will return to surveillance in Phase I with no additional swabs.

If a volunteer participating in the collection of vaginal/rectal swabs returns to surveillance after Phase IB (they were determined not to be infected), swab collection only continues if they remain in the four-month window of swab surveillance.

Should compliance with collections be under 50% after offering enrollment to 20 participants at a given site or analysis of the swab samples show no HIV nucleic acid signal among these first 20 participants, the collections will be stopped.

36. Added new Section 4.7 Entry into Phase Ib (Stutter Phase):

    Very early HIV infection may have low amounts of HIV RNA or intermittently negative RNA tests. In addition, some HIV infected individuals control HIV infection very effectively and have very low levels of HIV RNA or may be negative even with the most sensitive tests. Hence, negative test results do not exclude the possibility of HIV infection. Positive tests are not conclusive evidence of HIV infection either. Some false positive results may be due to mis-labeling, contamination or other error. Hence, diagnostic testing and post-test counseling are provided using larger blood volume sample test results at the 24-weekly clinic visits. HIV uninfected participants with a new reactive Aptima test from a valid run will prompt a return visit at the earliest opportunity to either enter stutter at Visit 1 or collect a single, 8.5 mL tube of ACD blood. In addition, a 2ml heparin tube can be collected. ACD cells and plasma will be processed in a single cryopreserved PBMC vial and plasma as 1 mL aliquots (3 to 4). The heparin anti-coagulated whole blood will be used to inoculate a SMARTube. The diluted plasma from the SMART tube will be processed as 1 ml aliquots (3) and PBMCs as a single cryopreserved vial. An SBV will be drawn at the same time as the visit 1 stutter SOE blood collections or the 8.5 mL ACD either by finger stick or from the venipuncture. An APTIMA test will be performed on this newly collected SBV plasma using the 1:5 dilution in PBS schema. If the follow-up sample APTIMA test is reactive, the participant is placed into phase IB. If the result is non-reactive, the participant returns to surveillance. If the volunteer returns to surveillance and has a second reactive APTIMA within 2 weeks, the volunteer is placed into phase IB. If the volunteer has a second reactive APTIMA after 2 weeks, then they are treated as if this were a first new reactive result, i.e. a single 8.5ml ACD tube is collected as soon as possible (as above).However, if the participant is western blot positive at visit 1 or 2 (Fiebig IV, V, VI), the participant will move directly to SOE 3 and will skip remainder of stutter phase.

37. Section 4.8 Overview of Volunteer Activities (Phase II-Incident HIV Infections): Added the following to the end of the 2nd paragraph:

    Upon entry into phase II the participant will be administered a short questionnaire (Appendix XI), which asks about symptoms, treatment, if any, received and the circumstances under which the participant thinks they were infected with HIV. However, this information could be collected from interviews and exams, documented in the source docs, from phase IB visits.

38. Section 5.2: Inclusion Criterion #10d: Eliminated "recent sexually transmitted disease" as an inclusion criterion as this has not been captured within ACASI in a sufficiently specific manner and is very common.

39. Section 5.2 Inclusion Criteria: Added the following footnotes:

    Any modification to the three African sites enrollment criteria will be described in the site-specific addenda.

1. A known or suspected HIV positive partner of an eligible participant may be permitted enrollment into phase II. Enrollment will be for 1 visit only (Visit 11), and will be at the discretion of each site. There will be no contact and tracing of the participant's partner. It is up to the participant to notify the partner and bring them in, if they so choose.

40. Section 7.5 Exit Visit: Added the text: "If the LBV occurred within 2 weeks prior to the exit visit, no blood will be collected and no ACASI will be taken during the exit visit."

41. Section 7.7 Transition to Phase II, 2nd paragraph: Added 2nd sentence *Phase II participants may continue in phase I surveillance for masking purposes. And* Removed the following language (**where bold)**:

Any HIV incident volunteer who refuses to enter phase II ***will have an exit visit conducted to insure a diagnosis is established accurately and*** *may continue follow-up in phase I of the study for the remainder of their visits.*

42. Section 7.9 Behavior Data Collection--added the following to end of section:

    In addition to the ACASI questionnaire, two questionnaires will be administered to participants. One questionnaire will ask questions pertaining to vaginal and anal washing methods (Vaginal/Anal Practices (VAP) Questionnare--Appendix IX) and another will be ask questions pertaining to frequency and type of sexual acts (Receptive Risk Questionnaire--Appendix X).

43. Section 7.10 Mucosal Specimen Collection: revised as follows (where bold):

    All acutely infected individuals, including those in the intermittent, low level viremic or "stutter phase" (SOE #2) of HIV infection, will be offered the opportunity to participate in the mucosal sample collection arm. Mucosal specimens will be collected as indicated on SOEs #1 and 3. Other mucosal collections, if appropriate, can be collected including breast milk, oral secretions, urine and semen.

    For each site, at the time of each incident case that is identified, an HIV seronegative volunteer will be matched to the incident case for age and gender, and will be offered the opportunity to provide mucosal specimens. *For this, they will sign a separate consent form and they will receive extra compensation, as the visit will take longer than regular visits. In addition, they will continue to be followed in their phase I schedule, including SBV. If any of these become infected, they will be replaced with another* ***control.***

    In addition*, female participants will be offered a Papanicolaou smear. Abnormal results will be referred for further evaluation and treatment.*

44. Section 7.10 Mucosal Specimen Collection, 1st paragraph, last sentence—removed window.
45. Section 7.10 Mucosal Specimen Collection, 2nd paragraph—revised so that controls no longer be preselected at Visit B, but instead will be matched at the time each incident case is identified.
46. Section 8.3 Storage—corrected ACD to read acid citrate dextrose.
2. Section 8.4 Shipping--In order to be consistent with the consent, the protocol has been revised to clarify that samples shipped for HLA typing and host genetics may be stored in Rockville as follows (where italicized):

    All samples for HLA typing and host genetic characterization, viral sequencing, viral isolation, neutralization, and Fiebig staging assays will be shipped to *and stored at* the MHRP laboratories in Rockville, Maryland, United States of America (USA) by dry shipper or on dry ice accompanied with all the required export and import documentation

47. Figure 7 Flowchart: Revised
48. Section 9.1 HIV Test Results—Added as last sentence:

    Informal support group sessions and/or focus groups may be utilized by the study participants to further discuss their concerns and provide a support network for those testing positive for HIV.

49. Section 10.0 Compensation of Volunteers—Clarified compensation for volunteers completing more than one type of visit on the same day (e.g. SBV and LBV) by adding the following language as paragraph #2:

    A volunteer is compensated for the time and inconvenience associated with the type of visit he or she is completing. If, by coincidence, the volunteer completes study activities for more than one type of visit on the same day, the volunteer will be compensated for each type of visit completed.

50. Section 12.1 Hardware and Software, 2nd paragraph--revised first sentence to read (where bold):

    The Data Coordinating and Analysis Center (DCAC) ***will manage data into a validated password-protected data management system.***

51. Section 13.1 Risks—removed the following statement in 4th paragraph "At no time will the standards of the American Red Cross of 450 ml limit per 8 weeks be exceeded." And replaced with the following:

    Thus far, no safety issues have arisen from the blood volumes collected during the conduct of ECHO. Hemoglobin is monitored throughout the period of blood collection and if anemia develops or is worsened, we will adjust phlebotomy accordingly. In view of the safety experience to date and the great scientific value of samples collected early during acute viremia, the total volume for the 8 week period during phase IB has been increased modestly from 448 mL to 472 mL.

52. Section 13.8 Management of Vulnerable Volunteers—added last paragraph, clarifying how incarcerated volunteers will be managed:

    Volunteers who have returned to the clinic after a period of incarceration will be counseled again about the potential social risks of being identified as a participant in the study. A note to the effect that the counseling was done will be written in the progress notes in the volunteer's binder. Any volunteer who is incarcerated for more than 6 months will be re-consented to include taking and passing the TOU.

53. Section 14.0 Policy Regarding Research-Related Injuries—updated section with current language:

    *If a participant is hurt or gets sick because of this research study, medical care will be provided at a hospital or clinic free of charge. The participant will only be treated for injuries that are directly caused by the research study. The Army will not pay for transportation to and from the hospital or clinic. If the participant has questions about this medical care, they may talk to the principal investigator. If the participant pays out-of-pocket for medical care elsewhere for injuries caused by this research study, they should contact the principal investigator. If the issue cannot be resolved, the principal investigator will contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221***.**

D. Added Vaginal/Rectal Washing Questionnaire as Appendix IX.

E. Added Receptive Risk Questionnaire as Appendix X.

F. Added Seroconverter Questionnaire as Appendix XI.

G. SOE #1

1. Added visit window for all LBV visits in Phase I (± 42 days)
2. Corrected footnote #1 to read (where bold): "After **6 months,** the volunteer will be seen every 3 months--for at least 50 months"
3. Revised footnote #2 to read (where bold): "Visit B may occur within 7 **to 42 days** of Visit A**; all other visits have a visit window of ± 42 days. If a volunteer "misses" a large blood volume visit (LBV), the blood draw and questionnaire will be completed at the earliest opportunity. However, 2 LBVs should not occur within 6 weeks of each other and the next LBV may therefore need to be rescheduled within its window accordingly. If the volunteer returns only at the time of the next LBV, the missed LBV is recorded as missed, but blood and questionnaire will be completed for the missed visit.**"
4. Added footnote #8 to describe unscheduled visits for confirming a new reactive Aptima test. "Stutter phase entry should be followed as described in Section 4.7 of the protocol. <u>These visits will be tracked as follows: the first unscheduled visit after a new reactive Aptima test is Visit 1.</u> Additionally, if any new reactive tests occur, the second round would follow as Visit 1a, the third round as 1b, etc."
5. Added footnote #18:"At every SBV, for 4 months, self-administered vaginal or rectal swabs will be collected, depending on the most recent sexual practice of the individual. If the participant has not had sex since the previous swab, there will be no swab collected."

6. Added 10 ml fresh whole blood for intracellular cytokine staining.
7. Added fresh whole blood (4ml) for on-site fresh HIV antigen specific flow cytometry to be done at visit B, F and J
8. Added the collection of the vaginal/rectal washing questionnaire to all LBV visits.
9. Added the collection of the Receptive Risk Questionnaire to all SBV visits for a period of 4 months.
10. Modified the collection of SBVs to be for one year from Visit B, so that only LBVs are performed from visit G to study exit. The footnote (#9) has been revised as follows (where bold):
    [9] *Capillary blood tube collection will be collected via capillary puncture or venipuncture on a twice-weekly basis for one year* ***starting at visit B and continuing through visit G. Thereafter, they will continue on a monthly basis.***
11. Modified to increase volume of PBMCs preserved at visits B, D, F, H, J study exit
12. Added footnote #13: Sites may conduct Aptima at Visit A and in case of an unscheduled visit in between the monthly visit
13. Added footnote #16: Controls for mucosal collections will be solicited at the time new incident cases are identified. After that, mucosal collections will vary by site and incident infection number. A pregnancy test will be performed prior to mucosal collection for safety considerations. In addition, each site will be permitted to collect baseline samples on 20 women and 20 men. If the participant does not move out of phase I, their sample may be used as a control or discarded, based on the PI's discretion
14. Added footnote #14: malaria will be tested for only if participant is febrile
15. Added T cell immunophenotype to be done at visit B.
16. Revised footnote #5 to read: Receptive Risk Questionnaires will be collected at every SBV.
17. Added footnote #20: Hepatitis C testing may be done on archived samples from any time point

H. SOE #2

1. Added mucosal back to stutter phase at baseline and 7-10 days as an option
2. Add visit window for stutter phase visits (-1 day to +2)
3. Updated footnote #3 to read: Samples may be collected twice during stutter at visit 2 or 3 and again at the first visit after EIA reactivity is identified. Other mucosal collections such as breast milk, oral secretions, urine and semen can be collected at 2 time points.
4. Added ACD tube of 7.5 mL at every visit for Plasma/PBMC for Immunoassays.
5. Added clot tube of 4 mL at every visit for humoral assays.
6. Added Smart Tube collection (2 mL) at Visit 1 and Visit 3.
7. Added footnote #8: malaria will be tested for only if participant is febrile
8. Revised footnote #9 to state that if at any time, the participant tests HIV positive on the EIA and WB, they will go straight to Phase II. (previously stated if test EIA/WB positive at visit 1 or 2)
9. Added footnote #10: Hepatitis C testing may be done on archived samples from any time point
10. Added additional clinical flow timepoints (in row labeled T-cell immunophenotype) in stutter phase at visits 2, 3, 4, 7. No extra blood will be drawn.
11. Corrected errors in the SOE and added time points for viral load testing at Vist 6. The blood volume total was not changed, but the 10ml for PCR was added for visit 6 and NB was put in for HIV diagnostics. At visit 8, an NB was added for HIV diagnostics, and for visit 9, HIV PCR was revised from 10 ml to NB (to make up for adding 10 ml at visit 6) and HIV diagnostics was revised to NB.

I. SOE #3

1. Added the following visit windows:
   Visit 10,11: ±3 days

Visit 12: ± 7 days

Visit 13: ± 14 days

Visits 14-17: ± 28 days

2. Added CD4 counts to be done at Visits 11, 13, 15 and Exit.
3. Removed syphilis/HSV testing at all visits except visit 11. Eosinophils deleted completely b/c this is part of CBC with diff.
4. Added footnote to malaria, that malaria would only be tested for if participant were febrile
5. Added Smart Tube collection (2 mL) at Visits 13 and 14.
6. Removed HIV Diagnostics for all visits except visit 11.
7. Added NB to visit 18 for T cell immunophenotyping
8. Added footnote #6: Hepatitis C testing may be done on archived samples from any time point

J. Addition to Phase I ICF

1. Added to end of Section 4: "If you become HIV infected, we would like you to participate in a sub-study, and there will be additional information provided and consent forms to participate in that portion of the study"
2. Section 8--Test of Understanding: The term 'simple' has been removed
3. Section 8-- 1st Paragraph, second to last sentence. Changed from 'Visits B-J will take about 1-2 hours' to 'all other visits will take about 1-2 hours'. Also, the Please move the table of visits up to follow this paragraph
4. Cervical cancer screenings may be offered to all female volunteers as a benefit to the study whether they enroll in the mucosal sub-study or not. If planning on doing this it will be added to the site-specific ICF.
5. Added text to ICF to inform volunteer that we will be storing their information generated by their fingerprint along with their name and address in a secure database for the purpose of identifying participants.
6. Added text to ICF to inform volunteer that with their permission (as indicated on the Contact Form) we will contact them to let them know information about future research that they may be interested in.
7. Revision of text from will to may in the following sentence: "If the test result on any subsequent visit shows that you may be infected with HIV, you may be scheduled for 9 additional visits which will be Phase Ib study." This change will allow for someone who goes to Phase Ib and is determined to be a "false positive" Aptima to return to Phase I.—Thailand consent only.

K. Addition to Phase II ICF

3. Updated blood volume collected to be consistent with new volumes in SOE #3.
4. Added the following under "Blood Specimen Collections and Tests, Other"

   Your saliva will also be collected in a cup after chewing on dental wax.

L. Additions to Female Mucosal Consent form

1. The folloiwng three paragraphs were added for additional collections:

   Mucosal Collection from Rectal Sponge:
   You may also be asked to provide a rectal sponge sample. If so, you will be required to refrain from any kind of sexual activity, douching, and inserting anything into you rectum for at least 72 hours prior to collection. The nurse will insert a short hollow tube into your anus, which will allow access to your colon. An anal sponge will then be inserted into your anus and let it rest inside you for 1 to 2 minutes. The nurse will then remove the sponge and scope and send the sponge to the lab.

   Other Collections:

You may also be asked to provide a urine sample, a breast milk sample or have your mouth swabbed.

What will happen to my samples?
All of these fluids will then be taken to our research lab for processing, then sent to the United States and/or Thailand for testing. You will receive compensation as described below for each of these visits. You may discontinue participation at any time without penalty.

M. Addition to Mucosal Consent—Male

1. Added semen collection

   Semen Collection:
   You will be required to refrain from ejaculation for two days.

2. Added information on rectal sponge collection

   Mucosal Collection from Rectal Sponge:
   You will be required to refrain from any kind of sexual activity, douching, and inserting anything into you rectum for at least 72 hours prior to collection. The nurse will insert a short hollow tube into your anus, which will allow access to your colon. An anal sponge will then be inserted into your anus and let it rest inside you for 1 to 2 minutes. The nurse will then remove the sponge and scope and send the sponge to the lab.

   All of these specimens will then be taken to our research lab for testing.

3. Added information on other collections that may occur

   Other Collections:
   You may also be asked to provide a urine sample or have your mouth swabbed

4. Added risk of bleeding after rectal sponges if participant has hemorrhoids

N. Addition of New ICF and enrollment criteria to permit a known or suspected HIV-positive partner to be enrolled into the study under phase II SOE.

O. Addition of self-administered vaginal/rectal swabs consent for Phase IB participants

1. Self-administered Vaginal/rectal swabs to be added to all Phase IB visits at all sites until such time that a participant is removed from Phase IB.
2. We will test these for HIV by either APTIMA or Roche assay.
3. Compensation for vaginal/rectal swabs will be equivalent to $2 USD.

P. Addition of self-administered vaginal/rectal swabs consent

1. Self-administered Vaginal/rectal swabs to be added to SBV visits at all sites for a period of 4 months at each site.
2. We will test these for HIV by either APTIMA or Roche assay.
3. Compensation for vaginal/rectal swabs will be equivalent to $2 USD.

Q. Edits to RV217 Baseline Questionnaire for Amendment (Version 0.91 to Version 1.4)

1. Skip pattern for Qb4 is fixed.
2. Added underlines for clarification to questions regarding times and number of partners.
3. Added underlines to receive money in D7 and pay/give money in D8
4. Removed STI (criteria #4) from eligibility report
5. Added a Title, Date, and Time Completed to report
6. Added the following definitions for sex and unprotected sex before starting Section D:
7. For all questions, "sex", "sexual contact", "having sex" or "playing sex" refers only to vaginal or anal sexual intercourse (receptive or insertive). "Oral sex", or "mutual masturbation" are not

considered "having sex" or "playing sex".For all questions, "unprotected sex" is sex without a barrier such as a condom

R. Edits to RV217 Follow-up Questionnaire for amendment (Version 0.1 to Version 0.4)

1. Removed Day number from question 3.
   Added the following definitions for sex and unprotected sex before starting Section D:
2. For all questions, "sex", "sexual contact", "having sex" or "playing sex" refers only to vaginal or anal sexual intercourse (receptive or insertive). "Oral sex", or "mutual masturbation" are not considered "having sex" or "playing sex". For all questions, "unprotected sex" is sex without a barrier such as a condom.
3. Added printed report to include study number and date completed

S. Changes to CRFs

1. A global update was done to the footer from Ver. 1.3 dated 10Mar2010 to Ver. 1.4 dated 14Dec2011.

2. Three new CRFs were added at the end of the file: The Pregnancy, Birth and Long Term Birth Reports.
3. The code from "0. Unknown" was changed to "8. Unknown" on the TOS CRF (Primary Cause of Death).

PROTOCOL RV 217

HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand

Short Title: Early Capture HIV Cohort (ECHO)

Study Conducted By
U.S. Military HIV Research Program, Rockville, MD, U.S.A.
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Mbeya Medical Research Program (Mbeya Referral Hospital, Mbeya Regional Medical Office, National Institute of Medical Research, Department of Infectious Diseases and Tropical Medicine, University of Munich and U.S. Military HIV Research Program), Mbeya, Tanzania
Department of Retrovirology, USAMC-AFRIMS, Bangkok, Thailand
and Royal Thai Army AFRIMS, Bangkok, Thailand

Study Funded By
Division of AIDS (DAIDS)
U.S. Military HIV Research Program

RV 217/WRAIR #1373 Main Protocol
VERSION 7.1
DATE 23 March 2009

Summary

This is a multi-center, non-randomized clinical observational study to be conducted in two parts, a pilot study to assess feasibility (Part A) and the full study (Part B). The purpose of the study is to characterize recruitment, retention, human immunodeficiency virus (HIV) prevalence, HIV incidence and biological characteristics of acute HIV infection in high-risk volunteers in Africa and Southeast Asia. The first part of the trial, Part A, is a small pilot study to establish and optimize the recruiting, retention and study compliance at each participating site. Based upon this pilot, the study will expand to full enrollment as planned (Part B) with operational procedures and study event schedules adjusted as needed with a corresponding amendment prior to initiating Part B. The study itself, as conducted in both Parts A and B, incorporates two phases. The main study activity, or phase I, is the observational cohort or surveillance activity. In contrast to standard cohort studies which are designed with regular visits at monthly to six monthly intervals, this study proposes to collect very small blood samples via finger stick collections similar to diabetic monitoring of blood glucose. These "small blood volume" (SBV) visits will occur twice weekly and afford the opportunity to diagnose HIV infection prior to the advent of detectable antibody by the most sensitive techniques available. The acceptability and feasibility of twice weekly monitoring is unknown and for this reason, a pilot part of the study is planned to optimize methods and engage target communities effectively (Part A). Participants with incident HIV infections observed during phase I of the study will be asked to enter long-term follow-up in phase II of the study. Phase II of the study observes HIV infected individuals for an extended period to evaluate early events, chronic viral burden and early disease progression. The protocol is organized into a base protocol, which describes both Parts A and B as well as both phases I and II and the scientific rationale and analysis plan. The site-specific addenda describe in greater detail implementation plans for the study at each site. Protocol team members for each of the four enrollment sites are listed in the relevant site-specific addenda.

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TABLE OF CONTENTS

SCHEMA

Title

HIV-1 Prevalence, Incidence, Cohort Retention, and Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand

Objectives

PRIMARY OBJECTIVES

1) Define the risk behavior, prevalence and incidence of HIV infection and retention of a high risk cohort of adults in Thailand, Uganda, Kenya and Tanzania.
2) Obtain 150 acute HIV infections (AHI) with at least 30% captured within Fiebig stages I and II to support the full characterization of host responses and viral dynamics.
3) Observe an incidence of 3.7% with at least 30% of the incident cases identified prior to the advent of detectable antibody.

SECONDARY/EXPLORATORY OBJECTIVES

1) Assess and optimize HIV diagnostic strategies in HIV primary infection across multiple subtypes and risk groups.
2) Define the genetic diversity and evolution of HIV-1 in the prevalent and incident HIV cases with particular emphasis on characterization of acute, primary HIV infection.
3) Characterize immune activation, innate and adaptive cellular immunity in the early acute HIV-1 infection.
4) Characterize B cell responses in peripheral and mucosal compartments arising in early acute HIV-1 infection.
5) Characterize genetic polymorphisms in genes controlling host restriction, innate and adaptive Immunity, and their influence on HIV acquisition and early control of HIV infections.
6) Characterize clinical events including endemic infection as risk factors for HIV acquisition.

Study Design

Phase I: Non-randomized, cohort, prospective, 24-month observational study to be conducted in two parts. Part A is a pilot study to establish and optimize operations and study design features to meet study objectives prior to opening the study to full enrollment at all sites in Part B. The study will conduct a screening visit and initial

follow-up in all enrolled volunteers. This will include both HIV negative and HIV prevalent cases. Prevalence is estimated to be high in these populations and as many as 1000-1500 volunteers will be enrolled and evaluated in the first two visits with an enrollment target of 500 HIV negative, high-risk volunteers per site. Subsequently, only HIV negative volunteers will be followed for a period of approximately 24 months, except for a small number of HIV positive individuals for masking to minimize risk of stigmatization. After the initial two visits, volunteers will be seen at the research clinic approximately every 3 months. In addition, at locations that are convenient to the volunteers, small blood volume samples will be collected using microvettes twice weekly. These samples will be analyzed and results returned to the site within 48 hours to permit new HIV positive participants to be identified while they remain antibody negative, HIV nucleic acid test positive. All newly infected participants will be referred to phase II of the study.

Phase II: study of HIV incident cases arising within phase I. HIV incident volunteers identified at any follow-up visit will be asked to consent to participate in phase II to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of at least 50 months from the time of entering the AHI phase. Very frequent visits using a more intensive collection of samples initially will occur to characterize acute HIV pathogenesis and then at regular three month intervals for the duration of the study.

Participants

The study population will include men and women, aged 18-50 years old, who may be members of the following high risk groups: female and male sex workers (SWs), barworkers (BWs), sexually transmitted infection clinic attendees (STIAs), motorcycle passenger transporters (Boda-Boda), transgenders (TG), and men who have sex with men (MSM). Although these populations are known to be among "Most at-Risk Populations" or MARPs, not all members of these occupational groups engage in equivalent risk behavior. Enrollment will therefore engage members of these populations whose behavior places them at higher risk.

Part A (pilot study), will enroll primarily SWs and BWs in Africa and SWs, TGs and MSM in Thailand. Each site will enroll up to 200 volunteers. Part B (full study) will enroll all of the MARPs to attain enrollment of 300 HIV negative, MARPs volunteers at each site in the following groups.

Kenya: SW; STIA

Tanzania: BW

Uganda: SW; STIA, BW and Boda-Boda

Thailand: SW, MSM and TG

Study Duration

Duration of enrollment will vary by site according to HIV prevalence and volunteer interest. Approximately nine months will be required for enrollment into Part A at all

sites and assessment of protocol operations based upon retention and compliance characteristics will require an additional 12 months. Assuming no design modifications are required as the study opens Part B, an additional 12-18 months will be required to complete enrollment and all HIV negative, high-risk volunteers participate in phase I for 24 months. Thus complete enrollment and follow-up for phase I, the surveillance component of the study will require 57 months (21 months for Part A and 36 months for Part B). Incident HIV infections will be followed for a minimum of 50 months and total duration from first enrollment, follow-up and data analysis will require at least 10 years.

Study Funded By

Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)

US Military HIV Research Program (USMHRP)

Study Clinical Sites

Kampala, Uganda (Protocol RV 217a)

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LIST OF ABBREVIATIONS AND DEFINITIONS

TERM	DEFINITION
ACASI	Audio Computer Administered Self-Interview
ACD	adrenocortical dysplasia homolog (mouse)
ADCC	antibody dependent cellular cytotoxicity
AFRIMS	Armed Forces Research Institute of Medical Sciences
Ag/Ab	antigen/antibody
AHI	acute HIV infection
AI	associate investigator
AIDS	Acquired Immunodeficiency Syndrome
ALT	alanine transaminase
APOBEC	apolipoprotein B-editing catalytic polypeptide-like subunit
Boda-Boda	motor cycle passenger transporters
BW	barworkers
CAB	community advisory board
CAP	College of American Pathologists
CBC	complete blood count
CCR5+	chemokine receptor 5
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence interval
CLSI	Clinical Laboratory Standards Institute
CNV	copy number variation
CRF	case report form
CRR	continuing review report
CTL	cytotoxic T lymphocyte
CVL	cervicovaginal lavage
DAIDS	Division of AIDS
DC	dendritic cells
DCAC	Data Coordinating and Analysis Center
DHSP	Division of Human Subjects Protection
DLDM	Department of Laboratory Diagnostics and Monitoring
DMO	data management officer
DMS	data management supervisor
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DoD	Department of Defense
DSMB	Data Safety Monitoring Board
EDTA	ethylenediaminetetraacetic acid
EI	exposed infected

EIA	enzyme immune assay
ELISA	enzyme-linked immunosorbent assay
EM	expectation maximization
env	envelope
ERC	ethical review committee
EU	exposed uninfected
FL	full-length
FSW	female sex worker
GALT	gut associated lymphoid tissue
GCP	Good Clinical Practice
GEIS	Global Emerging Infections Surveillance
GLP	Good Laboratory Practices
GWAS	Genome-wide association studies
HAART	Highly Active Anti-Retroviral Therapy
HEPS	highly exposed persistently seronegative
HISIS	HIV Superinfection Study
HIV	human immunodeficiency virus
HIVNET	HIV Network
HJF	Henry M. Jackson Foundation for Advancement of Military Medicine
HLA	human leukocyte antigen
HPTN	HIV Prevention Trials Network
HSR	hypersensitivity reactions
HSV-2	Herpes Simplex Virus Type 2
HTA	high transmission area
HW	Hardy-Weinberg
ICF	informed consent form
IFNγ	interferon gamma
Ig	immunoglobin
IgG	immunoglobin G
IgM	immunoglobin M
IP	internet protocol
IRB	institutional review board
KEMRI	Kenya Medical Research Institute
KIR	killer immunoglobulin receptor
LBV	large blood volume
LD	linkage disequilibrium
LFT	liver function tests
LFU	lost to follow-up
LLV	lower level viremia
LMU	Ludwig-Maximilians-University of Munich
LPS	lipopolysacharide
m	monomeric
MARPs	most at-risk populations

MEPS	Military Entrance Processing Stations
MHA	multi-region hybridization assay
MMRP	Mbeya Medical Research Programme
MO	Medical Officer
MOP	Manual of Operations
MoPH	Ministry of Public Health
MSM	men who have sex with men
MSW	male sex worker
MUWRP	Makerere University Walter Reed Project
MVA	modified vaccinia virus Ankara
NAAT	nucleic acid amplification technologies
Nab	neutralizing antibodies
NAD	nicotinamide adenine dinucleotide
NCCLS	National Community for Clinical Laboratory Standards
NCR	natural cytoxicity receptors
NGO	non-governmental organization
NHS	normal human serum
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	natural killer
OE	Onto-Express
OHRP	Office for Human Research Protections
p	polymeric
PAMP	pathogen-associated molecular patterns
PAM-R	prediction analysis of microarrays in the R workbench
PAVE	Partnership for AIDS Vaccine Evaluation
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEP	post-exposure prophylaxis
PEPFAR	President's Emergency Plan for AIDS Relief
PHSC	Public Health Service Center
PI	principal investigator
PrEP	pre-exposure prophylaxis
RCC	Regulatory Compliance Center
RDS	respondent driven sampling
RLU	relative luminescence units
RNA	ribonucleic acid
RPR	rapid plasma reagin
RR	rate ratios
RSS	Research Support System
RTA	Royal Thai Army
RT-PCR	reverse transcription polymerase chain reaction
s	secretory

SAM	significance analysis of microarrays
SBV	small blood volume
SC	seroconverter
SE	southeast
SGA	single genome amplification
SIV	simian immunodeficiency virus
SN	seronegative
SNP	single nucleotide polymorphisms
SOE	schedule of events
SRV	steadily rising viremia
STI	sexually transmitted infections
STIA	sexually transmitted infection clinic attendees
SW	sex worker
TG	Transgender
TLR	toll-like receptors
TOU	Test of Understanding
TRIM5	TRIpartite Motif-5
UNAIDS	The Joint United Nations Programme on HIV/AIDS
US	United States
USA	United States of America
USAMC	United States Army Medical Component
USAMRMC	United States Army Medical Research and Materiel Command
USAMRU-K	United States Army Medical Research Unit-Kenya
USFDA	United States Food and Drug Administration
USG	United States Government
USMHRP	United States Military HIV Research Program
VCT	voluntary counseling and testing
VRC	Vaccine Research Center
VTN	Vaccine Trials Network
WB	Western Blot
WBC	white blood cells
WDC	withdrawal of consent
WGA	whole genome amplification
WRAIR	Walter Reed Army Institute of Research
WRP	Walter Reed Project

1.0 INTRODUCTION

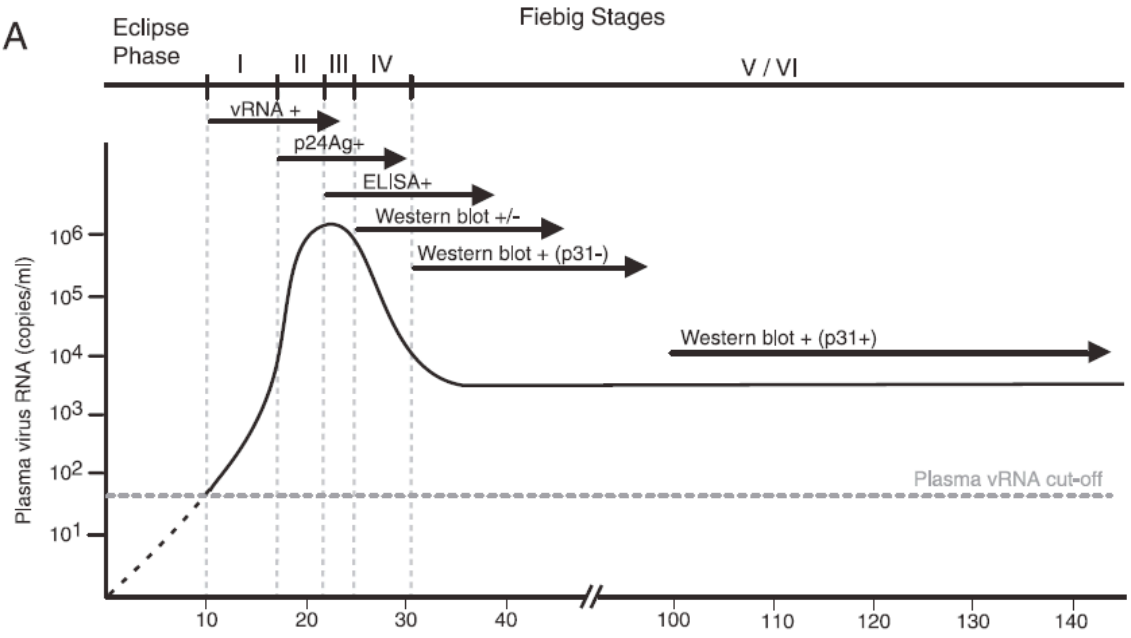
The HIV vaccine field has been roiled by the results of STEP, the Merck/NIH-VTN phase IIB "proof of concept" efficacy trial (Science 2007). The failure of this candidate vaccine to prevent infection or afford discernable control of viremia early after infection has called into question the merits of any vaccine strategy focused entirely upon induction of T cell mediated immune responses. Further, the apparent increase in transmission rate in a subset of participants not only alters the risk to benefit analysis of prophylactic vaccine trials but underscores our limited knowledge of transmission events and factors that modulate transmission efficiency. A consensus has formed that a more fundamental understanding of the basic pathogenesis of HIV infection and disease is required (Science 2008, Walker 2008). Further, the design of future "proof of concept" efficacy trials will need to carefully consider designs which limit experimental vaccine exposure to the minimum number of volunteers required.

HIV disease progression rates vary substantially and correlate with early viremic set-point (Mellors 1997; Lyles 2000). Individuals who exert poor control over viral replication in this early phase of infection are destined to progress rapidly to immune deficiency and death and conversely those who are able to substantially control viral replication enjoy relatively prolonged survival. Rarely, individuals are able to suppress viral replication below levels of detection for extended periods and appear to remain healthy for an indefinite period (Deeks 2007). Recent work has shown that the gut associated lymphoid tissue (GALT), the predominant reservoir of CD4+ T cells, suffers massive depletion of memory CD4+ T-cells during the first weeks following SIV infection of macaques (Veazey 1998; Mattapallil 2005) or HIV infection in humans (Guadalupe 2003; Brenchley 2004). Further, the primary target for HIV in the acute infection are memory T cells with specificity for HIV antigens (Douek 2004; Lichterfield 2007; Strapans 2004). The extent to which the GALT is preserved correlates with survival in non-human primate models receiving candidate preventive SIV vaccines (Mattapallil 2006). In addition to the critical role of early viral replication and the viral set-point, it has been shown that T cell activation levels independently are associated with CD4+ T cell decline and that this parameter is also established as an early set point as humans emerge from the acute phase of infection (Deeks 2004). A recent report finds a strong correlation between viral load in the acute phase of illness and viral load set point (Kelley 2007). This study also showed a higher acute viral burden was associated with more symptomatic primary HIV illness. Individuals with symptomatic primary infection have higher viral load set-points and higher mortality rates (Henrad 2000; Lavreys 2006).

These observations suggest that the long-term course of HIV disease progression is established in the first weeks of infection. It is critical to understand the early events associated with GALT T-cell depletion and the mechanisms responsible for the variable control of viral replication. This work in humans has been limited to individuals who have passed the critical time points where the dynamics of host-viral interaction dictate the level of acute viral replication and influence the ultimate steady state of chronic viremia. It is hoped that understanding what events distinguish the elite controllers from those with a typical course of HIV infection or from those who progress rapidly will afford insights leading to novel prevention and treatment strategies. It is apparent that the events that define these differences occur very early after infection.

The current classification of acute infection relies upon the hierarchical humoral immune response and the dynamic characteristics of HIV detection as defined by Fiebig et. al., (Fiebig 2003), and outlined below in Figure 1(Salazar-Gonzalez 2008).

Figure 1 Fiebig Stages



The earliest phase of infection, the eclipse phase, precedes HIV ribonucleic acid (RNA) detection and is estimated to last from a few days to 11 days (Kahn 1998). Fiebig stage I follows with detection of viral RNA only. Fiebig stage II is characterized by an increased RNA signal and detection of p24 antigen. Fiebig stage III is similar to stage II but includes a positive sensitive enzyme immuno-assay (EIA) identifying HIV specific antibodies. Fiebig stage IV, V and VI ensue with an indeterminate Western blot, followed by a positive Western blot absent a p31 signal and finally a fully positive Western blot, respectively.

The duration of each Fiebig stage for non-B clade HIV infections is not known. The relationship of the eclipse phase and subsequent Fiebig stages, in terms of duration or amplitude of viral load, to the viral load at set-point and the long term clinical course is not known. It is plausible that innate immunity and genetically determined host defense mechanisms are critical determinants to preservation of the GALT and the long-term effectiveness of the host adaptive response to HIV. This study seeks to identify acute infection in stages I and II prior to establishing peak HIV viremia and potentially to characterize the eclipse phase to better define the early pathogenic events, characterize host-viral interactions and explore their relation to long term outcomes in terms of viral set-point and immune system integrity.

1.1 State of USMHRP Vaccine Research

The primary mission of the United States Military HIV Research Program (USMHRP) is to develop strategies to prevent HIV infection and the major objective of this effort is establishing a vaccine for prevention of HIV infection across the globe. There are two obstacles that arise towards the achievement of this objective: 1) the determinants of protective immunity to HIV are unknown; and, 2) the role of genetic diversity in limiting protective immunity is unknown. The USMHRP is advancing a vaccine strategy for efficacy testing in Thailand to address the first obstacle in an environment with a single subtype of HIV in circulation (subtype E). Clinical testing of vaccines in East Africa permits an evaluation of the role of genetic diversity as subtypes A, C and D are contributing in varying proportions to the HIV epidemic in the region.

Together with partner organizations, USMHRP has identified at least one site in each of the East African countries of Uganda, Tanzania, and Kenya, and another in Thailand that could serve as vaccine efficacy trial sites. The study proposed here is part of a series of studies aimed at developing Phase IIb-III clinical trial capability through identification and preparation of cohorts for possible phase III HIV vaccine efficacy trials. Efforts in each country are independent collaborations between the USMHRP, local partners, academic centers and/or host country medical research institutes. However, an attempt it made to integrate activities at all sites. By combining capabilities, training and experience from each site, USMHRP seeks to achieve a sufficiently cohesive organization to conduct a single trial at all the sites in each of the countries or to contribute collaboratively as a network to larger multi-national clinical trials.

As noted above, there is a need for further basic research defining early events in HIV infection, which might guide vaccine development. To this goal, the proposal addresses both basic cohort definition and a series of exploratory objectives aimed at discovery of correlates of protection from HIV infection or disease.

The USMHRP has three vaccine products in clinical development. These are the ALVAC/gp120 prime/boost clade B and CRF01 AE vaccines currently in efficacy testing in Thailand, the Vaccine Research Center (VRC) deoxyribonucleic acid (DNA) prime/recombinant Adenovirus type 5 boost which have been evaluated at USMHRP in East African sites and will be undergoing efficacy testing on a small scale in 2009 and finally a DNA prime, modified vaccinia virus Ankara MVA boost vaccine under development within USMHRP. Results from the Thai trial of the ALVAC/gp 120 study will be available in 2009 and, if positive, would utilize the cohorts developed through this proposal for further efficacy testing. The VRC candidate vaccine may also be tested in its current formulation in these cohorts should the small efficacy trial commencing in 2009 show interim safety. It is expected that the USMHRP DNA and MVA product will be ready for efficacy testing in about five to six years. Clearly, identifying and characterizing high-risk cohorts is needed for anticipated efficacy trial needs eventuating over the next three to six years.

The feasibility and acceptability of working in these high-risk populations with frequent visits to ascertain very early HIV infection will be established through conduct of RV 217 parts A and B. If successful, it is clear that this population would be suitable for a number of intervention studies aimed at reduction of secondary transmission, alteration of long term prognosis and potentially, radical cure of HIV. These studies may include behavioral, anti-retroviral and

vaccine interventions alone or in combination. The investigators are very interested in these studies and applying lessons learned from the proposed study to the optimal design and execution of acute HIV infection intervention research.

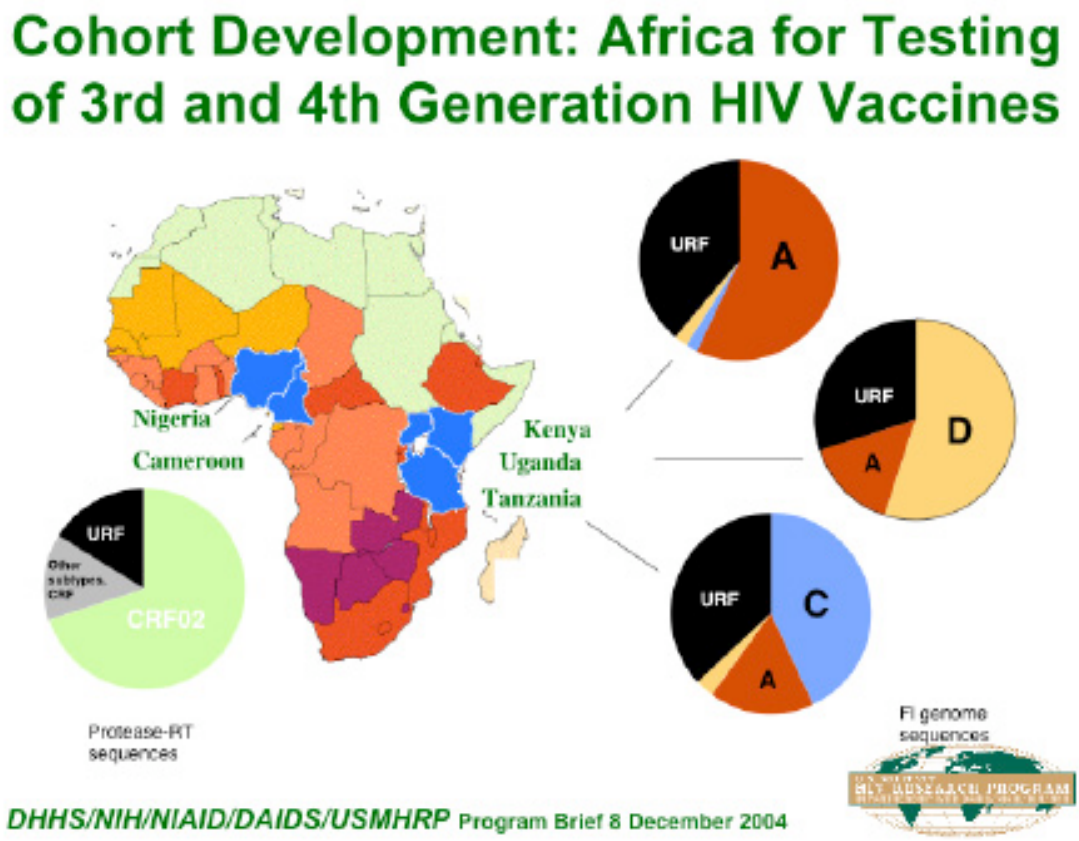
1.2 Cohort Development Activities

The USMHRP has sought long-term partnerships to conduct HIV vaccine research in keeping with the goals, processes and considerations discussed above. Initial cohort development activities in Thailand were conducted to support evaluation of the ALVAC/gp 120 prime-boost vaccine strategy. These efforts included seven studies at various sites within Thailand. The highly commendable AIDS control program in Thailand has limited potential populations for the conduct of further efficacy trials to high-risk populations, such as sex workers, MSM, and transgendered individuals. To further achieve strategic requirements, populations with high incidence of multiple HIV subtypes must be identified. A review of the prevalence rates and distribution of genetically defined subtypes indicates that Africa provides a setting with severe epidemics of multiple subtypes. In view of the need for political stability and will to address HIV/AIDS, the presence of an infrastructure and cadre of trained scientific collaborators as well as other considerations, the USMHRP has expanded its cohort development activities to support future efficacy trials in three African countries: Uganda, Tanzania, and Kenya. As shown below, these sites provide the opportunity to evaluate vaccines within communities with four of the prevalent sub-types of HIV (A, C, D and CRF02_AG (IbNG), along with the two sub-types prevalent in Thailand (B and CRF01_AE).

The epidemic in all three East African participating countries is substantial and in most respects similar among the three (UNAIDS 2007). Within each of these three countries the USMHRP is currently conducting vaccine cohort development activities that have thus far identified populations with prevalence and incidence sufficient to contribute to HIV vaccine trials with reasonable efficiency (12-month incidence greater than 1.3% - 1.8%).

In addition, the sites offer an important difference in respect to the molecular epidemiology of the epidemic at each site based upon full-length sequence data. The distribution of subtypes is shown below in Figure 2. More detailed analysis identifies the major subtype in each region contributing disproportionately to the recombinant forms found in that region. For example, in Kenya, pure A subtype is most common and most recombinants include substantial subtype A genetic contributions.

Figure 2: Cohort Development: Africa for Testing of 3rd and 4th Generation HIV Vaccines



Of critical importance, the incidence and follow-up characteristics have been evaluated in the context of closed cohorts. To some extent, experiences at the three African sites have varied. At the Tanzania site, relatively high follow-up rates were identified in all three of the site's cohorts and in the general population cohort no decline in incidence was observed over time. By contrast, the tea plantation cohort in Kericho has observed steadily declining incidence over time and had low follow-up rates early but have shown considerable improvement over time with adjustments to compensation, follow-up schedules, and research study staff participation and study "ownership."

RV 217 seeks to not only define the cohort characteristics of Most at-Risk Populations (MARPs), but also seeks to identify very early HIV infections. It is possible to model closed cohort characteristics in an open cohort design. The retention of these MARPs is not known and replacement enrollments for loss to follow-up and non-compliance may be needed to acquire the number of acute infections specified. Details of the procedures and circumstances for additional enrollment are noted in the protocol Manual of Operations (MOP).

1.3 Most At-risk Populations (MARPs)

Current Phase IIb/III HIV vaccine efficacy study protocols are focusing upon populations with annual incidence of at least 2% and preferably 3% with yearly loss to follow-up of 10% or less. Although current USMHRP sites may reach these metrics with respect to a 2% incidence and 90% annual follow-up, when age constraints are imposed to identify the higher risk elements of these cohorts, it is considered necessary to explore MARPs as potential participants along with our established cohorts. The purpose of this study is to develop data for key parameters used to assess the suitability of these additional populations.

In addition, this cohort activity represents an opportunity to determine if such MARPs are interested in participating in HIV vaccine research and provides a vehicle for positive community engagement as well as HIV education, prevention, and treatment referrals where applicable in MARPs often in need of such services.

The populations to be included in this study have been the subjects of considerable research and their increased risk for HIV infection is well established (Estebanez 1993). Each site has assessed likely incidence of study sub-populations based upon direct experience, published information or personal communication from those performing studies in these groups. Incidence rates are shown below:

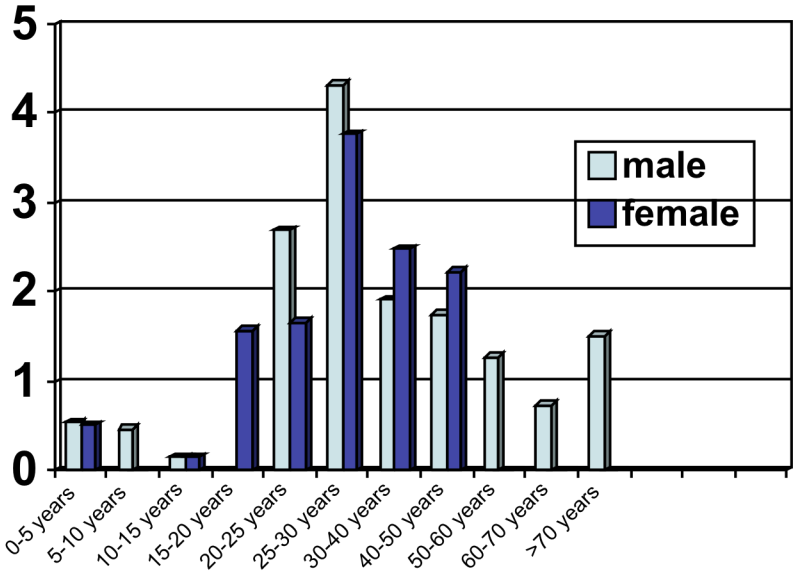
sex workers (SW)/barworkers (BW): Kenya 3%,
SW only: Uganda 4%
BW, motor cycle passenger transporters (Boda-Boda): Uganda 3%, Tanzania 7%
sexually transmitted infection clinic attendees (STIAs): Kenya and Uganda 3%
SW, men who have sex with men (MSM), transgender (TG): Thailand--similar cohorts in Bangkok suggest an incidence of 4% (3.5 – 5%);

Despite the high incidence in these MARPs, concern has been raised about the potential to include these groups in intervention studies due to high mobility and unacceptable rates of loss to follow-up. Recent work from the Tanzania group has successfully demonstrated an operational approach to one of these high risk groups, bar workers, which maintained an aggregate follow-up of 75% over three years (Hoffmann 2004). At that site, a cohort of 600 female BWs was established with the aim of studying their HIV-1 status, subtypes of HIV-1 and immunological and virological correlates of infection. Data collection based upon 3-month follow-ups included laboratory data for HIV and selected sexually transmitted infection (STI), clinical and behavioral data.

The HIV-incidence over a 3-year follow-up was 7 per 100 person-years (Hoffmann 2004). Newer unpublished data, obtained through the ongoing EMINI surveillance, a population based, observational cohort of 20,000 individuals, could confirm high prevalence and incidence rates for HIV in “high-transmission-areas”. In this study nine distinct geographical areas with a total population of 220,000 individuals were registered and a subset of 10% was randomly selected for the surveillance. Five of the nine sites are close to the transafrican highway and called “high-transmission-area” for HIV. We found HIV incidence rates of up to 4% in this study group. As this surveillance is done in the general population and does not focus on high-risk behavior or

occupations, we expect a higher incidence in high-risk groups, similar to the findings of the HIV Superinfection Study (HISIS) described above.

Figure 3 HIV Incidence in 5 "high-transmission-areas" in the Mbeya Region from 2006/2007 to 2007/2008



The overall HIV-prevalence was 68.0% in the 600 participants initially enrolled into the cohort. The yearly HIV-incidence was 15.2, 6.2 and 2.4 per 100 person-years in year 1, 2 and 3 respectively (Riedner 2006). The most difficult task concerning a cohort involving a MARP would be to take account of the mobility of the study participants. In the cohort described above the baseline survey determined that only 12% of the eligible population worked more than three years in the same workplace. The remarkable retention of 75% over three years (Hoffmann 2004) can be attributed to the trust built within the target group, their employers, and local authorities before the study participants were enrolled and that this trust was maintained throughout the study.

Other data support a high incidence in the populations targeted for enrolment in RV 217. Several studies have documented high incidence rates in female sex workers (FSWs) (3.2-9.8%), bar-workers (4.27-5%), and truck drivers (3-4%) in the African setting (Kaul 2004; Laga 1993; Van Loggerenberg 2008; Rakwar 1999; Jackson 1997; Riedner 2006; Watson-Jones 2008; Baeten 2000). In two studies, the temporal trend of infections identified a preponderance of incident cases arising in the first 6-12 months of study (Riedner 2006; Baeten 2000).

1.4 Clinical Events Including Endemic Infection As Risk Factors For HIV Acquisition

Results of the recent STEP study of the Merck Ad5 vaccine showed a correlation between pre-existing Ad5 antibodies and increased susceptibility to infection to an Ad5 based vaccine (Science 2007). One possible explanation for increased susceptibility is that the Ad5 vaccine vector boosted an existing primed-Ad5 adaptive memory immune response and the resultant secondary immune response predisposed volunteers to an increased susceptibility to HIV infection, perhaps by a mechanism as simple as stimulating the production of more chemokine receptor 5 (CCR5+) CD4+ T-cells, the primary target of early HIV infection. If a vaccine indirectly increased susceptibility to HIV infection by stimulating an immune response then it is reasonable to hypothesize that other non-vaccine stimulants of adaptive immunity would also increase HIV susceptibility. Since many diseases are known to induce robust humoral and cellular immune responses, we hypothesize that individuals with co-incident malaria, helminthes, or sexually transmitted infections will have an increased risk of HIV transmission. An increase in HIV replication has been documented in association with malaria infection *in vitro* (Xiao 1998; Frobel 2004) and *in vivo* (Hoffmann 1999; Kublin 2005), and recently, that acute HIV infection (AHI) is highly prevalent in patients suspected of malaria (Bebell 2008). To explore this issue, we propose to estimate the association of HIV infections with recent malaria infection, sexually transmitted infections, other infectious diseases as discerned by interim medical history, physical examination and laboratory markers of parasitic infestation, gut endothelial integrity and systemic immune activation.

1.5 Scientific Background

1.5.1 HIV Diagnostics

Primary HIV infection causes diverse non-specific clinical symptoms that typically do not lead to HIV diagnosis (Daar 2001), and the usual serological methods for laboratory diagnosis of established infection do not turn positive for weeks to several months (Horsburgh 1989, Ciesielski 1997). The seroconversion window duration has narrowed due to emergence of 3rd generation EIAs which detect Immunoglobulin M (IgM) as well as Immunoglobulin G (IgG), and 4th generation assays that detect both antibody and p24 antigen (Ly 2001). While these advances have incrementally narrowed the seroconversion window, little is known about relative performance of these assays using non-subtype B seroconversion panels. The majority of studies that allow for direct comparison of assay sensitivity during early seroconversion have utilized samples from patients in developed nations with subtype B infection, leaving questions about the generalizability of such data, particularly since most available assays are based on subtype B strains (Peeters 2003). Studies to date have assessed subtype detection using viral culture material or chronically infected individuals, whereas seroconversion panels tested have been from patients infected with subtype B (Ly 2001). Implementation of combination tests in many locations is constrained by cost and laboratory factors that would be at least partially addressed if antigen/antibody (Ag/Ab) rapid tests were available.

In less cost-constrained environments, early diagnostic strategies have focused on detection of either viral antigen (Fiscus JCM 2007) or nucleic acid (Busch AIDS 2005) for diagnosis of primary infection. Here again, studies comparing methodologies for detection of acute infections have been conducted using subtype B seroconversion panels (Busch Transfusion 2005). A study to evaluate methods of detecting clients with acute HIV infection in Malawi compared the Roche Amplicor Monitor RNA assay v1.5 to p24 antigen assays and several antibody tests (Fiscus JID 2007), but the methodology employed did not include serial specimen collection that would allow for assay-assay comparison.

A stereotyped pattern of sequential appearance of diagnostic markers has been defined for subjects with primary subtype B infection consisting of sequential appearance of RNA, p24 antigen, and anti-HIV antibodies (Fiebig 2003). While proper characterization of early infection stage affords the ability to describe viral and immune dynamics among infected individuals, the sequence and duration of laboratory stages are not known for patients from developing nations with non-B subtype infections.

Most HIV surveillance has relied on seroprevalence estimates using samples collected from sentinel populations. However, incident infection surveillance provides a more timely and proximate estimation of recent transmission, yielding much greater utility to prevention programs and site selection for interventions such as microbicides and vaccines. Major efforts have been underway to develop methods of incidence estimation using a single blood sample. All such approaches rely on the well-documented crescendo anti-HIV humoral immune response that occurs during the first several months of infection. The Centers for Disease Control and Prevention (CDC) has been the leader in the field, and currently considers three approaches worthy of further study. First, incident cases may be reactive using the sensitive EIA but non-reactive using a less sensitive assay (Gouws 2002; Janssen 1998). Secondly, CDC has been developing a strategy to estimate the proportion of anti-HIV-1 IgG in total IgG following seroconversion (Hargrove 2008; Parekh 2002). Finally, incidence may be estimated using HIV antibody avidity (Suligoi 2008; Loschen2008; Chawla 2007, Martro 2005; Suligoi 2003). Development of these strategies has been limited by lack of appropriate samples to allow for statistical analysis, which requires sequentially collected samples from the same individual including an HIV-negative test and positive tests <1 year after the last negative test, as well as three or more positive tests spaced at ≥ 3 month intervals (personal communication, Bernard Branson, CDC 02MAY08).

1.5.2 Viral Evolution

From the moment of infection, multiple lines of host defense impinge on HIV-1 (Goff 2004; Hilleman 2004). First, host restriction factors, such as co-receptor polymorphisms (Carrington 1999a; O'Brien 2000), Apolipoprotein B-editing catalytic polypeptide-like subunits (APOBECs) (Harris 2004; Malim 2006; Holmes 2007) and TRIM5-alpha (Kaumanns 2006; Speelmon 2006; Li 2007), control the establishment of a productive infection, influence the magnitude of the initial viremia, and have a continuing impact on the subsequent development of effective viral control. Next, innate immune mechanisms, already in place before viral infection occurs, take control of virus spread in the infected individual, using both cellular (NK) and humoral (antibody dependent cellular cytotoxicity (ADCC)) effector mechanisms to identify and lyse infected cells

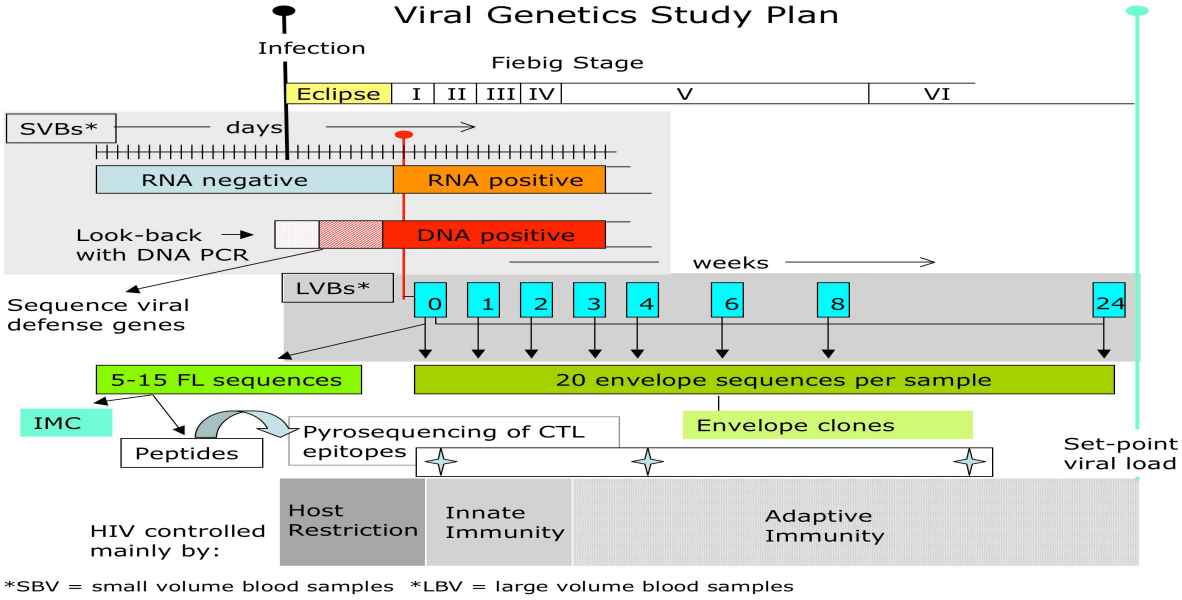
and provide for efficient viral recognition and clearance (Little 1999; Jacobs 2005; Boyton 2007). Concomitantly, the adaptive immune response is triggered, and its effectiveness is dependent on the outcome of the earlier interactions (da Silva 2003; Douek 2006; Davenport 2007; Nabel 2007). If host restriction and innate immunity are relatively ineffective, the adaptive responses may be mounted in an environment of massive immune system damage, dis-coordination, and unfavorable cytokine milieu, and the adaptive immune system is faced with a viral quasispecies that results from unbridled replication and a corresponding exponential increase in quasispecies diversity (Coffin 1996; Ferbas 1996; Liu 2002; Troyer 2005; Lemey 2007; Tebit 2007). In a positive feedback loop, poorly constituted adaptive responses lead to further uncontrolled viral replication, and diminish the long-term benefits that would ordinarily accrue from effective adaptive cellular and humoral responses in the years to come. The commonly used outcome measure for host-virus interaction, viral load set-point, is the aggregate result of all of these host defenses, is further modulated by the host genetic (Gottlieb 2008) background and the infecting viral strains, and is not established until an equilibrium has been reached between the virus and host, typically around 6 months post-infection. During the first six months of infection, significant changes occur in the diversity and neutralization susceptibility of HIV-1 envelope (Wei 2003; Derdeyn 2004; Gottlieb 2008; Liu 2008) and some cytotoxic T lymphocyte (CTL) responses have already generated escape variants that come to dominate the population (Karlsson 2007; Liu 2007; Loh 2008); investigation of the earliest responses to adaptive immune responses is a critical element of HIV vaccine development.

A significant gap in knowledge exists regarding the specifics, timing, range, and relative effectiveness of early control mechanisms for HIV-1. Barriers to knowledge have been both logistical, driven by the relatively short interval between introduction of HIV-1 into the human host and the onset of effective viral control (Busch 1997; Kahn 1998; Little 1999), and technical, related to the inherent difficulty of access to and efficient sampling a diverse viral quasispecies during the earliest time period. Here we propose both traditional and novel approaches to define the initial viral quasispecies, in order to gather direct evidence for selection by host restriction factors, innate immune mechanisms, and from the earliest manifestations through the complete development of adaptive cellular and humoral immunity. Novel features of this proposal include: 1) an exploratory look-back study for the presence and characteristics of proviral DNA in the clinical eclipse phase (Fiebig 2003), after infection but prior to the first detectable plasma RNA (Fiebig 2005); 2) serial, in-depth early sampling of the envelope quasispecies before, during, and after the ontogeny of high affinity antiviral antibodies; 3) application of pyrosequencing, capable of sampling at least 1000 templates from the initial viral quasispecies, to investigate the contribution of CTL escape variants, present before the adaptive cellular response is mounted, to early escape from CTL; and 4) use of the yeast-based recombination/cloning system to efficiently generate infectious molecular clones of HIV-1 representing the earliest accessible viral strain(s) and some of the selected variants arising early in infection.

The viral genetics of the approximately 150 acute seroconverters will be studied within a strong contextual framework of accurate and consistent Fiebig staging (Fiebig 2003), early intensive clinical follow-up of seroconverters, maintenance of large, relevant, exposed uninfected cohorts, host genetic analysis, evaluation of innate and adaptive immune responses and the early impact of HIV-1 infection on gene expression profiles in key target cells, evaluation concurrent infection with selected endemic pathogens and of generalized immune activation, and a nested

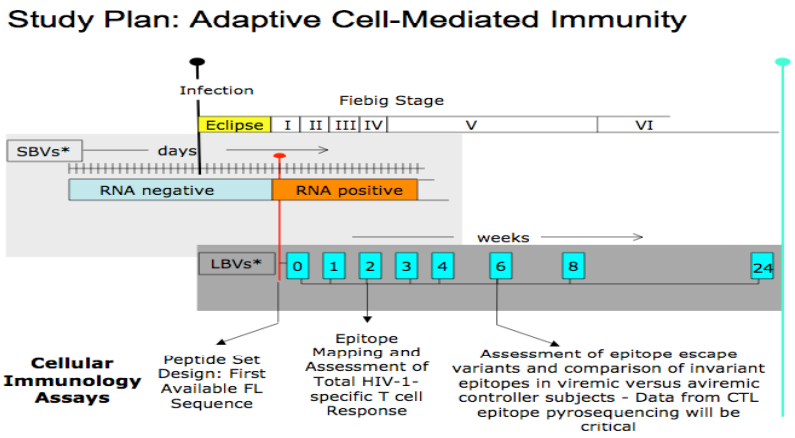
sub-study of mucosal damage and mucosal responses to HIV-1. Finally, the study will include four of six globally prevalent HIV-1 subtypes/ circulating recombinant forms (McCutchan 2006; Taylor, 2008) and two genetically distinctive human populations (Cao 2004) and (Kikak, G, unpublished data).

Figure 4 Viral Genetics Study Plan



1.5.3 Cellular Immunology

Figure 5 Adaptive Cell-Mediated Immunity



Adaptive T Cell Immune Responses to HIV-1

Defining the precise correlates of the cellular immune response to HIV-1 that are associated with control of HIV-1 replication has been the focus of intense study over the past two decades. The identification of such correlates has been pursued as part of a rational strategy to produce an HIV-1 vaccine capable of best mimicking the cellular immune response associated with control of HIV-1 viremia. Once defined, such correlates will also help establish the necessary benchmarks for candidate vaccine down selection and efficacy testing. While indisputable proof that $CD8^+$ T cell activity is the causative selective pressure that controls viral load in either acute or chronic infection is not available (Letvin 2006; Letvin 2007; Walker 2008), an overwhelming abundance of circumstantial evidence supports this notion: the association of $CD8^+$ T cell activity with virus clearance during acute infection (Borrow 1994; Koup 1994; Brander 2002); the clear association of certain human leukocyte antigen (HLA) class I alleles with delayed progression to Acquired Immunodeficiency Syndrome (AIDS) (Gao 2001; O'Brien 2001; Carrington 2003; Gao 2005; Altfeld 2006; Martin 2007) and the molecular imprinting of HLA-allele associated mutations in the viral proteome both at the population level and within infected individuals (Kiepiela 2004; Bhattacharya 2007; Brumme 2007; Rousseau 2008); the positive selection of virus escape mutations within, and proximal to epitopes targeted by $CD8^+$ T cells (Wilson 1999; Kelleher 2001; Draenert 2004; Leslie 2006), the transmission of these escape mutants to a new host (Goulder 2001; Leslie 2004; Leslie 2005; Goepfert 2008), and the subsequent reversion of these escape mutants in hosts that cannot make an immune response against them (Allen 2004; Feeney 2004; Leslie 2004; Allen 2005; Goepfert 2008). Furthermore, the quality of the HIV-specific $CD8^+$ T cells in controlled versus uncontrolled infection differs with respect to both their functional capacity and their surface phenotype. Described functional abnormalities of $CD8^+$ T cells in uncontrolled HIV-infection include a reduced capacity to proliferate which is linked to a reduction in both autocrine IL-2 production and surface CD28 expression (Trimble et al., 2000; Migueles 2002; Topp 2003; Lichterfeld 2004; Rethi 2005), reduced production of Interferon gamma (IFNγ), and a general decrease in the number of cells with a multifunctional phenotype (Betts 2004; Betts 2005; Betts 2006; Streeck 2008). The differentiation status of HIV-specific $CD8^+$ T cells is also compromised in HIV-infection, resulting from the disregulation of expression of a variety of surface molecules including, but not limited to, Immunoglobulin (Ig)-superfamily molecules, TNFRSF receptors and ligands and chemokine receptors (Day 2006; Trautmann 2006; Wang 2007; Elrefaei 2008; Holm 2008). Hence, it has become apparent that measuring any given parameter in a univariate approach does not provide a meaningful assessment of the quality of the adaptive cellular immune response against HIV-1. A systematic multi-factorial approach to studying the cellular immune response as early as possible after infection with HIV-1 would identify critical facets of the adaptive immune response associated with control or delay of initial peak viremia, and help establish important immune parameters that should be measured during vaccine trial assessment.

T-cell Immune Activation

HIV-1 infection is associated with immune activation that involves T-cell lymphocytes and is characterized by an increased expression of cell surface markers some of which include CD25, CD38, CD69, HLA DR, β-2-microglobulin and neopterin (Fauci 1993). The most common measure of activation is CD38, an ectoenzyme important to the metabolism of T lymphocytes, and HLA-DR, which measures a class II major histocompatibility complex antigen. The degree of immune activation for any HIV infected individual is widely variable but during chronic

infection is thought to be associated with level of viremia, CD4 T cell counts, and disease progression (Giorgi 2002; Barry 2003; Hazenberg 2003; Eggena 2005), and have prognostic value for individuals on antiretroviral therapy (Tilling 2002; Ondoa 2005). Moreover, it is thought that baseline activation is higher in HIV seronegative Africans due to other infections as compared to Europe and North America (Kassu 2001). Additionally, it would be useful to describe the effects of infecting HIV-1 subtype on the immune system as measured through activation and regulatory T cell surface markers. Hence, it would be informative to assess T cell activation pre-infection, and at successive time-points post-infection to follow the evolution of this phenomenon. This approach may also reveal pre-existing immune activation correlates that increase risk of infection or the rapidity with which the virus replicates early in infection.

The specific role of innate immunity during HIV-1 infection is poorly defined, but may offer some insight into control, clearance, and moderation of infection and disease progression. NK cells are a subset of bone marrow derived large granular lymphocytes that are responsible for anti-tumor or anti-viral target recognition, lysis, and cytokine and chemokine production during initial infection until the adaptive arm can fully respond (Yokoyama 2004). It is known that T cells are to some extent linked with controlling HIV-1 infection and certain functional profiles may be associated with slow HIV-1 disease progression in infected individuals (Betts et al., 2006), indicating the importance of a vigorous adaptive immune response. Despite the association of adaptive T cells with partial control of HIV-1, decline of peak viremia during acute infection is poorly understood. During the early events in acute HIV-1 infection before the adaptive arm can fully respond the innate arm of the immune response may play a critical role in initial viral control, driving the adaptive arm, and may influence disease progression. Rapid expansion of certain subsets of NK cells, with a preferential increase in cytolytic NK cells, occurs in acute HIV-1 infection and is reduced after the emergence of HIV-1 specific CD8 T cells (Alter 2007b). Additionally, NK cell cytolytic response to HIV-1 is as potent as CD8 T cells *in vitro* (Kottilil 2003) and thus could be a strong containment force during this initial phase of infection. NK cells are regulated by a vast array of stimulatory and inhibitory receptors designed for a non-antigen specific recognition of infection and divided into 4 classes: killer immunoglobulin-like receptors (KIR), C-type lectin receptors, the natural cytotoxicity receptors (NCR), and the toll-like receptors (TLR) (Biassoni 2001). Chronic HIV-1 infection has been associated with a switch from inhibitory to activating C-type lectin receptor expression (Mela 2005) and a down regulation of NCRs (De Maria 2003). More recently focus has been placed on certain KIRs that interact with HLA molecules on potential target cells. The activating receptor KIR3DS1 interacts with certain HLA-B alleles and is associated with delayed progression to AIDS (Martin 2002), decreased NK cell activation and increased function (Long 2008). Furthermore, interactions between KIR and HLA have been shown to directly influence the functional ability of NK cells to control HIV-1 replication (Alter 2007a). KIR2DL3 is associated with NK cell activation, loss of function and inversely correlates to CD4 percentage in perinatally HIV-1 infected children (Ballan 2007). There is also evidence that certain KIR and HLA interactions may affect the risk of HIV transmission (Jennes 2006).

Further studies are needed to understand how subsets of NK cells contribute to protection and pathogenesis of HIV-1 infection. Here we propose to study the frequency, function, and phenotype of NK cells in acute HIV-1 infection. Analysis of phenotypic changes will focus on subset distribution defined by CD56 and CD16 expression, and expression of inhibitory and activating receptors. Functional assessment of NK cells will be made based on degranulation,

cytokine, and chemokine production after stimulation with media, MHC^{null} K562 cell line, or PMA/ionomycin. The data on NK cells will be analyzed in relation to the immune responses detected, and potential associations with viral subtype, host genetic factors and disease progression will be determined.

Functional Genomics and HIV Surrogate Marker Exploration
Functional genomics is a powerful tool to investigate host responses to infection and therapy. The success of gene chips is due, in part, to their ability to interrogate the entire genome, offering the broadest and most comprehensive coverage of the transcriptome. Many genetic polymorphisms affect disease trajectory, drug response or propensity to develop drug toxicities. Known host determinants of HIV-1 disease progression include genes for chemokine receptors (eg, CCR5 delta 32) and HLA molecules (eg, B57 and B27), natural killer cell killer immunoglobulin-like receptor (KIR3DS1), and APOBEC3F and APOBEC3G proteins. There are likely more unknown host genetic determinants yet to be discovered. While the search for elusive correlates of protection from HIV disease progression continues, we propose to use global gene expression profiling because it offers the most comprehensive coverage of the human genome - especially those genes involved in immunity or response to viral infection. Global gene expression profiling of the earliest emergent adaptive HIV-specific T cells in acute infection provides the most likely approach to discovery of novel correlate(s) of protection. In combination with other novel and exploration assay systems it would from a central component of a systematic multi-factorial approach to identify critical facets of the adaptive immune response associated with control or delay of initial peak viremia.

1.5.4 Humoral Immunology

Historically, vaccines that induce antibodies have been the most effective strategy to combat viral diseases such as polio, hepatitis, measles, and influenza. While antibodies are known to play an important role in protection in these diseases, the importance of antibodies in human immunodeficiency virus type 1 (HIV-1) protection and pathogenesis remains to be further defined (Zolla-Pazner 2004; Srivastava 2005; McMichael 2006; Huber 2007). Despite this and because of knowledge gained from other successful vaccines, the design of a vaccine that will elicit functional antibody responses directed against multiple HIV clades continues to be an important goal in laboratories that are developing and testing vaccines. It has been proposed that, although the virus envelope (Env) proteins have evolved an extraordinary ability to evade neutralizing antibodies, a vaccine that can elicit protective antibodies remains the best hope for developing an HIV vaccine that confers sterilizing immunity. Vaccine strategies that exclusively stimulate T-cell immunity may at best generate persistent and broadly reactive T-cell responses that can suppress virus and limit damage caused by the virus, without preventing infection (McMichael 2006). Characterization of the development and function of HIV antibodies in patients infected with both B and non-B subtypes will therefore be critical for understanding the role of humoral responses in both sterilizing and non-sterilizing immunity. It will also be critical to determine whether or not HIV-1 clade has a direct influence on functional humoral responses, in order to effectively inform vaccine design.

In addition to the viral Env proteins, antibodies may be generated to several HIV antigens. Antibody responses to conserved structural proteins like Gag are thought not to have any antiviral function, but these responses may provide correlates of immunity. For example,

declining or absent Anti-Gag antibody responses are associated with disease progression (Forster 1987; Weber 1987). The kinetics of the antibody response also may correlate with overall immunity. In a study of antibody responses during structured treatment interruption, it has been demonstrated that those who have a robust and quick anti-Gag response showed decreased virus set point. In addition, the magnitude of the Gag antibody response correlated with specific CD4 T helper frequency (Trkola 2004). In human vaccine trials, the correlation between antibodies and CD4 help has been provocative to date and will garner more attention as more vaccines elicit both potent antibody and CD4 responses. Long-lived plasma cells are the ultimate goal for HIV vaccines in order to maintain the vaccine induced antibody responses once the appropriate antibody responses are identified. In recent human vaccine protocols (Goepfert 2007; Johnson 2005; McFarland 2006), durable antibody responses were detected many weeks after the last vaccination. This work has suggested that memory B cell responses may be developed as a result of vaccination. Analyses of the kinetics and strength of this durable antibody response in primary HIV infection could enable more informed vaccine design for elicitation of a strong memory response.

The impairment of development of memory B cell responses and the alterations to the humoral immune system in general, that occur in HIV-1 infection have been recognized since the beginning of the epidemic. One of the first observations was that some patients developed autoantibodies and other autoimmune phenomena (Kopelman 1988; Kaye 1989). Since then many investigators have noted the defective humoral response in patients with poorly controlled HIV-1 infection (De Milito 2004; Moir 2001) and that restoration of immune function through the use of Highly Active Anti-Retroviral Therapy (HAART) may (Moir 2008) but does not necessarily result in repair of the humoral responses (Bekker 2006). There is evidence of both a dramatic loss of memory B cells with infection (Titanji 2006) and a shift in the circulating populations of B cells in HIV-1 disease (Malaspina 2006). Most of these studies have been carried out in patients with chronic HIV-1 infection, and the exact nature of changes in acute HIV-1 infection has not been fully characterized. Some of the critical questions that therefore remain to be answered are: What are the alterations in B cell subsets among control subjects, acutely infected patients, and chronically infected patients, particularly in patients infected with non-B HIV-1 subtypes? What are the circulating B cells that can be detected with HIV-1 antigen-specific B cell reagents in these samples? From which subsets are these cells detected and are there differences in these subsets between epitopes and/or in the time course of these cells found in the periphery? If specific antibody populations are detected in functional assays, can we recover the specific B cells using the newer technologies to recover the antibody genes of interest for molecular analysis?

Related to the questions of B cell subsets are also questions regarding the antigen-specificity of the B cells during acute infection. Since the response to HIV-1 appears to be skewed against the production of broadly neutralizing antibodies until late in infection, if at all, the fate of B cells capable of reacting to epitopes that are targets of these antibodies is an open question. That such cells exist at some stage of B cell development is expected based on the diversity of the antibody repertoire, since 10^{10}-10^{14} potential antibodies can be generated via the genetic machinery of antibody gene rearrangement (Sanz 1991). Understanding the fate of these cells (i.e. whether they are deleted prior to emigration from the bone marrow, are deleted or tolerized in the periphery, or are present and anergic) may allow the generation of strategies to ultimately target these cells for the development of an effective vaccine.

Regarding the production of detectable antibodies in HIV-1 infection, previous studies have shown that HIV-1 seroconversion occurs in a range from 8 days to 8-12 weeks from onset of clinical acute HIV-1 infection (AHI) (Ho 1985; Carne 1985; Fiebig 2003). IgM reactive with virus-infected cells has been detected during the course of AHI (Cooper 1987), but the antigen specificity of these antibodies and the precise timing of the earliest antibody has not been determined. Early studies performed in cohorts developed by CHAVI indicate that gp41 antibodies develop very early; these studies have been possible thus far using samples from clades B and C infections. Following HIV-1 transmission, it has been estimated that there is a window of opportunity of time between transmission and establishment of the latently infected pool of CD4 T cells (approximately 25 days) for a preventive vaccine to work (Johnston 2007; S.B. Justin Wong 2007). Thus, critical questions are: What are the first antibodies that arise following HIV-1 transmission and are these antibodies the same across clades? The detailed analyses of binding antibodies to several HIV antigens at multiple, closely spaced time points post-HIV RNA detection in peripheral blood will allow us to address this question in cohorts where clades A, C, D, and CRF01_AE are prevalent. In addition, this protocol will incorporate a mucosal immunology substudy to allow us to address these questions in secretions of the genital mucosa.

Globally, infection with HIV-1 is primarily via the mucosal route. Defining the earliest mucosal immune events following HIV-1 infection is of central importance for characterizing precise virus-host interactions that must be altered by vaccine-induced immune responses. However, the kinetics, quality and quantity of mucosal antibody development in acute HIV-1 infection remain to be fully characterized. The protective role of antibodies in the prevention of mucosal HIV-1 infection has been demonstrated most convincingly in the macaque-SHIV model, where systemically administered monoclonal virus-neutralizing IgG antibodies protected the animals from vaginal viral challenge (Baba 2000; Mascola 2000). It is still uncertain whether some of this protection was by HIV-1 specific IgG mediating antibody-dependent cell-mediated cytotoxicity (ADCC). Mucosal antibodies of the IgM and IgA isotype have not been well characterized for their protective capability in HIV-1 infection.

Multiple mechanisms of HIV-1 infection via the mucosa have been proposed (Miller 2003). However, modulation of infection by mucosal immune responses (adaptive and/or innate) has not been fully defined. It has been proposed that HIV-specific IgA in the vagina may play a role in the resistance of highly exposed persistently seronegative (HEPS) subjects to HIV-1 infection (Beyrer 1999; Kaul 1999; Mazzoli 1997). However, other reports have not supported this observation (Belec 2001; Skurnick 2002). Mucosal and systemic compartments of the immune system display some independence. In chronic HIV infection, HIV-specific IgG is readily detected in mucosal secretions, and is the predominant isotype in both serum and mucosal compartments. HIV Env-specific IgG has been reported to be dominant to specific IgA in all compartments (Mestecky 2004). Levels of HIV-specific IgG and IgA were comparable between cervicovaginal lavage (CVL) and semen, although the former were approximately 2 $\log_{10}$ higher than the latter. The ratio of serum and seminal plasma IgA1: IgA2 were equivalent (85:15%), whereas in the CVL, the levels of the two subclasses were similar, with a marginal increase in IgA2 around the time of ovulation (Mestecky 2007). In semen and CVL, secretory (s), polymeric (p) and monomeric (m) IgA are present in roughly equal proportions, unlike serum, where p-IgA and m-IgA predominate. The finding of HIV specific sIgA in CVL and semen suggests local production (Artenstein 1997). There have been extensive studies of the mucosal

immunology and virology of HIV-1 infection in subtype B prevalent infections (Mestecky 2007; Artenstein 1997; Wright 2002; Belec 1995); however, little is known about the mucosal immunology of acute HIV-1 (especially non-B subtypes) infection in humans. Additionally, many previous studies focused on the mucosal response to HIV-Env and not other HIV antigens (Artenstein 1997; Belec 1995; Raux 2000).

Following the detailed characterization of binding antibodies in both peripheral and mucosal compartments, it will be important to investigate the functional consequences of binding of these antibodies to HIV. The functions that have been described include virus neutralization (anti-Env), ADCC (Ahmad 1996; Battle-Miller 2002), opsonization, inhibition of bystander apoptosis, direct virolysis and other possible unknown consequences. ADCC eliminates HIV-infected cells through the action of specific antibodies that bind to target cells expressing antigens on their surface. Effector cells bearing Fc receptors are linked to the infected cells via the Fc portion of the antibody and the target cell is killed. Natural killer cells expressing CD16 and CD56 are the major effectors of ADCC, although a broader range of cells including monocytes (Murayama 1990) and granulocytes (Gale 1975) have been implicated. The precise role of ADCC in HIV infection is unknown, but ADCC activity has been inversely associated with HIV viral load (Forthal 2001) and high ADCC activity has been associated with non-progression in humans (Baum 1996). Cervical fluids collected from women with cervicovaginal ADCC had lower genital viral load than women with no cervicovaginal ADCC (Nag 2004). While there have been studies of ADCC activity in the CVL of chronically HIV-infected women, to date there have been no studies of ADCC activity in either the CVL or semen obtained during acute HIV infection.

To study the breadth and potency of functional antibodies and to model some of the extensive genetic variation of HIV-1, several laboratories have produced panels of virologic and serologic reagents from HIV-1 patients from multiple geographic regions (Brown 2005; Li 2005; Li 2006). Early studies performed in several countries indicated that cross-neutralization of HIV-1 isolates using polyclonal sera showed no relationship between viral genotype and neutralization "serotype" (Kostrikis 1996; Kostrikis 1996b; Moore 1996; Nyambi 1996; Weber 1996; Nyambi 2000). In these early cross-clade neutralization studies, the data were complicated by the use of viruses and sera from subjects infected with recombinants, and by autologous virus/serum pairs, which often yield negative results. In a recent study, using full-length sequenced pure clade reagents, we have demonstrated a trend towards preferential neutralization of homologous clade viruses using pooled clade-specific plasma. These relationships were most evident when the PBMC based neutralization assay was used; the clade C plasma pool also appeared to exhibit superior breadth and potency, especially in the pseudovirus assay (Brown 2008).

Several previous reports highlight the notion that clade-specific differences may indeed have an impact on Env immunogenicity or sensitivity to neutralization (Li 2006; Brown 2008; Derdeyn 2004; Frost 2005). A significant effort has been expended by both cellular and humoral HIV immunologists to obtain envelope (env) clones or sequences of very early, CCR5 coreceptor-utilizing isolates from acutely infected individuals at all Feibig stages (Fiebig 2003). This effort is based on the hypothesis that these viruses will best represent the infecting strains that seed an acute infection, and thus are the isolates that vaccines should be targeted against. While logical, there is no published data to support this hypothesis. For the studies proposed here, envs will be cloned from both peripheral blood and mucosal compartments at the earliest timepoints available

and at subsequent intervals, under the viral genetics section of Dr. Francine McCutchan. This will allow us to assess pseudoviruses that are from most or all Feibig stages and from the four major clades prevalent at the four study sites.

1.5.5 Host Genetics

The barriers imposed by the host to the establishment of a productive HIV infection are multiple and can be schematically divided into mechanisms of host restriction (Lama and Planelles, 2007), innate immunity (Alter and Altfeld, 2006), and adaptive immunity (Stephens, 2005). Genetic variation in the genes controlling these factors has been documented and used to explain, at least in part, the observation that rates of HIV acquisition and disease progression are not uniformly distributed in human populations (Dean et al., 1996).

Understanding the role of genetically determined responses to HIV infection and HIV treatments or preventative interventions, (e.g. vaccines) is critical to development of new approaches to prevention and treatment of HIV. For example, a vaccine that appears to be unsuccessful in efficacy testing may prove to have been effective in a subset of participants who possessed a particular host immune response gene allele. Exploring this insight may allow for re-design of the vaccine to achieve that response seen only in the genetically defined subset in the broader, general population as well.

Host restriction can be exerted to prevent viral entry, or through the interference with key post-entry steps in the viral replication cycle. Polymorphic variants of these host restriction factors have been identified in human populations and have been associated with variation in rates of HIV acquisition and disease progression, probably reflecting variation in their intrinsic antiviral activity and their capacity to avoid the viral counter-mechanisms aimed at antagonizing them(Alvarez, Lopez-Larrea, and Coto, 1998; Amara et al., 1997; Bashirova et al., 2006a; Bleiber et al., 2005; Brettle et al., 1996; Carrington et al., 1999b; Cohen et al., 1998; Colobran et al., 2005; Dean et al., 1996; Gonzalez et al., 2005; Gorry et al., 2002; Hendel et al., 1999; Javanbakht et al., 2006; Kaslow et al., 1996; Kijak, 2007; Leslie et al., 2004; Liu et al., 2003; Reiche et al., 2007; Schinkel et al., 1999; Smith et al., 1997; Speelmon et al., 2006; Valcke et al., 2006).

The second line of host defense is represented by innate immunity, whose major cellular effectors against viral infection are the NKs. Their capacity to lyse virally-infected cells without the need of prior antigen sensitization makes them vital elements for the control of the initial spread of incipient viral infections (Khakoo et al., 2004). NK cytolytic functions are mediated by their membrane KIRs, which recognize specific ligands on target cells. The complex KIR locus contains various KIR genes, whose products exhibit an intricate pattern of binding specificity/affinity for their ligands, and they also vary in their ability to generate stimulatory/inhibitory signals (Bashirova et al., 2006b). KIR genes are polymorphic in human populations and so are their ligands, and their epistatic interaction can affect the rate of HIV acquisition or disease progression (Carrington et al., 1999; Jennes et al., 2006; Martin et al., 2002). Polymorphism in DC-SIGN lectins on dendritic cells (DC) can also affect initial viral spread, probably by limiting the trans-enhancement of HIV infection of T-lymphocytes. TLRs, expressed on immune cell rich tissues provide innate recognition of viral nucleic acids and

pathogen-associated molecular patterns (PAMPs) (Takeda, Kaisho, and Akira, 2003). TLRs have a crucial role in initiating innate immune responses and determine the secretion profile of cytokines and interferons, thus shaping the subsequent adaptive immune responses (Meier and Altfeld, 2007). Their polymorphism has been associated with different rates of HIV disease progression (Bochud et al., 2007; Ferwerda et al., 2007).

The third line of defense is represented by adaptive immune responses, which require antigen sensitization, delaying their onset until the HIV infection has already been established. While the underlying genetic basis of the elicitation of anti-HIV antibodies has not been elucidated (Haynes and Montefiori, 2006), the efficacy of ADCC is associated with polymorphic variants of the Fc-γ receptors IIa (CD32) and IIIa (CD16a) (Forthal et al., 2007a), which are expressed on the surface of macrophages and NKs, respectively. These polymorphisms affect the specificity and affinity of the receptors towards different subclasses of IgG (Bredius et al., 1994; de Haas, 2001; Koene et al., 1997; Parren et al., 1992) and have been associated with accelerated disease progression (Brouwer et al., 2004; Forthal et al., 2007a; Forthal et al., 2007b). The genetic basis of cellular adaptive immune responses has been long acknowledged (Liu et al., 2003). Class I HLA genes are the most variable ones in the human genome and condition the focus of the responses from cytotoxic T-lymphocytes (CTLs), by restricting the nature of epitopes that can be presented by infected cells. Some HLA alleles have documented delayed courses of infection whereas other alleles are associated with accelerated disease progression (Brettle et al., 1996; Carrington et al., 1999; Dorak et al., 2004; Dorak et al., 2003; Farquhar et al., 2004; Flores-Villanueva et al., 2003; Gao et al., 2001; Hendel et al., 1999; Kaslow et al., 1996; Keet et al., 1999; Leslie et al., 2004; Liu et al., 2003; MacDonald et al., 2001; Martin et al., 2002; Moore et al., 2002; Tang et al., 1999; Tang et al., 2002; Trachtenberg et al., 2003). By their interaction with KIR on NKs, HLA molecules bridge the innate and adaptive arms of cellular immunity(Alter and Altfeld, 2006).

Our knowledge of the impact of host genetic variation on HIV acquisition comes mostly from predominantly subtype-B settings, and in most cases it is unclear if the same principles apply to other HIV clades or non-Caucasian genetic backgrounds. The host genetic basis of variation in disease progression rates proceeds mostly from the comparison of plasma viral load set-points among chronic HIV infections. A major obstacle for the establishment of the contribution of genetic variation in host restriction, innate and adaptive immunity arises from the fact that the effectiveness of each of these lines of defense is highly dependent on the success of the preceding one.

The current study design allows for the comparison of HIV sero-prevalent individuals, exposed sero-incident cases and matched controls to establish the polymorphisms in factors of host restriction, innate and adaptive immunity that confer varying risks for HIV acquisition in East Africa and Thailand, in the setting of non-B clade infections. This affords the opportunity to determine the principal components of host genetic variation in these lines of defense that can distinguish the different levels of viremic control.

The human host genetic information collected in this study is obtained for the sole purpose of understanding the control of HIV infection as it relates to acquisition of HIV and the long-term prognosis of HIV disease course. None of these associations have any clinical relevance at this time. As an example, the known genetic host factor which reduces susceptibility to HIV

infection, the homozygous deletion of a sequence within CCR5 does not prompt genetic counseling to inform the participant that they have a lower risk of HIV infection than others. This is because the risk is lower for these persons than the general population but not zero and the message of behavioral risk reduction is not altered by this information. For the vast majority of genetic markers under consideration in this analysis, no known human disease has been established and the information has no impact upon risk behavior counseling or therapeutic decisions. In the rare instance where a disease association exists, there is no standard for counseling participants who are otherwise healthy and have no manifestations of the disease. Finally, the methods used here to ascertain human genetic information are not licensed or approved medical testing methods for determining medically relevant genetic information. They are research methods designed to permit rapid, high throughput information to address scientific questions but may not meet standards needed to support medical diagnoses. In consideration of these issues, the research team does not feel the host genetic information should be provided on a routine basis to participants.

1.6 Description of Partners

Makerere University, founded in 1922 is Uganda's premier higher education institute. With over 20 schools/faculties/institutes, Makerere University offers day, evening, and external study programs to over 22,000 undergraduate and 3,000 graduate students. Located in Kampala, the capital of Uganda, Makerere is a hub for resources and intellectual resources, making it an ideal nexus for conducting research in many fields of study. HIV infection related research at Makerere University has been conducted in collaboration with a variety of North American and European institutions (including Case Western Reserve University, Johns Hopkins University, University of California at San Francisco, Columbia University, University of Medicine & Dentistry of New Jersey, the London School of Hygiene and Tropical Medicine, etc) for over ten years. Among others, HIV infection related research at Makerere University has included the Partnership for AIDS Vaccine Evaluation (PAVE), the HIVNET program, the HIV Prevention Trials Network (HPTN) and the successful completion of Africa's first preventative vaccine trial – ALVAC vCP205 (HIVNET 007). In addition, Makerere University has been actively participating in initiatives directed at the clinical epidemiology, diagnosis, pathogenesis, treatment and prevention of HIV/AIDS since its identification in East Africa in early 1984.

Makerere University Walter Reed Program (MUWRP): MUWRP is a non-governmental, non–profit HIV research Program that was established in 2002 by Makerere University, The U.S. Military HIV Research Program (USMHRP), and the Henry M. Jackson Foundation for Advancement of Military Medicine of the United States. Clinical studies were initiated in early 1999 in Uganda through Rakai Project. The focus of the Project has been on development of infrastructure, definition of vaccine research cohorts, acquisition of appropriate products for evaluation in the region, and clinical evaluation of these products. The core of the Project's efforts is to accomplish all activities required for initiation of phase III trials in the region over the next seven years.

MUWRP's main facility, located at Plot 42 Nakeasero Road in Kampala, Uganda, includes administration, data, logistics and the clinic. The College of American Pathologists (CAP)-

certified laboratory is located at the Makerere University School of Medicine, approximately 1.6 kilometers from the main complex.

U.S. Army Medical Research Unit-Kenya (USAMRU-K): USAMRU-K is a Special Foreign Activity of the Walter Reed Army Institute of Research (WRAIR), Washington, DC. USAMRU-K is affiliated through a Cooperative Agreement with the Kenya Medical Research Institute (KEMRI). The unit was activated on a temporary basis in 1969 at the invitation of the Government of Kenya to study trypanosomiasis. The success of that initial venture led to the establishment of a permanent activity in 1973. Over the past 32 years, research has been conducted on malaria, trypanosomiasis, leishmaniasis, entomology, HIV/AIDS and arboviruses, with more than 250 manuscripts published.

In addition to laboratories housed in Nairobi (where anti-malarial drug resistance, trypanosome biology and Global Emerging Infections Surveillance (GEIS) are studied), USAMRU-K has other field sites in western Kenya (Kisumu, Kisian, Kombewa and Kericho) that are mainly concerned with malaria and HIV/AIDS.

The United States Army Medical Research Unit of Kenya (USAMRU-K) HIV program is located in Kericho at the Kenya Medical Research Institute (KEMRI) / Walter Reed Project (WRP) Clinical Research Center. Within Kenya's largest tea plantations in the African Highlands of the southern Rift Valley Province, Kericho is a rural city with a population of approximately 500,000. The center was originally established for malaria research in late 1990s. The WRP HIV Program was established in Kericho and began focusing upon HIV research in early 2000 given the recognized breadth and depth of HIV disease in Kenya. The WRP Kericho HIV Program is led by one Department of Defense (DoD) civilian and Kenyan professionals. As part of the USMHRP, USAMRU-K's mission in HIV research is the contribution to the development of a globally effective HIV-1 vaccine and testing and evaluating HIV vaccine candidates. The USAMRU-K HIV program currently has several ongoing, complimentary HIV research and care and treatment activities, all contributing to the USMHRP HIV mission of vaccine development.

Mbeya Medical Research Program The Mbeya Medical Research Programme (MMRP). MMRP is a partnership between Tanzanian health authorities represented by the Mbeya Regional Medical Office, the Mbeya Referral Hospital, the National Institute of Medical Research (NIMR), the Department of Infectious Diseases & Tropical Medicine at the University of Munich (LMU) and the USMHRP. Recently a Memorandum of Understanding has been signed by all MMRP collaborating partners to enable MMRP to become a NIMR-MMRP research centre. With this Memorandum the new NIMR-MMRP research centre has now full legal status. MMRP has expanded its research into the three major infectious disease challenges for Tanzania: HIV/AIDS, malaria and tuberculosis. The center has a CAP certification of its laboratory acquired in October 2007. The shared mission of MMRP is to evaluate new interventions for these diseases utilising vaccines, drugs or diagnostics.

Royal Thai Army, Armed Forces Research Institute of Medical Sciences (RTA-AFRIMS): RTA AFRIMS is operated under the Phramongkutklao Medical Center of the Royal Thai Army Medical Department. RTA-AFRIMS has collaborated with USAMC-AFRIMS in areas of HIV

vaccine research for more than 15 years. RTA-AFRIMS provides support in areas of clinical, field and laboratory research.

U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS): USAMC-AFRIMS is the U.S. Army Medical Component of a Royal Thai Army – U.S. Army joint command on the grounds of the Phramongkutklao Medical Campus in Bangkok, Thailand. It is operated under the Walter Reed Army Institute of Research (WRAIR), Washington, D.C.

The Department of Retrovirology was established in 1992 and serves as the USMHRP's forward platform in Southeast Asia for HIV vaccine development. The AFRIMS has conducted six phase I/II HIV vaccine trials, multiple vaccine preparatory cohort studies, and have stewarded the largest HIV vaccine trial to date-the RV144 phase III prime-boost vaccine trial which began in 2003.

AFRIMS capabilities include a highly-experienced clinical and laboratory staff, with a laboratory that is CAP-accredited, and is registered with the NIH AIDS Research and Reference Reagent Program and the Division of AIDS, NIAID. The research lab is capable of cellular and humoral immune assays as well as viral genotyping and sequencing. Additionally, specimen processing, archiving, and other clinical laboratory support for clinical trials can be done at two specimen processing facilities, one in Bangkok and the other in Chon Buri Province (currently serving the Phase III HIV vaccine trial).

The U.S. Military HIV Research Program (USMHRP), is a multi-dimensional research project headed by the Walter Reed Army Institute of Research (WRAIR) in collaboration with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) and sponsored by the United States Army Medical Research and Material Command (USAMRMC). The mission of this project is to prepare and protect the U.S. military forces so they are ready for the challenges and opportunities of deployment and peacekeeping operations in the future. This program strives to develop effective vaccines to protect U.S. military forces from infections and also bring under control the international proliferation of HIV. It is through cooperative relationships that scientific ideas are exchanged and progress made in fighting the HIV/AIDS epidemic.

The Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) is a private not-for-profit organization dedicated to improving military medicine and public health. The mission of HJF is to advance medical research and education in the military medical community by providing scientific and management services to improve health worldwide. HJF supports a wide variety of research programs ranging from small bench top programs to complex multi-site programs. HJF was chartered in 1983 by U.S. Congress to support military medical research and education. HJF was named in honor of Henry "Scoop" Jackson, the late Senator from Washington State, and embodies his long-standing dedication to military medicine and public health.

The Walter Reed Army Institute of Research (WRAIR) conducts research on a range of military relevant issues, including naturally occurring infectious diseases, combat casualty care, operational health hazards, and medical defense against biological and chemical weapons. WRAIR provides an essential link between troops in the field and research in the laboratory with

a mission to conduct biomedical research that is responsive to the DoD and U.S. Army requirements and delivers life saving products including knowledge, technology, and medical material that sustain the combat effectiveness of the war fighter. Despite a focus on soldier related research, many non-military medical problems around the world have been solved and lifesaving and life enhancing discoveries made.

Division of AIDS (DAIDS)

As protocol sponsor, DAIDS has direct responsibility for human use protections and oversight, which is accomplished through protocol development by the active involvement of the Vaccine Clinical Research Branch, and particularly the assigned Medical Officer in all aspects of protocol development. The draft protocol is subject to internal DAIDS review at the "Prevention Sciences Review Committee" which provides a comprehensive scientific, medical, statistical, ethical and regulatory review. Subsequently, the final draft protocol is subject to a second level regulatory/ethical review at the Regulatory Compliance Center of Regulatory Affairs Branch, DAIDS prior to submission to the WRAIR and local institutional review boards (IRBs) for approval. Prior to initiation of a protocol at a specific site, the Regulatory Compliance Center (RCC) goes through a protocol initiation review to verify that the final, translated consent is faithful to DAIDS and United States Government (USG) guidelines and the sample consent. In addition, this step involves verification that all required regulatory documents and approvals are present at the site prior to initiation. All amendments to the protocol will be reviewed on behalf of DAIDS by the Medical Officer (MO) prior to submission to RCC and approved by both RCC and the MO prior to submission to local IRBs.

2.0 HIV PREVALENCE AND INCIDENCE IN MOST AT RISK POPULATIONS IN STUDY LOCATIONS

2.1 Uganda Target Populations

The MARPs in Uganda will target three populations that are believed to have high-risk behavior. The populations to be targeted will include, but are not limited to, sex workers (SWs), individuals diagnosed with sexually transmitted infections who are clinic attendees (STIAs), bar workers (BWs) and motor cycle passenger transporters (Boda-Boda). These populations and known HIV epidemiology in these populations are described in the site-specific addendum (Protocol RV217a).

During Part A of the study, the investigative team in Uganda will mainly recruit and enroll SWs, but individuals from any of the other three population groups specified above that may respond to the recruitment drive and meet the inclusion criteria may be enrolled.

During Part B of the study, the investigative team in Uganda will recruit and enroll from among all four target population groups including SWs, STIAs, BW and Boda-Bodas.

2.2 Kenya Target Populations

Kenya will target two MARPs, SWs and STIAs, which are both estimated to have an incidence of 3% or greater. These populations and known HIV epidemiology in these populations are described in the site-specific addendum (Protocol RV217b). For Part A of the study, only SWs will be recruited while in Part B, recruitment will be from all the groups mentioned (SWs and STIAs).

2.3 Tanzania Target Populations

The Mbeya Medical Research Programme (MMRP) in Tanzania site has one target population. The BW cohort has been the subject of much study and multiple recent publications. This population and known HIV epidemiology for this population are described in the site-specific addendum (Protocol RV217c).

2.4 Thailand Target Populations

Overlapping high-risk groups have been identified in Thailand. SWs, both female and male continue to be a group at high risk, along with transgender individuals (TG) and those men who have sex with men (MSM) who are living a high-risk lifestyle in the entertainment areas. These populations and known HIV epidemiology in these populations are described in the site-specific addendum (Protocol RV217d).

3.0 STUDY OBJECTIVES

The research collaboration outlined in phase I of this protocol will define the epidemiology of HIV in a volunteer cohort drawn from high-risk populations in East Africa and Thailand. The primary objective of this study is to estimate HIV-1 incidence and retention in a volunteer cohort established to test vaccine strategies. This study will also define the prevalence of HIV-1 in this volunteer cohort, determine the distribution of HIV-1 genotypes and different host genetic backgrounds, assess the range of CD4 and viral load in HIV-1 infected volunteers, characterize behavioral and other risk factors associated with HIV-1 infection, and augment HIV-1 prevention and education programs, human resources, and laboratory infrastructure to support future vaccine trials.

Individuals with incident infections during observation will be invited to enroll in phase II, a sub-study to evaluate the dynamics of viral burden, diversity, possible impact of host genetics, and adaptive immune responses in early HIV infection. In order to optimize the scientific value of these observations, phase I of the study will be conducted with frequent, small blood volume sampling of the participants to diagnose HIV infection very early, ideally, prior to the advent of detectable antibody using extremely sensitive diagnostic Enzyme-linked Immunosorbent Assays (ELISAs).

Very frequent surveillance as proposed here has not been conducted in the HIV field. It is possible that participants will either be unavailable this frequently or unwilling to participate. To ensure that protocol procedures are going to be successful in meeting the audacious goals of the study, a pilot study will be conducted (Part A). Only after establishing feasibility of the proposed design in Part A would enrollment open fully in Part B. Should compliance with the demanding study visits regime prove unsuccessful in Part A, the study would be re-designed and modified accordingly through an institutional review board (IRB)-approved amendment.

Thus, broadly speaking, there are two sets of objectives as noted below and two parts of the study: Part A) pilot feasibility study and Part B) full study implementation. All Part A participants and data will be used to satisfy the study objectives along with the Part B participants. Part A has limited objectives pertaining to feasibility but will also contribute to the objectives of the fully implemented study (Part B).

3.1 Part A (Pilot Study)

3.1.1 Primary Objectives

1) Determine the ability to recruit up to 200 high-risk volunteers at each study site in 9 months
2) Determine the ability to achieve at least 50% compliance for study visits
3) Observe an incidence of 3.7% with at least 30% of the incident cases identified prior to the advent of detectable antibody

3.2 Part B (Full Study)

3.2.1 Primary Objectives

1) Define the risk behavior, prevalence and incidence of HIV infection and retention of a high risk cohort of adults in Thailand, Uganda, Kenya and Tanzania

2) Obtain approximately 150 acute HIV infections (AHI) with at least 30% captured within Fiebig stages I and II to support the full characterization of host responses and viral dynamics

3.2.2 Secondary/Exploratory Objectives

1) Assess and optimize HIV diagnostic strategies in HIV primary infection across multiple subtypes and risk groups

2) Define the genetic diversity and evolution of HIV-1 in the prevalent and incident HIV cases with particular emphasis on characterization of acute, primary HIV infection

3) Characterize immune activation, innate and adaptive cellular immunity in the early acute HIV-1 infection

4) Characterize B cell responses in peripheral and mucosal compartments arising in early acute HIV-1 infection

5) Characterize genetic polymorphisms in genes controlling host restriction, innate and adaptive Immunity, and their influence on HIV acquisition and early control of HIV infections

6) Characterize clinical events including endemic infection as risk factors for HIV acquisition

4.0 DESIGN AND METHODOLOGY

4.1 Hypothesis Being Tested

Two formal hypotheses will be addressed under objective #1: 1) lower bound of the 90% confidence interval around the aggregate incidence will be in excess of 3 per 100 person years; and, 2) 90% of the aggregate cohort will be retained through one year of follow-up.

4.2 Summary of Methods

The study will be conducted at three locations corresponding to the three East African vaccine research sites of the US Military HIV Research Program. These are Kampala, Uganda (Protocol RV 217a); Kericho, Kenya (Protocol RV 217b) and Mbeya, Tanzania (Protocol RV 217c). A fourth study area has been identified in Thailand (Protocol RV 217d).

This is a multi-center, non-randomized clinical observational study to be conducted in two parts (Parts A and B). The purpose of the study is to characterize recruitment, retention, HIV prevalence, HIV incidence and biological characteristics of acute HIV infection in high-risk volunteers in Africa and southeast (SE) Asia. The first part of the trial, Part A, is a small pilot study to establish and optimize the recruiting, retention and study compliance at each participating site. Based upon this pilot phase of the study, the study will expand to full enrollment as planned (Part B) or operational procedures and study event schedules will be adjusted with a corresponding amendment prior to initiating Part B. The study itself, as conducted in both Part A and B, incorporates two phases. The main study activity, phase I, is the observational cohort or surveillance activity. The evaluation of the incident infections arising during phase I is an intensive evaluation of the interaction of HIV virus and host response and constitutes phase II of the study.

Phase I: Non-randomized, closed cohort, prospective, 24-month observational study to be conducted in two steps. Part A is a pilot study to establish and optimize operations and study design features to meet study objectives prior to opening the study to full enrollment at all sites in Part B. The study will conduct a screening visit and initial follow-up in all enrolled volunteers. This will include both HIV negative and HIV prevalent cases. Prevalence is estimated to be high in these populations and (in Part B) approximately 1000-1500 volunteers will be enrolled and evaluated in the first two visits with an enrollment target of 300 HIV negative, high-risk volunteers in Part B. In Part A, the enrollment target of HIV negative, high-risk volunteers is 200. Subsequently, only HIV negative volunteers will be followed for a period of approximately 24 months, except for a small number of HIV positive individuals for masking to minimize risk of stigmatization and to provide positive controls for laboratory assays. After the initial two visits, volunteers will be seen at the research clinic approximately every 3 months. In addition, at locations that are as convenient to the volunteers as feasible, small blood volume samples will be collected using microvettes twice weekly. These samples will be analyzed and results returned to the site within 48 hours to permit new HIV positive participants to be identified while they remain antibody negative, HIV nucleic acid test positive. All newly infected participants will be referred to phase II of the study.

Phase II: study of HIV incident cases arising within phase I. HIV incident volunteers identified at any follow-up visit will be asked to consent to participate in phase II to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of approximately 50 months from the time of entering phase II. Very frequent visits using a more intensive collection of samples initially will occur to characterize acute HIV pathogenesis and then at regular 3-month intervals for the duration of the study.

4.2.1 Part A (Pilot Study)

Part A will be a feasibility study, which will include up to 200 participants per site for a total of 800 participants overall.

At each site approximately as many as 300-450 volunteers will undergo a briefing presentation describing the trials and its risks and benefits provide written informed consent and undergo other activities as noted in Part B below. The endpoint for enrollment is identifying 200 HIV uninfected, high-risk participants per site who are willing to be followed for the next 24 months to determine the ability to recruit in these high-risk groups as well as retention of volunteers. In addition, approximately 20 HIV infected participants will be recruited to a) provide masking of HIV status for the population as a whole and b) provide HIV positive samples to serve as controls for study assays.

HIV incident volunteers identified at any follow-up visit will be invited to participate in phase II, to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of at least 50 months from the entry into phase II.

All Part A volunteers will contribute to analyses for Part B and to total enrollment goals.

4.2.2 Part B (Full Study Implementation)

At each site approximately 1000-1500 volunteers (total includes those enrolled in Part A) will undergo a briefing presentation describing the trial and its risks and benefits. The volunteers will then complete a written informed consent. The endpoint for enrollment is identifying 2000 HIV uninfected, high-risk participants (up to 500 at each site), (participants in Part A will contribute to the total) who will be followed for the next 24 months to establish incidence and retention rates. Recruitment methods and activities are directed at each group separately to minimize stigma risks and for operational efficiency. Volunteers will receive HIV risk reduction counseling, a briefing regarding HIV and HIV vaccine research in general, a medical history and physical, a behavioral risk questionnaire, and provide a blood sample to characterize general health (e.g. complete blood count (CBC), chemistries and liver function tests (LFTs)), diagnose HIV status and characterize both host genetics and viral characteristics. All volunteers will be seen in follow-up within approximately 30 days of Visit A to review laboratory and clinical findings from the screening visit. Those testing HIV positive or indeterminate at the first visit will provide a blood sample to verify HIV status at the second visit and PBMC and serum to contribute as positive controls for other study Aims. The extent of participation in the study is determined by HIV status at Visit A. Those enrollees who test HIV positive at Visit A will be discontinued from study participation after visit B, with the exception of a small number of HIV

prevalent cases who will continue follow-up to afford some masking to the HIV infected participants and decrease the risk of stigmatization. In addition, the positive samples derived from their participation contribute to quality control of the diagnostic and other assay platforms.

Incidence and retention rates among this cohort will be defined at each site and for the study population as a whole for the 24-month period of follow-up. Participants will be seen at 3-month intervals for HIV post-test and risk reduction counseling and every 6 months standard HIV diagnostic labs, and the presence of co-infections will be assessed. Exposure risk will be carefully documented by a questionnaire administered at baseline and every 6 months.

To ascertain acutely infected volunteers in a timely fashion and begin their intensive observation in the AHI phase, it is necessary to obtain at least weekly and preferably twice weekly samples for diagnosis. This will be accomplished by collecting up to two microvettes of blood via capillary puncture or venipuncture. The serum sample will be analyzed for RNA and any individual reactive for RNA (and previously known to be negative, seronegative volunteers) will be counseled at the next encounter (planned twice weekly) and entered into the AHI phase. The microvettes collected on each newly infected individual prior to the first RNA reactive sample will be evaluated for HIV specific DNA to define the eclipse phase of infection. We expect to detect 150 acute infections during the study with at least 30% of those being Fiebig stage I or II using this approach to diagnosis. Seroincident infections will be defined as acute HIV infection cases while the remainder of the volunteers completing 24 months of follow-up will be defined as exposed uninfected controls. This will afford the ability to capture the ascending and peak portions of plasma viral RNA in these participants. Approximately 1,850 exposed uninfected controls will be available for comparison to the AHI population as well as approximately 2000 seroprevalent cases acquired at study entry.

The study will employ an open cohort design to accommodate loss of participants due to death, pregnancy, and departure due to non-compliance. For the primary objectives of the study, the initial enrolled population will be treated as a closed cohort for the purposes of analysis.

4.3 Laboratory Methods

4.3.1 HIV Diagnostics

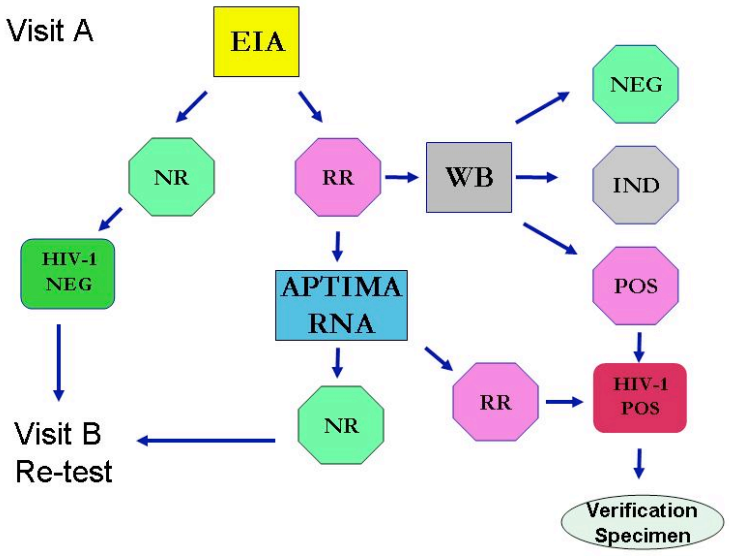
All clinical/translational research diagnostic support will be conducted in CAP accredited diagnostic laboratories using performance verified instrumentation/platforms and assays validated in accordance with Clinical Laboratory Standards Institute (CLSI) standards (formerly the National Committee for Clinical Laboratory Standards (NCCLS)).

Blood will be drawn for: 1) hematological evaluations (CBC, differential, platelets) and for clinical chemistry (Creatinine and alanine transaminase (ALT)). In addition, co-infecting pathogen screening (syphilis, hepatitis B, Herpes Simplex Virus Type 2 (HSV-2) and other endemic infections with viral vectors), eosinophil determination as a surrogate for helminth infestation and plasma lipopolysaccharide (LPS) determinations will be performed to assess the

influence of co-infection and microbial translocation on immune activation. Assays will be performed per manufacturer's specifications.

HIV testing for cohort enrollment will be by anti-HIV antibody using a 3rd generation EIA, Genetic Systems HIV-1/HIV-2 Plus O EIA (BioRad Laboratories, Redmond, WA), or equivalent and by Aptima HIV-1 RNA Qualitative Assay (Aptima: Gen-Probe). Repeatedly reactive EIA samples will be tested in parallel using Genetic Systems HIV-1 Western Blot (WB) (BioRad Laboratories, Redmond, WA) and Aptima HIV-1 RNA Qualitative Assay (Aptima: Gen-Probe) (see Figure 6 below). The enrolled prevalent participants will also be concurrently screened using the capillary microvette sampling method described below as concordant detection of HIV-1 prevalent cases by microvette sampling and traditional EIA/WB methods will provide a critical internal control for the small blood volume sampling methodology proposed.

Figure 6 HIV Screening Algorithm*



*NEG = negative, POS = positive, IND = indeterminate, RR = repeatedly reactive, NR = non-reactive

Participants non-reactive by both EIA and/or by qualitative RNA assay will be considered non-infected for purposes of the study and will enter AHI surveillance by twice weekly small volume capillary blood sampling. Rapid identification and transition of participants to the AHI protocol is essential if eclipse and early Fiebig stage I-II infections are to be captured. One serum and one ethylenediaminetetraacetic acid (EDTA) microvette sample will be acquired at each visit with each participant serum microvette sample processed and tested in real-time for the presence of HIV-1 RNA by Aptima HIV-1 RNA Qualitative Assay (Aptima: Gen-Probe). This assay was chosen over mini-pooling RNA detection strategies (Pilcher 2005), or detection by p24 antigen EIA (Fiscus JCM 2007) due to lack of lower limit analytical sensitivity achieved by these approaches, and in the case of min-pooling, time/labor constraints imposed by

construction/deconvolution of positive pools. Aptima RNA detection at ~90-100 cp/ml (98.5%, confidence interval (CI) .973-.992) to as low as ~20 cp/ml (82.6%, CI .796-.853) can be effected (personal communication, Dr. Tom Nugent, Gen-Probe). This approach will mark a critical advancement in AHI surveillance as it will permit detection of the lower level viremia (LLV) phase of primary infection, defined as viremia of <100 cp/ml which occurs post infection, but prior to the RNA ramp-up phase (Fiebig 2005). This study evaluated 69 samples from 15 patients that were collected before the period of steadily rising viremia (SRV) above 100 cp/mL. Using an ultrasensitive assay (2 mls plasma) with an analytical sensitivity of 1.4 RNA cp/mL (50%) and 4 RNA cp/mL (95%), 23/69 (33.3%) samples had detectable LLV with a median of 18 days (range 9-25 days) prior to SRV. More over, this approach will allow us through associated studies (viral genetics, host genetics, immunological, genomic) to redefine the window period in which pre-existing immunity controls the virus prior to expansion – a critical period for vaccine intervention. The EDTA microvette sample will be processed for plasma (~150 ul) and peripheral blood mononuclear cells (PBMCs) (~0.15-0.3 x 10^6) and will be archived for look back studies for proviral DNA and LLV associated with the eclipse phase (Fiebig 2005).

The first Aptima RNA reactive sample will immediately transition participants into the AHI (Phase IB) study where multiple large volume blood draws will be acquired at 3-4, then 7-14 day intervals (see SOE #2 and #3). This approach will provide valuable highly exposed uninfected (EU) and exposed infected (EI) samples with well defined lineages for construction of matched panels of negative, pre-seroconverter, and seroconverter sample sets of which ~50% are anticipated to be in eclipse to early Fiebig stages I-II infection. These panels will provide material for laboratory staging, characterization of incident estimation/recent infection methodologies, assessment of novel technology advances in molecular and serological diagnosis, and the associated studies referenced herein. Laboratory staging studies and novel technology assessments will be conducted in the USHMRP Reference Laboratory within the Department of Laboratory Diagnostics and Monitoring (DLDM).

Laboratory staging assignments of AHI are dependent upon the sensitivity of assays employed. While staging by Fiebig et. al., (2003) employed the most sensitive/specific United States Food and Drug Administration (USFDA) approved screening methods available at the time, state-of-art screening/diagnostic platforms are now available which further narrow the detection window for RNA and HIV antigen/antibody with higher sensitivity and specificity for diverse subtypes (Swanson 2006; Schumacher 2007; Guendin 2007; Weber 2004). Staging will be performed on large blood volume samples (LBVs) using 1) ultrasensitive HIV-1 RNA qualification (Aptima HIV-1 Qualitative Assay, Gen-Probe) and quantification (Abbott HIV-1 Real Time, Abbott Diagnostics) nucleic acid amplification technologies (NAAT), 2) HIV-1 DNA polymerase chain reaction (PCR), and 3) serological assays (p24 antigen EIA [Perkin Elmer or equivalent] 3rd generation IgM sensitive EIA (Genetic Systems HIV-1/HIV-2 Plus O [BioRad Laboratories], 4th generation p24 antigen, anti-HIV antibody EIA, HIV-1 WB [BioRad Laboratories]). Estimations of the emergence of HIV viremia, p24 antigenemia, anti-HIV antibody, and WB reactivity in these subtype diverse cohorts will be generated. As there are no USFDA approved 4th generation EIAs marketed within the United States (U.S.), we have initiated collaboration with Dr. Kathleen Shriver of BioRad Laboratories to test the company's new 4th generation EIA, Genetic Systems HIV Combo HIV Ag/Ab EIA. This assay is scheduled to enter USFDA clinical trials late Summer/Fall FY08. Alternative NAAT or serological assays may be employed if data suggests

that subtype diversity impacts detection sensitivity/specificity or if technology advances precede initiation of this study.

Identification of the first RNA reactive will trigger a look back of archived small blood volume samples (SBV) PBMC for detection of proviral DNA for an estimation of the duration of the eclipse phase and of SBV plasma to investigate the dynamics of low level viremia preceding RNA ramp-up (Fiebig et. al 2005). This effort will leverage against Viral Genetics Section studies: whole genome amplification (WGA) for detection of low-proviral DNA and single genome amplification (SGA) for full-length (FL) viral sequence analysis. Samples will be scored for presence of proviral RNA and detection/quantification of RNA. If the WGA approach fails, ultrasensitive nested PCR will be employed, which can detect as few one proviral integration events of 1/100 – 1/100,000 cell.

As qualitative and quantitative NAATs for detection of HIV RNA and HIV Ag/Ab tests are continuously advancing, panels constructed from laboratory staging studies will be used to assess the most promising. One such novel technology is the BioHelix Corp IsoAmpTM "On Demand" Manual Molecular Analyzer- a manual platform for HIV viral load determinations consisting of a manual sample-prep, isothermal amplification (30-60 min), and instrument-free rapid detection (5 min) reported as a visual qualitative result. This technology, which is in beta development, shows great promise for deployment in resource-constrained settings. We will also assess the Biohelix IsoAmpTM "Quan" Realtime Molecular Analyzer - low cost and portable instrument that integrates sample-prep, isothermal amplification, and quantitative RNA detection steps for plasma specimens, whole blood, dried blood spots and serum specimens. Primers are targeted at the gag gene. Preliminary results are promising for HIV-1 subtype performance post primers redesign for detection of C, A and A/G subtypes. The current lower limit of detection is ~500 cp/ml. We have developed collaborations with Bertrand Lemieux, Director Technology Development, BioHelix Corp, to assess both of these technologies. Biohelix's IsoAmp MRSA Screening Assay has a sensitivity of 5 copies of MRSA. The Ebola RNA Rapid RT-HDA can detect 50 copies in 30 minutes.

Studies to evaluate methods for estimation of incidence/recent within populations of diverse subtype/circulating recombinant forms will be conducted in collaboration with Drs Steven Ethridge and Bernard Branson, CDC. This work will be conducted under an investigative new device (IND) application (USFDA IND 8193). Assessment of newer assay platforms in diverse non-B populations is critical if refinement of current methods is to be accomplished. Matched seronegative and seroconverter panels from samples acquired at 3, 6, 12, 18, and 24 months post detection of the first RNA positive sample will be constructed. Panels will be used to evaluate 1)IND BED-Capture EIA (Trinity Biotech, PLC), 2) two 3rd generation sensitive/less sensitive (detuned) EIAs, the fully automated enhanced chemiluminescence Vitros ECi HIV1/2 (Ortho Clinical Diagnostics) and the automated GS HIV-1/2 Plus 0 (BioRad Laboratories), and 3) one 3rd generation automated avidity assay, the Vitros ECi HIV1/2.

Serum LPS levels in LBV plasmas will be assessed using the QCL-1000 Chromogenic LAL Endpoint Assay (Lonza) which has an analytical sensitivity of 0.1 EU/ml-1.0 EU/ml. Co-infection induced immune activation is known to influence HIV acquisition and disease progression. Presence of malarial infection will be assessed at enrollment and at 6-month intervals by standard giemsa stain smear microscopy. Archived EDTA anti-coagulated whole

blood samples or will be screened for the presence of low level parasitemia by Real-Time PCR using modifications of methods of Mangold et. al., 2005. Analytical sensitivity of 1-5 parasites per microliter of blood can be attained by this approach (Snounou 1993; Mangold 2005; Boonma 2007).

HSV-2 serology status will be determined using HerpeSelect 2 enzyme-linked immunosorbent assay (ELISA) IgG (Focus Technologies, Cypress, CA).

Syphilis testing will be performed using a non-treponemal screen (Rapid Plasma Reagin (RPR), Wampole RPR, Wampole, Inc.) followed by treponemal supplemental confirmatory testing.

Malaria testing will be performed by DNA PCR on thawed whole blood lysates using modifications of Johnston et al (2006) and Snounon et al (1993b).

Samples will be reposed for potential testing of antibodies to HIV vaccine vectors.

4.3.2 Viral Evolution

Figure 4 shows the timing of samples to be used for viral genetics (Fiebig 2003) and describes the nature and scope of the analyses proposed. The earliest attained viral RNA positive sample will trigger a look-back into earlier plasma samples for the presence of low-level viral RNA (Fiebig 2005) and in the primary PBMC, collected twice weekly for the previous 3 months, for the presence of proviral DNA, in order to retrieve viral variants that were transmitted but never replicated to a detectable threshold in the viral RNA population. The approach will be random, WGA of the extracted DNA, which may derive from as few as 100,000 cells, followed by nested PCR for the presence of viral genome. Using very short, well-conserved target gene segments, conventional first round PCR will be followed by second round, real-time PCR in the presence of a specific fluorescent probe within the amplicon. This exploratory study will be pursued on the first 10 seroconverters and, if any are positive, a second stage will be initiated to recover the viral defense genes *vif* and partial *gag*, which interfere with apolipoprotein B-editing catalytic polypeptide-like subunit (APOBEC) and (TRIpartite Motif 5 (TRIM5α), respectively. This will help to determine if the successful viral variants represent escape variants from host defense genes. If no sample is DNA positive, or of insufficient material is present to recover viral defense gene sequences, this approach will not be pursued further.

The first RNA positive small-volume sample will transition the participant to the AHI study, where a large volume blood draw schedule begins 3-4 days later. The first large RNA positive blood sample will be the source of several crucial materials: viral RNA for 5-15 complete genome sequences, depending on the complexity of the quasispecies observed, a complete viral proteome sequence for the synthesis of autologous peptides to be used for identification of recognized CTL epitopes, reagents for production of an infectious molecular clones representing the earliest attainable virus, the first 20 envelope sequences, and an in-depth study of the later identified CTL epitopes. FL viral sequences will be attained by SGA. Five FL genomes will be generated initially and, if two or more distinct variants are observed, ten additional genomes will be generated. We expect 80% of samples to the homogeneous and 20% heterogeneous, based on current reports (Salazar-Gonzalez 2008; Keele 2008). A consensus sequence of the viral proteome will be generated and provided to the cellular immunology group for use in the

synthesis of autologous peptides for evaluation of CTL epitopes. The full genome PCR products will be used to generate an infectious molecular clone by recombination in yeast. This reagent will be used for biological characterization of the earliest viral strain, for studied of virus neutralization, and to generate autologous infected target cells for certain cellular immune studies.

Envelope sequencing will be performed at closely spaced intervals in very early infection. We will again use SGA of RNA extracted from plasma, generating approximately 20 envelope sequences from the first RNA positive sample and at 1, 2, 3, 4, 6, 8, and 24 weeks post-infection, starting with 10 acute infections initially to explore the utility of the frequent sampling approach. Functional envelope clones will be generated from every sample for evaluation of the evolution of neutralization susceptibility during the elaboration of innate and adaptive humoral effector mechanisms.

From the study of recognized CTL epitopes and their early escape variants, we will select 5 for in-depth longitudinal analysis for each study subject. Viral RNA from the first RNA positive sample, and from 1 and 4 months post-infection, will be subjected to pyrosequencing to interrogate the viral quasispecies in great depth. This technology provides a more complete description of the population of viral genomes present in a given sample, and can permit identification of variants present as low as 0.5% of the quasispecies.

4.3.3 Cellular Immunology

The aim of this study is to take advantage of a unique opportunity to comprehensively evaluate the early and pre-seroconversion HIV-1-specific $CD4^+$ and $CD8^+$ T cells and NK cell repertoire in the setting of four different genetic subtypes of HIV-1 in Uganda (subtype D, A, A/D), Tanzania (subtype C, A, A/C), Kenya (subtype A), and Thailand (subtype CRF_01 AE, B). Previous studies of the role of cellular immunity in the earliest time-points after HIV-1 infection have focused upon only single parameters of the cellular immune response such as cytolytic capacity or cytokine expression and secretion, have usually relied upon sporadic capture of acute seroconverters, have suffered from a small sample size and have concentrated upon subtype B and to a lesser extent subtype C. The proposed study is designed to take a systematic approach to studying early and pre-seroconversion cellular immunity by first identifying the earliest emergent HIV-specific T cells using multiparametric flow cytometry and then use novel and exploratory assay platforms to reveal potential cell-mediated correlates of initial viral control. Perturbations in the innate immune response will be studied through assessment of the NK cell repertoire using multiparameteric flow cytometry to measure expression patterns of activating and inhibitory surface receptors. Pre-existing and generalized immune activation will also be studied throughout the course of early infection and correlated with clinical parameters such as the serum LPS evaluation. The comprehensive analysis of viral diversity that is proposed in the viral genetics study will be used as a guide for all reagents and peptide sets used to screen for adaptive cellular immune responses, while the KIR-typing, HLA-typing and other host genetic factor assessed in the host genetics study will provide a genetic background for studies of both innate and adaptive cellular immune responses. Taken together this study will provide a unique opportunity to evaluate the adaptive and innate cellular immune response at the earliest possible time-point after infection in the setting of four different genetic subtypes of HIV-1.

Adaptive Immune Response Analysis:
Guided by the full-length sequencing data from the earliest time of detection of plasma virus peptide sets based-upon the autologous virus sequence will be designed. Between 5 and 10 subjects will be selected for this analysis depending upon the reliability of patient follow-up. Peptide sets will consist of approximately 400 peptides, 16-18 amino acids in length, overlapping by 11-12 amino acids and spanning each ORF of the entire HIV-1 proteome. Peptides will be: 1) kept as individual aliquots; 2) pooled into master pools representing each parent protein (e.g. Gag or Env), and 3) pooled in a matrix format for epitope mapping. According to SOE #2, PBMC drawn at 2 weeks post the first RNA-positive SBV will serve as the source of cells for detecting the earliest emergent adaptive T cells. Instead of relying solely upon the IFNγ Elispot to detect antigen-specific T cells, we propose to use multiparametric flow cytometry to detect 5 important functional markers of T cell effector activity – intracellular trapping of IFNγ, TNFα, IL-2 and MIP1β, and CD107 surface translocation. Bulk responses against a particular gene product will first be identified using the master pools of peptides, and the individual epitopes identified using a matrix approach and screening in an Elispot format assay. 10×10^6 PBMC will be required for each of these screening steps. The epitope recognition data (also the CD4 and CD8 lineage information) will be co-analyzed with the sequencing data from later time points to identify potential epitope escape variants. The data will be further stratified according to the viral load during this period. We plan to rank epitopes according to their "viral pressure index" and "escapability index". Epitopes that mutate rapidly will be assumed have placed selective pressure upon the virus but have readily escaped immune recognition - these epitopes would therefore have a high "viral pressure index" and a high "escapability index". Epitopes that remain unchanged in the presence of high viral load would have a low "viral pressure index", while those that remain unchanged in the presence of low viral load would have a high "viral pressure index" and a low "escapability index". We predict this analysis will reveal the epitopes, and importantly the variants of these epitopes, which might be the best to include in a vaccine formulation. In addition, we will gain direct insight into the breadth and depth of cellular immune responses that control initial peak viremia.

NK cell Immunophenotyping and Immune Activation Marker Analysis:
Cryopreserved PBMC will be thawed and used for measurement of NK cell frequency, phenotype and activating and inhibitory receptor expression. The cells are washed and stained in flow panels consisting of core antibodies to differentiate NK cell and subsets. The core panel will distinguish NK cells based on expression of CD3, CD4, CD19, CD14, CD16, and CD56. NK subsets are defined by CD16 and CD56 expression. Each panel will also look at different activating and inhibitory receptor expression for, but not limited to KIR2DL1, KIR2DL2/3/DS2 (clone DX27), KIR2DL3, CD94, NKG2A, NKG2C, KIR3DL1 (DX9), KIR3DS1/DL1 (z27), CD161, NKp30, NKp44, and NKp46. For study of immune activation, the core panel outlined above (CD3, CD4, CD19, CD14, CD16, and CD56) will be used and will have a selection of the following markers added to it: CCR5, CD25, CD38, HLA-DR, CD69 and Ki67. Samples will be acquired on an LSRII and analyzed using Flow Jo software. For functional analysis of NK cells, samples will be thawed, washed and activated using media, MHCnull K562 cell line, or PMA/ionomycin. These samples will be incubated with CD107a, Brefeldin A, and monensin overnight (18 hours). The samples will then be stained for expression of CD3, CD4, CD19, CD14, CD16, and CD56 to determine NK cell frequency and subsets. Additionally, MIP1β and IFNγ will be stained intracellularly and compared to other functional markers of interest. The

samples will be acquired on an LSRII and analyzed using FlowJo software. We anticipate that immune activation markers will be generally higher in the populations studied (especially east African) and that perturbations in the NK cell repertoire will be detectable early in infection.

Pilot Studies of Gene Expression Profiling and Novel Assay Platforms:
Samples for gene expression analysis will be taken at the earliest stages of HIV infection in order to elucidate those host gene expression patterns indicative of the status and duration of the Fiebig stages. PBMC samples for expression analysis will be identified in close co-ordination with those used in the Host Genetics sections and from those generated by flow cytometric analysis and sorting of subsets of cells of interest with respect to assessment of the cellular immune response. RNA samples will be prepared for analysis an Affymetrix Gene-chips following well-established procedures. In the first year of the study we will perform several small pilot experiments to determine the feasibility of gene profiling of small numbers of cells. Ideally we would like to sort or purify only the antigen-specific T cells. However, these will be limiting in number and we would need in the order of 20,000 cells at least, to perform the analysis. In collaboration with other groups we will be exploring novel assay strategies that may reveal underlying, and as yet undefined, mechanisms of T cell mediated inhibition of HIV propagation in *in vitro* culture systems. A modified assay for direct measurement of antiviral $CD8^+$ T cell activity against autologous $CD4^+$ T cells infected with select strains of HIV-1 will be applied. This assay has the advantage of directly measuring antiviral activity, without the need for a pre-determined surrogate marker of immune function. $CD8^+$ T cells displaying effective inhibition of viral growth can be further studied using gene profiling and multiparametric flow cytometry in an attempt to identify the function, or pattern of functions associated with antiviral activity. Other novel assay platforms, such as CFSE monitoring antigen-specific T cell proliferation will be employed as these assays become applicable and deployable.

4.3.4 Humoral Immunology

Binding antibody assays
The antigens utilized in the assessment of binding antibodies are versatile and can mirror the diversity of the vaccine immunogens for the clade of the infection cohort studied. HIV-1 Env and HIV Gag Proteins are the predominant antigens utilized to characterize the antibody response. In addition, peptides specific for certain regions of the HIV proteins can be utilized to fine map the specificity of antibody responses.

A customized multiplex assay (using Luminex technology) for the detection of IgM, IgA1, IgA2, IgG1, IgG2, IgG3, IgG4 antibodies with specificities against HIV Env gp140 and gp41, Gag p55, Tat, Nef and Env epitopes (ie. MPER) will be assessed in longitudinal samples. Autoantibody responses will be assessed including a rheumatoid factor test. Samples will be separated into IgG and non-IgG fractions for analysis. Positive and negative controls are included in every assay. Fully characterized samples in terms of antibody type and specificity will then be assessed for functional activity. The formation immune complexes (antibody-virion) will also be measured using developmental antibody capture assays to assess the possible role of antibodies complexed with virus in acute infection.

Mucosal Specimen Collection
Mucosal secretions for this study are defined as CVL and semen.

We propose to assess the following in mucosal secretions from subjects with acute HIV infection:

1) Qualitative and quantitative determination of the evolution HIV-specific binding antibody to include, but not limited to Gag and Env and characterization of the antibody isotype using techniques described below.
2) Measurement and evolution of HIV-specific neutralizing antibody to primary isolates of HIV-1.
3) Determination of the immunoglobulin isotype(s) of neutralizing antibodies and their temporal evolution.
4) Correlation between HIV-specific binding and neutralizing antibodies.
5) Determination and evolution of ADCC activity against HIV Env from diverse subtypes and the isotype(s) of the ADCC antibody if present

Methods

Specimen Collection: All specimens will be processed within 8h of collection

HIV-1 acutely infected study participants will be asked to provide mucosal specimens as indicated on SOEs #1 and 3.

Specimens will be collected on a subset of HIV seronegative subjects (N=20 males and 20 females at each site) at study entry to assess specificity of the assays and ensure that their performance is comparable across the different sites. For each HIV acute infection in the study, one of the "base-line" HIV seronegatives will be matched on age and gender, and specimen collection will follow the schedule for HIV acute infection (Phase II), with a collection window of +/- 10 days. Should that seronegative convert during the study, another HIV seronegative will be asked to enroll in the mucosal study and follow the same visit schedule as the HIV acutely infected subject. CVL and semen will be collected according to the procedures detailed in "The Manual for Collection and Processing of Mucosal Specimens; AVEG Mucosal Immunology Laboratory, University of Alabama at Birmingham, Alabama; Feb 1999). Mucosal collections from females must be accompanied by a serum tube collection (3ml) for hormonal testing as this may affect levels of immunoglobulin in the CVL.

Collection of CVL:
Exclusion criteria:
- Abnormal vaginal discharge
- Sexual intercourse without a condom (ejaculate was deposited into vagina within 72 prior to collection – monitored using the Sema kit
- Douching within 72h prior to collection
- Blood contamination- CVL will be tested for Occult blood using the Hemoccult test

Briefly, 3ml sterile saline will be flushed several times over the cervix and around the external cervical os. Material will be collected and placed into a test tube on ice, aliquoted (0.4ml) into cryovials, and protease inhibitors added at 1/10th the specimen volume. Samples will be stored at $< -70^{o}C$.

Collection of Semen:

Exclusion criteria:

- Symptoms of infection – e.g. abnormal penile discharge
- Semen samples do not liquify following refrigeration
- Any ejaculation within 48h prior to collection

Briefly, the volunteer is asked to masturbate without lubricant, and the ejaculate collected into a sterile specimen container. Ejaculate is stored at 4 ^{o}C for 1h, diluted with an equal volume of PBS and centrifuged at 1200g/10 minutes. Protease inhibitor solution is added at 1/10th the final volume, the supernate aliquoted at 0.2ml and stored at $< -70^{o}C$, and the cell pellet frozen in a single vial at $< -70^{o}C$.

The protease inhibitor solution contains PMSF, aprotinin, leupeptin, antipain and pepstatin.

ADCC, Neutralization, and other functional assays.
These assays will be performed on frozen plasma or mucosal samples collected at all indicated time points post-HIV-1 RNA detection. Assays for a single subject will be tested in the same assay to reduce inter-assay variability and the same positive and negative controls will be used for all assays to allow normalization of the data. The positive and negative controls will be sera from HIV infected and uninfected subjects, and in the case of neutralization assays, additional specific reference mAbs. The plasmas will be assessed first in the TZM-bl assay using autologous and well characterized heterologous env clones, and later, available IMC and infectious isolates will be characterized in a subset of PBMC assays for comparisons and to assess clade-specific relationships. There will be no paucity of reagents for neutralization studies; however, antigens available for ADCC studies may be a technical challenge. Our laboratory at AFRIMS in Bangkok has extensive experience performing ADCC using subtype B and CRF01_AE gp120; we will purchase gp120 of subtypes A, C, and D from Immune Technology, Inc, and will also test vaccinia-expressed o-gp140 from the strains used in our A, C, D and CRF01_AE vaccines.

The systematic evaluation of both binding and functional antibodies directed against autologous and heterologous viruses will contribute to our knowledge of the cross-clade responses within peripheral and mucosal compartments and against non-B HIV-1 subtypes from East Africa and Thailand, and specifically at potential vaccine cohort sites.

Characterization of HIV envs and isolates from acute infection.
Envelope clones will be prepared from both the mucosal and plasma samples at multiple early time points, and these envs will be tested for function (formation of infectious pseudovirus through transfection of 293T cells and infectivity assessment in the TZM-bl cell model). Functional envs will be assessed for neutralization phenotype using reference panels of poly- and monoclonal antibodies. The env clones will be assessed first in the TZM-bl assay, and later,

available IMC and infectious isolates prepared from samples obtained at or near the first RNA positive sample, will be characterized in a subset of comparative PBMC assays. These data will provide extensive characterization of the biotype of envs from acute infection for subtypes A, C, D, CRF01_AE and some novel recombinants.

Characterization of Antigen-Specific B Cells

The laboratory of Dr. Tony Moody, a CHAVI investigator at Duke University School of Medicine (see attached letter of collaboration) has developed a B cell phenotyping panel that is based on a series of core markers for positive and negative selection of B cells and the definition of core subsets, as well as a series of rotating markers for the characterization of additional B cell subsets. The core set is based on early work by Banchereau (Pascual 1994) and additional work by Bohnhorst (Bohnhorst 2001). The core panel consists of negative markers (CD3, CD14, CD16, CD235a) to eliminate cells not of interest and the B cell marker CD19. The CD19+ve population is then further subdivided by into six subsets that divide cells into pre- and post-class switching and into mature and naïve groups. Circulating plasma cells are then grouped into this scheme. A set of several additional markers are rotated in different FACS channels are to identify and enumerate numerous cell populations. A set of epitope-specific B cell tetramer reagents is also available to identify antigen-specific B cells. Comparisons of cells that express immunoglobulins reactive with epitopes of well known broadly neutralizing antibodies, or epitopes to which antibodies are commonly made, will inform our understanding of the fate of these cells and potentially offer clues as to the rescue of these cells by vaccination strategies.

4.3.5 Host Genetics

We propose both the targeted analysis of genetic polymorphisms within genes controlling host restriction, innate, and adaptive immunity factors that can influence HIV acquisition and disease progression and GWAS-derived associations with peak and set-point viral load. We will, respectively, apply a real-time PCR platform at our Rockville laboratory and the Illumina Infinium Whole Genome Human1M BeadChip within the Duke IGSP genotyping facility to accomplish these goals. The Human1M BeadChip combines an unprecedented level of content for both single nucleotide polymorphisms (SNP) and copy number variation (CNV) analysis, along with additional unique, high-value genomic regions of interest, as the MHC region. This chip is therefore especially promising in a population with African ancestry. A series of quality control procedures will be carried out to check for: 1- Infinium BeadStudio Raw Data Analysis quality, 2- minor allele frequency consistency to assess data handling accuracy, 3- mismatches between clinical and genetically inferred specification of gender, 4- cryptic relatedness, 5- pattern of missing genotypes, 6- low minor allele frequency, 7- Hardy-Weinberg Equilibrium violation, and 8- visual inspection of genotyping quality for top SNPs. Core association analyses will focus on single-marker genotype trend tests of the quality-control passed SNPs using linear regression. To control for the possibility of spurious associations resulting from population stratification, a modified EIGENSTRAT (Price, 2006) method will be used. Other covariates considered in the regression model will include age and gender. To assess significance, the suitability of a straight Bonferroni correction will be evaluated by comparing this to the results of permutation testing to assess the appropriate cutoff. If a straight Bonferroni correction is used, the conservative P cutoff will be 5×10^{-8}.

The targeted analysis of pre-identified host restriction factors will be directed at those polymorphisms that occur within HIV co-receptors, the natural cytokine ligands of these co-receptors, and host defense systems interfering with crucial post-entry events of the viral replication cycle, that have been reported to be associated with different rates of HIV acquisition and disease progression. Commercial reagents will be used when available, and otherwise, reagents published in the literature will be adapted to the real-time PCR platform.

The analysis of innate immunity factors will be targeted at KIR genes and their ligands, and polymorphisms in DC-SIGN and TLRs, that that have been reported to be associated with different rates of HIV acquisition and disease progression. The targeted analysis of humoral adaptive immunity factors will be directed at polymorphisms in Fcγ receptors that affect binding specificity and affinity for the diverse subclasses of IgG. The targeted analysis of cellular adaptive immunity factors will be directed at alleles in the Class I HLA-A, -B, and –C loci, which restrict CTL responses. Population-based comparative analyses of frequencies of the targeted polymorphisms will be conducted between exposed HIV sero-negative vs. HIV sero-prevalent individuals derived from the same socio-demographic setting. The comparison between exposed HIV sero-negative and HIV sero-incident cases will be performed in a case-control format.

Among HIV sero-incident cases, the following associations with targeted polymorphisms will be explored:

- patients' genotypes vs. the duration of the different Fiebig stages
- patients' genotypes vs. timing and level of peak HIV viremia
- patients' genotypes vs. timing and level of set-point HIV viremia
- patient's host restriction genotypes vs. dynamics of HIV genetic variation (e.g., APOBEC3s vs. *vif*, TRIM5α and Cyclophilin A vs. *gag*, co-receptors and their ligands vs. *env*, tetherins vs. *vpu*)
- patients' KIR/ligand genotypes vs. dynamics of HIV genetic variation, to identify early NK-escape viral variants
- patients' Fcγ receptor genotypes vs. dynamics of anti-HIV ADCC responses

The HLA genotyping information from HIV sero-incident cases will support the work of the functional cellular immunology sub-project, and will be used to design reagents to ascertain CTL responses to autologous and near-autologous viral sequences, identify immunodominant and subdominant epitopes, design tetramer or pentamer reagents.

The HLA testing that will be used in this study is a research tool that has not yet been validated for clinical use. The HLA typing will be essential for the analysis of adaptive cellular immune responses in early HIV-1 infection and their subsequent effects on viral evolution and they will be among the host factors that will be evaluated for their contribution to protection from HIV-1 infection and early control of the virus. The methodology that is proposed for HLA typing is adequate for these purposes, but is intended for research use only. To provide this information to study volunteers, the new HLA typing approach would have to be validated, and the testing would have to be done by an accredited clinical lab. Further, we are advised by colleagues in the field that even the strongest HLA effects are not used in current practice to dictate treatment or

other clinical care options, because they represent population-level probabilities, not necessarily predictive for any given individual. To meet the research needs, the HLA typing that is proposed would be done unlinked to any personal identifiers, and results reported in aggregate for the populations studied.

The GWAS approach will be limited to associations with viral load peak and subsequent viral load set-point in the anticipated 150 subjects who develop incident infections. Associations with peak viral load will be completely novel while associations with viral load set-point, while not completely novel, will provide substantial new information from individuals with subtype A, C, D, CRF01_AE, and intersubtype recombinant infections. Exploration of associations between incident infections and the anticipated 1850 subjects who are exposed and uninfected using GWAS would be of great scientific value, but are beyond the scope of this current proposal. Additional funding will be sought from other sources to execute this specific aim.

4.4 Acute HIV Infection Phase Design

All HIV positive volunteers identified at any time after a negative HIV test at the first follow-up visit will be asked to participate in an HIV incident follow-up phase IB. This is integral to phase I of the study and does not require re-consent and allows definitive diagnosis of HIV status. Those who are confirmed as HIV infected incident volunteers will be asked to consent to further participation in phase II. Those who decline to participate in phase II will continue follow-up within the main protocol if they so wish. The HIV incident follow-up schedule in phase II will assess viral diversity, host immune responses, host genetics and early expression makers, which will be related to viral burden and characteristics of host immune response. AHI cases will be followed intensively with blood collection for cellular and acellular specimens in the first several months following infection and will then be followed every 3 months for at least five years (see attached Schedule of Evaluations #3). All HIV positive volunteers will be referred to a President's Emergency Plan for AIDS Relief (PEPFAR) funded clinic or to an appropriate medical facility for care and treatment. CD4 counts and viral burdens derived from the research will be available to the clinic at the direction of the volunteer at no charge.

4.5 Susceptibility to HIV infection from Co-Incident Infections Design

Study volunteers will be tested for malaria, Hepatitis B (if appropriate), HSV-2, eosinophils, LPS upon study entry, at 30 days (Visit B) and subsequently every 6 months during the course of the study. If volunteer becomes HIV(+) they will be tested at the time of phase II enrollment for malaria, syphillis, HSV-2, LPS, and eosinophils. Self-reported STIs will be assessed at every scheduled visit and queried every 6 months by questionnaire. EDTA whole blood will be reposed to assess subclinical malarial infection by PCR. Self reported STIs will be confirmed by voluntary physical exam and syphilis will be diagnosed by RPR followed by confirmatory testing. Full CBC differentials will be obtained to quantify absolute eosinophils. Plasma will be collected to assess LPS levels.

4.6 Overview of Volunteer Activities (Phase I - Surveillance)

Recruitment methods and activities are directed at each group separately to minimize stigma risks and for operational efficiency. Volunteers will receive HIV risk-reduction counseling, a briefing regarding HIV and HIV vaccine research in general, a medical history and physical, a behavioral risk questionnaire, HIV testing and provide a blood sample to characterize general health (e.g. CBC, chemistries and LFTs), and HIV status. All volunteers will be seen in follow-up no later than one month after visit A (within 7 to 21 days of Visit A) to review laboratory and clinical findings from the screening visit.

Those testing HIV positive or indeterminate at visit A will provide a blood sample to verify HIV status at visit B. Only healthy and non-HIV infected volunteers will continue follow-up after the second visit, with the following two exceptions:

1. Participants will be enrolled contemporaneously and consecutively in a 1:10 ratio of HIV positive to HIV negative participants.
2. For those volunteers who are considered acute seroconverters based on testing indeterminate at Visit A and positive at follow-up can be enrolled into the RV 217 phase IB at the discretion of the site PI.

All other volunteers who are not eligible for participation due to HIV infection or other chronic illness will be referred to a PEPFAR funded care and treatment activity or to an appropriate medical facility for care.

Eight follow-up visits will occur at 12-week intervals after the second visit and include a directed medical history and physical and post-test counseling for all HIV results and risk reduction counseling. At every other visit (every 24 weeks) a behavioral and knowledge questionnaire, and blood draw to ascertain HIV status will be conducted. All volunteers will be asked to return to a site as convenient to their work place or home as feasible for small blood volume collections collected in a microvette. These samples will be delivered to the lab for analysis. Study volunteers will be tested every 24 weeks and at the time of enrollment into phase II for syphillis, Hepatitis (if appropriate), HSV-2 LPS, and eosinophils. Self-reported STIs will be assessed at every scheduled visit and queried every 24 weeks by questionnaire. In East Africa sites, malaria will be tested every 24 weeks and at enrollment into phase II. Malaria will be diagnosed by a thick and thin blood smear. Self-reported STIs will be confirmed by voluntary physical exam and syphilis will be diagnosed by RPR followed by confirmatory testing. Full CBC differentials will be obtained to quantify absolute eosinophils. Plasma will be collected to assess LPS levels.

Very early HIV infection may have low amounts of HIV RNA or intermittently negative RNA tests. In addition, some HIV infected individuals control HIV infection very effectively and have very low levels of HIV RNA or may be negative even with the most sensitive tests. Hence, negative test results do not exclude the possibility of HIV infection. Positive tests are not conclusive evidence of HIV infection either. Some false positive results may be due to mis-labeling, contamination or other error. Hence, diagnostic testing and post-test counseling are provided using larger blood volume sample test results at the 24-weekly clinic visits. Further, any HIV uninfected participants with a new reactive Aptima test will shift to phase IB and follow SOE 2 to confirm HIV diagnosis. All confirmed HIV incident cases will be referred for entry

into phase II. The evolution of antibody response will eventually confirm all assignments as HIV infected and its long-term absence will confirm an assignment of HIV negative.

Should a volunteer at any stage of participation indicate their desire to leave the study due to re-location or other reason, the team will attempt to arrange an exit visit to establish HIV status at the time of discontinuation.

4.7 Overview of Volunteer Activities (Phase II – Incident HIV Infections)

Volunteers who are confirmed to be newly infected with HIV are referred to the clinic at the next visit or as soon as possible to offer enrollment in the HIV acute infection (phase II). Confirmation of HIV status will be completed through phase IB.

Among volunteers who consent to participate in phase II, the blood volume and sample collection schedule specified in Appendix VI-SOE #3 will be applied and serve to provide all samples needed to confirm the diagnosis. Participants in phase II will have an initial assessment of safety labs, HIV diagnostic labs and collection of PBMC and plasma to meet the scientific discovery objectives of the study. The first visit in phase II will follow the last visit in phase IB by one week and relative to the first visit in phase II, subsequent visits will occur one week, two weeks, four weeks and then every 12 weeks for at least 50 months.

Should a volunteer at any stage of participation indicate their desire to leave the study due to re-location or other reason, the team will attempt to arrange an exit visit to establish HIV disease status at the time of discontinuation.

All HIV positive participants will be referred to an affiliated PEPFAR clinic or other care provider to manage HIV infection including provision of HAART as indicated.

5.0 STUDY POPULATION

5.1 Study Population

A description of the sites' study populations can be found in section 2.0. Part A (pilot phase), will enroll primarily SW and BW in Africa and SW, TG and MSM in Thailand. Each site will enroll up to 200 volunteers. Part B (full study) will enroll all of the MARPs to attain a total enrollment of 300 HIV negative volunteers at each site.

It is customary to provide the exact inclusion and exclusion criteria in the volunteer consent. In order to minimize the risk of stigmatization of participants who must engage in high-risk behaviors to enter the study, the specific behavioral elements for inclusion will only be summarized in the consent as follows: "Based upon information provided in the questionnaire, your behavior places you at greater risk for HIV infection than the general population."

5.2 Inclusion Criteria

A participant must meet all of the following criteria:

1) Able to demonstrate understanding of the study by passing score of 90% on test of understanding within 3 attempts to take the test
2) Ability and willingness to sign/mark/thumb print the informed consent form
3) Man or woman aged 18-50 years
4) Available for follow-up for a total study duration of 24 months
5) Willingness to consent to HIV counseling and testing
6) Willingness to receive HIV test results
7) Willing to provide location or contact information
8) Willing to have picture taken for the study ID, or provide a photo ID or fingerprint
9) Must understand English or local language as approved by IRB
10) In the last 3 months prior to inclusion in the study, the volunteer must meet one or more of the HIV risk criteria as follows:
    a. has provided vaginal or anal intercourse in exchange for money, goods, or services
    b. has had unprotected vaginal or anal intercourse with one or more known HIV-positive partners
    c. has had unprotected vaginal or anal intercourse with three or more partners of known or unknown HIV status
    d. has a new sexually transmitted disease

5.3 Exclusion Criteria

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

1) Any significant condition (including medical and psychologic/psychiatric disorder) which in the opinion of the study investigator might interfere with the conduct of the study
2) Unwillingness to give social history, medical history, undergo medical examination and provide blood specimen
3) Has ever received an HIV vaccine

Note: When the protocol is submitted to a local regulatory agency for approval, the site may note that it will be limiting enrollment to a subset of the risk criteria based upon relevant epidemiological data or prior site cohort research.

6.0 STATISTICAL CONSIDERATIONS

6.1 Power and Sample Size

The study is expected to generate approximately 150 seroconverters over the course of 24 months of follow-up, and 1850 are expected to remain seronegative as shown in Table 1 (assuming no loss to follow-up). On average therefore, at least 37 seroconverters are expected to be observed at the end of the 24 month follow-up, at each of the four participating country sites. We expect roughly 66% of infections (100 AHI) would be observed in the first 12 months (Riedner 2006; Baeten 2000) and of these at least 50% would have been compliant with surveillance and therefore identified in the first 2-3 weeks of HIV infection (Fiebig stages 1 and 2).

Of primary importance will be the ability to determine the incidence rate of HIV-1 infection and the rate of participant retention during the approximately 24-month study. With regard to the incidence rate, based upon prevalence and incidence estimates from previous research with these and related populations, and given the approximate sample size proposed for this study, we estimate an aggregate HIV-1 incidence rate of 3.7/100 person-years of follow-up, with 90% confidence intervals around this estimate of 2.9/100 person-years to 4.6/100 person-years. The sample size anticipated for this study also provides sufficient power (>90%) to detect a retention rate that is + 3% or more of 90%.

The main body of Table 1 shows the power to detect given rate ratios (RRs) between HIV-1 sero-converters (SCs) with a given risk factor of interest at baseline ranging in frequency from 10% to 50%; versus HIV non sero-converters (SNs) with the same risk factor of interest.

The following assumptions were made in the calculations:

- A total of at least 150 HIV incident cases would be observed over the 24 month follow-up period
- A loss to follow-up of 10% by the end of the 24 month follow-up period, thus 150 seroconveters, and 1650 non sero-converters by the end of the 24 month follow-up
- A level of significance (α) of 5%

Table 1 Power to detect a given Rate Ratio

Risk Factor proportion at baseline, among non sero-converters (SNs)	Rate Ratio*				
	1.8	**1.9**	**2.0**	**2.1**	**2.2**
0.1	77.8%	85.3%	90.7%	94.4%	96.7%
0.2	98.5%	99.0%	> 99%	> 99%	>99%
0.3	>99.0%	> 99%	> 99%	> 99%	>99%
0.4	> 99.0%	> 99%	> 99%	> 99%	>99%
0.5	> 99.0%	> 99%	> 99%	> 99%	>99%

**Rate Ratio = rate of HIV infection among participants with risk factor of interest at baseline, versus the rate of HIV infection among participants without the risk factor of interest.*

We note from Table 1 that the study will have more than 80% power to detect a rate ratio of at least 1.9 if the baseline prevalence of any risk factor of interest among HIV non sero-converters (SNs) is at least 10%. All study power calculations were performed using the PS (Power and Sample Size Calculations) Software Version 2.1.31 (Dupont and Plummer 1990, 1998).

6.2 Analysis of Phase I Primary Objectives

Brief descriptions of statistical analysis methods to be applied to address the objectives of this study are as follows:

Prevalence - Baseline prevalence will be defined as the proportion of individuals testing positive at baseline divided by the total number of individuals who are test for HIV.

Incidence – Aggregate and interval-specific HIV-1 incidence rates will be calculated using person-time analysis, with the numerator being the number of seroconverters over a given time period and the denominator being the cumulative number of person-years contributed by the total number of individuals in the study at the beginning of the same time period. HIV-1 negative individuals will contribute person-time to the denominator beginning with the time they enter the study until the study concludes or they are censored (lost to follow-up, die). The respective 90% CI of the HIV incidence rate will also be calculated. We will then establish if the overall lower bound of the 90% CI of the HIV incidence rate is greater than 3.0%, as hypothesized.

Seroconverter case definition: for purposes of this study, incident HIV cases/seroconverters will be defined as any volunteer testing HIV positive at any encounter (scheduled or unscheduled) after enrollment by a standardized testing algorithm consisting of sequential ELISA and confirmatory WB testing who also test positive on a subsequent, repeat phlebotomy and verification HIV testing. Volunteers testing HIV positive and defined as seroconverters as

above who are not able to return for verification HIV testing (i.e. refuse to return; lost to follow-up) will also be considered in analyses as incident HIV cases/seroconverters due to the high positive predictive value of the ELISA, ELISA, Western Blot algorithm.

For HIV incident case/seroconverters, the date of seroconversion will be calculated as the midpoint between the visit dates of the last negative test and first positive test. Seroconverters will contribute zero person-time from their calculated date of seroconversion to the visit corresponding to their first positive test. Ninety-percent confidence intervals will be calculated around point estimates.

Retention-Retention over approximately 12 and 24 months of follow-up will be calculated as the number of all study participants completing the approximately 12 and 24 months of follow-up at all of the country sites, divided by the total number of participants enrolled in the cohort at baseline at all of the three country sites. From this, we will establish if the overall retention rate is greater than 90% as hypothesized.

Risk Factors –Chi-square or Fisher's Exact Test will be applied to compare differences in proportions for categorical variables. Student t-test will be used to compare differences among continuous variables. RRs and their 95% CIs relating individual risk factors to incident HIV-1 infection will be calculated. Univariate and multivariate Poisson regression analysis will be used to assess the associations between these risk factors and HIV-1 infection. P-values for all tests will be two-sided, with a p value <0.05 considered statistically significant.

6.3 Data Analysis for Secondary/Exploratory Objectives

Host Genetic Factors- Phase I and II: Descriptive statistics will be generated to summarize the frequency and distribution of polymorphic variants at loci for host restriction, innate and adaptive immunity, among both uninfected and infected subjects at baseline. Allele frequencies will be determined by direct counting of the occurrences of each variant and statistics on genetic analyses will be computed using modules implemented in the Pypop version 0.6.0 (Lancaster, Nelson et al. 2003) and Arlequin version 3.1 (distributed by L. Excoffier, CMPG, University of Berne, Switzerland) software packages. Deviations from Hardy–Weinberg (HW) proportions will be tested using chi-square or the exact tests of Guo and Thompson (Guo and Thompson 1992). The Ewens-Watterson homozygosity test of neutrality (Ewens 1972; Watterson 1978) will be conducted as implemented by Slatkin (Slatkin 1994; Slatkin 1996). The significance of linkage disequilibrium (LD) among loci and the corresponding haplotype frequencies will be assessed by a permutation test using an expectation-maximization (EM) algorithm (Slatkin and Excoffier 1996). Two measures of overall LD will be determined: D′, which weights the contribution to LD of specific allele pairs by the product of their allele frequencies (Hedrick 1987), and Wn, which is a re-expression of the chi-square statistic for deviations between observed and expected haplotype frequencies (Lancaster 2004). For each inferred haplotype, individual D′ values will be calculated as previously reported (Cao, Moormann et al. 2004) to investigate deviations from random association of alleles, and to account for differing allele frequencies at the loci.

Phase II: The frequency and distribution of host genetic factors will be compared among the following groups: subjects who are uninfected and remain uninfected for the duration of the study, subjects who are HIV infected at baseline, and subjects who become HIV infected during the course of the study. Exploratory descriptive analyses of association of host genetic factors with susceptibility to HIV infection will be performed. We will compute unadjusted odds ratios for HIV infection in the presence of different alleles and extended haplotypes of host restriction factors and HLA, using variance estimates and 95% confidence intervals to evaluate the significance of any associations observed

HIV Viral Genetic Analysis- Descriptive statistics will be generated to summarize both baseline prevalent and incident HIV infections including frequency and distribution of each viral subtype. Exploratory descriptive analyses including unadjusted univariate analyses will be performed to explore potential associations between risk factors and subtype. The study of viral genetics will be performed in a descriptive manner, as the anticipated number of HIV infections accrued during the study will be too low to perform any meaningful statistical test. However, any associations or significant trends will be followed up.

Humoral Immune Responses- ELISA and Neutralizing antibody titers will be compared at various stages of disease using non-parametric statistical analyses. The evolution of antibody responses will be characterized through a comparison of ELISA titers for different Ig subclasses (against several HIV antigens) at various time points post-seroconversion and between the various Feibig stages at which the patients enrolled in the AHI protocol are captured. Differences in the magnitudes of titers in early versus late Feibig stages, as well as in chronic infection, may be compared by Wilcoxin-Rank sums analysis, the Mann-Whitney U-test, or other appropriate measures. The agreement between titers derived from different assay platforms will be analyzed by linear regression to assess comparability of different methodologies and to identify antibody populations for which discordance exists.

HIV Diagnostics Analysis –Descriptive statistics and group comparisons to include stratified Mann-Whitney and stratified Kruskal-Wallis tests will be calculated for Fiebig Staging. Samples will be stratified, and the intervals of assay specific window periods will be projected using a parametric model. Analyses will evaluate 1) aggregate analysis of reactivity of participant panels, 2) relationship between HIV laboratory stage and HIV-1 RNA quantification, 3) model relationship between HIV-1 RNA level and p24 antigen signal-to-cutoff ratio, estimate interval of window period closure relative to Fiebig staging, 5) estimate probability of presentation due to high risk exposure, and 6) multivariate/univariate analysis of stage vs results of viral genetics, host genetics, immunological, and genomic studies.

Cellular Immunology Data Handling and Analysis- All flow cytometric studies will utilize a Becton Dickenson LSRII flow cytometer equipped with three or four lasers for simultaneous acquisition of data in 12-15 channels. Datasets will be analyzed using FlowJo™ software. The 12-parameter panels for the surface immuno-phenotyping will utilize a subset of the following surface markers: CD3 (or Tetramer), CD8, CD27, CD28, CD45RA, CD45RO, CD62L, CD127, CCR5, CCR7 and PSGL-1. A viability marker will be used in a discrete channel and a "dump" channel will be used to gate out non-T cells from the datasets. The 12-parameter panel for the functional immuno-phenotyping will utilize the following panel: CD3, CD8, CD28, CD95, IFNγ, TNFα, IL-2 and MIP1β expression, while degranulation will be studied by analysis of CD107

surface expression. Hierarchical clustering analysis will be used for the phenotypic analysis and definition of the different T cell subsets. Defined T cell subsets will be assessed in terms of absolute numbers and enrichment for HIV-1 antigen-specific cells. Boolean gating strategies will be used, particularly in the case of the cytokine gene up-regulation assays, to discern the different patterns of cytokine gene expression. Data will first be analyzed in terms of descriptive statistics, which will include calculation of the median and interquartile (25^{th} and 75^{th} percentiles) and extreme values (highest and lowest values). Non-parametric statistical tests (e.g. Mann-Whitney and Kruskal-Wallis Tests) will be used to compare and compute statistical differences among the data sets. The epitope mapping studies will utilize the interferon-gamma Elispot assay where the widely accepted cut-off for a positive response of 55 spot forming cells per 1 million input PBMC is to be applied.

7.0 PROCEDURES

7.1 Volunteer Recruitment

Volunteers will be recruited using methods in accordance with 32 US Code of Federal Regulations (CFR) 219 and all other policies and regulations of the United States Government and host nation. In addition, host national guidelines will be adhered to for conduct of the research study in each country.

Recruitment will be done using a combination of methods after approval by the WRAIR Institutional Review Board (IRB) and local IRBs. IRB-approved flyers/posters and public announcements will be used to recruit potential volunteers. The recruiting efforts will depend heavily on direct interaction with members of the target community, their community leaders, support groups and NGOs active in these communities. Information seminars will be organized in strategic locations within the targeted communities. At the end of each information seminar, potential volunteers will be referred to the clinical research sites for screening and enrollment. Details on recruiting and advertising are included in each site-specific protocol addendum.

In addition, sites may employ a respondent driven survey (RDS) method that is known to be effective in "hidden" populations subject to legal or cultural opprobrium. In this setting, initial "seed" respondents recruited by the methods noted above are given two coupons to recruit at most two individuals to the study. The new recruits may obtain appointments for study briefing and enrollment with presentation of the coupon. The coupon will identify the "seed" using a code known only to the research staff and if the recruit is qualified for enrollment, the participant providing the recruit via the coupon will receive a very modest compensation. The recruiting participant and the recruited participant will not have further interaction, i.e. the participant who referred the new recruit is not in any way responsible for the recruited volunteer's compliance.

7.2 Study Visit A

Potential participants will receive a briefing, consent, "test of understanding" (TOU) and questionnaire. Visit A commences with a detailed briefing provided by the site principal investigator (PI), the associate investigator (AI) or their designee (qualified staff such as, a study medical officer, study nurse, or counselor). The briefing will review some general information about HIV, HIV research, and vaccines as well as the study purpose, design, risks, benefits, compensation, volunteer rights and other topics included within the written consent form. A question and answer period will follow the briefing.

Interested participants will then undergo a detailed review of the consent form (found within Appendix I: Site-Specific Addendum) by research study staff and will be asked to provide consent to participate in the study by signing the Informed Consent Form (ICF). In addition to the consent to participate, participants will be asked to provide consent to store their samples for future use on a separate "future use" consent (also found within Appendix I). Volunteers will receive a verbal, brief TOU (Appendix II) that is used as a tool to insure that key elements of

consent and participation are understood. Volunteers will be permitted three successive attempts to pass (passing score defined as 90%) and those not passing will not be permitted into the study.

After volunteers have given consent to participate in the study, volunteers will proceed for study number assignment and preparation of a study identification card that may be used for identification in study follow-up. The identification card may contain the volunteer study number or contain the photograph, but not both together. Further details of volunteer identification can be found in the site-specific addenda.

After successful completion of the consent and TOU, the volunteer is administered a questionnaire using the Audio-Computer Administered Self Interview (ACASI) or if preferred by the participant, the questionnaire will be administered by research study staff. Behavioral risk information will be collected through ACASI to determine eligibility for continued study participation. All consenting participants will undergo a full questionnaire. Only those participants whose behavior categorizes them as members of a MARP will continue the study. To provide masking, i.e. to obscure the stigmatization of participants who continue the study due to high-risk behaviors, a small percentage of eligible volunteers may be randomly excluded. All volunteers who are categorized as MARP will then undergo an entry medical history and physical and blood collection. This visit may be conducted over two days to allow the time necessary to complete the study procedures. Study procedures by visit can be found in the SOE #1 (Appendix IV).

Contact information will be collected from the volunteer at either study visit A or B. The volunteer may be asked to provide their contact information using a contact information form. This form may include the volunteers' name, phone numbers, if applicable, date of birth, age, address of residence (or best description), review of national identification and/or personal identification card, fingerprint and acceptable person(s) to contact including address and phone number, if applicable. The volunteer will be asked to specify which methods of contact are acceptable and will sign giving approval for acceptable methods. The personal identifiers are included on the contact information form for purposes of verifying the identity of the volunteer at each study visit. Furthermore, the contact information will be useful for contacting volunteers who may not return for their scheduled visits, or those who may move away from their places of residence.

7.3 Study Visit B

The second study visit will provide an opportunity to review initial test results with the volunteer. Those who are found HIV negative who remain otherwise eligible have blood drawn as described in SOE#1 and are then scheduled to begin their twice-weekly small blood volume collections (SBVs) within the next seven days after Visit B. They are also scheduled for Visit C.

Individuals found HIV positive at Visit A will have a full Visit B blood draw. They will be scheduled for a test result session (see Section 7.6) later for result disclosure and counseling. If they are confirmed infected, they will be referred to care and treatment programs without further follow-up in the study. A small number of HIV positive volunteers will be retained in phase I to provide both masking to minimize risk of stigmatization and to provide positive controls to study

assays. The retained HIV positive participants will be selected at random as each group of ten seronegative volunteers are recruited and consented.

7.4 LBV and SBV Visits

Phase I of the study, the surveillance of HIV uninfected volunteers for early infection, is comprised of two types of visits: 1) clinic visits with large blood volume collections (LBV) and surveillance visits with small blood volume collections via microvette collection (SBV). To easily distinguish activities and locations they will be referred to as LBV and SBV even though counseling, questionnaires and other activities may occur at each.

The purpose of the SBV visits which occur twice weekly at locations as convenient as feasible to the volunteer, is to identify HIV infection prior to the advent of detectable levels of HIV antibody. The project plans to analyze these samples within 48 hours and deliver results to the study team to allow a volunteer to be referred to the clinic for entry into phase IB of the study and once HIV infection is confirmed to enter phase II of the study for acutely infected individuals. The currently recognized diagnostic criteria for HIV world-wide depend upon antibody responses.

The study will identify individuals in advance of detectable antibody. A positive test detecting HIV nucleic acid is also an acceptable standard for establishing a diagnosis and is commonly employed in the diagnosis of HIV infection in infants. In the study proposed here, individuals are contributing diagnostic samples very frequently and may have an initial positive HIV nucleic acid test which is not positive again for a few weeks (Fiebig 2005). This era of low level, intermittent viremia is referred to as a "stutter phase" and has been documented up to 21 days after an initial positive sample. Thereafter, the nucleic acid test signal is generally strongly positive. Therefore an initial positive sample from SBV is not diagnostic but prompts entry into an accelerated schedule (SOE #2) where samples will be collected that will verify the initial sample results and lead to definitive diagnosis.

At each LBV clinic visit after and including visit B (occurring at six-month intervals), blood is collected for HIV testing in an attempt to identify individuals using standard diagnostic methods to as soon after infection as possible.

7.5 Exit Visit

The exit visit is performed for cases that are not willing or able to continue in the study and do not withdraw their consent. For those who have recently (within the last six weeks) had an LBV, the "Exit Visit" column in SOE#1 is followed. For all other cases, the procedures for their upcoming LBV may be performed as their "exit visit". For those participants who withdraw their consent, no further research procedures may be conducted (see Section 9.10 for more information).

7.6 Test Result Sessions and HIV Test Counseling Visits

During phase I, HIV tests are done on all LBV visit samples (Visit A, B, D, F, H, J and Exit). For each of these, pre-test counseling is done at the time of the visit. Post test counseling for Visit A is conducted at Visit B. All other test result sessions for LBV are done in "test result sessions" following the LBV, conducted outside of the protocol schedule of events. These visits collect no study data and are not counted as visits (unless it coincides with an SBV) although they are documented in source documents. The site may give compensation up to half the amount of a regular visit.

HIV tests on the SBV samples are done twice weekly. HIV pre and post-test counseling visits take place every three months. More frequent counseling sessions would be time-consuming and would potentially dilute the counseling message.

Volunteers who are found HIV positive will be handled as described in Section 9.1 on HIV Test Results.

All counseling is done by research study staff with training in HIV test counseling, and routine supervision. The post-test counseling sessions will be conducted in a private setting, with great attention paid to the preservation of volunteer confidentiality. To receive their HIV test results, volunteers must identify themselves to the research staff as outlined in the site-specific addenda. Counseling guidelines for each country will be applied.

All HIV sero-positive volunteers will have in-depth counseling regarding the nature of HIV disease progression and the availability of interventions, support groups and follow-up counseling. Follow-up counseling and support will be available.

7.7 Transition to Phase II

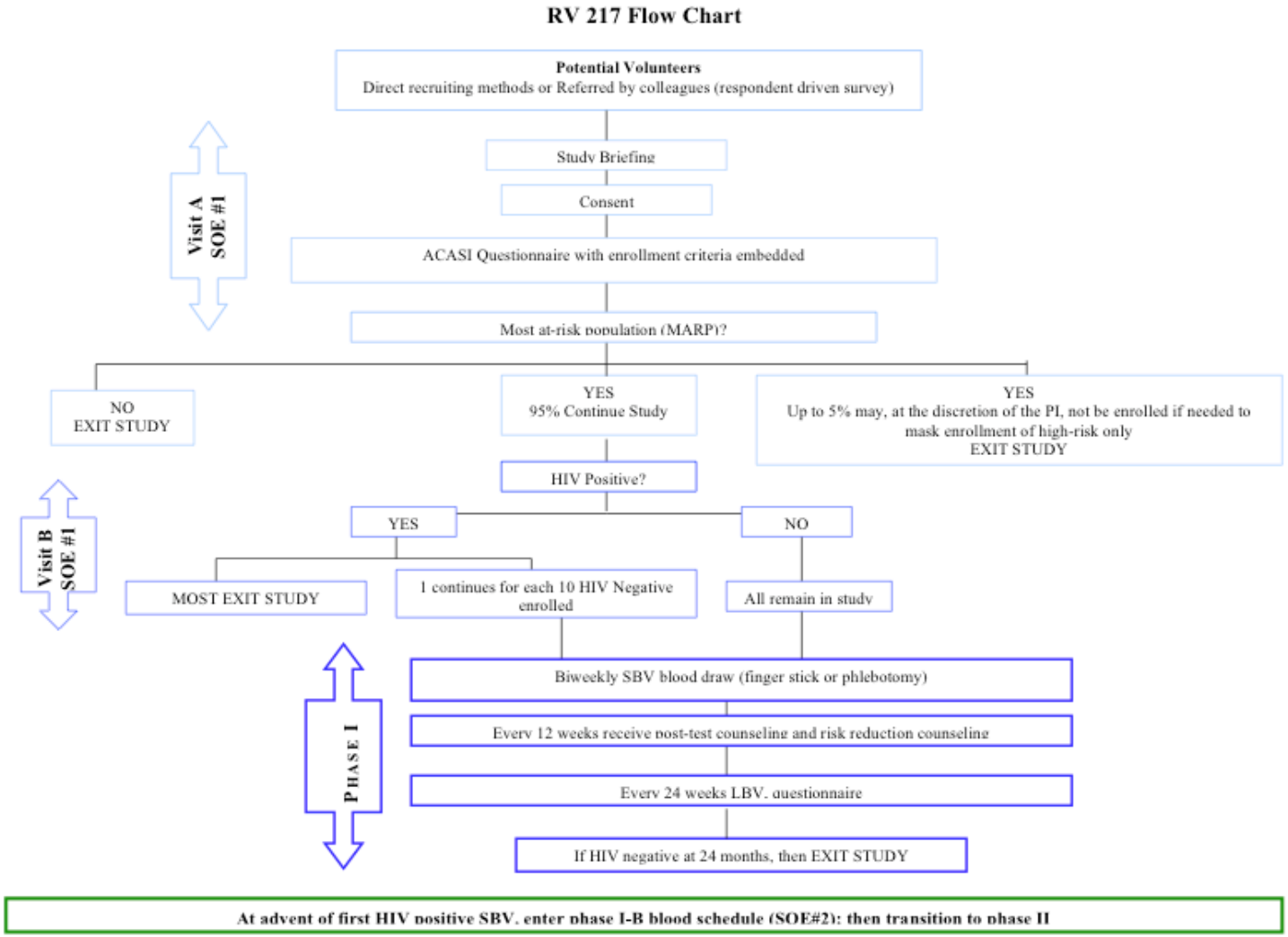
All participants who have a positive test on the RNA assay (as described in Section 9.1) will be transitioned to the IB schedule (SOE #2) as soon as possible and ideally on the day of notification. They will receive LBV twice weekly for one month. At the end of this period, if they are determined to be HIV negative, they will return to the phase I schedule. If they are determined to be infected, they will be offered participation in the phase II schedule as well as the mucosal sample collection arm of phase II. Those phase II volunteers who agree to participate in the mucosal sample collection arm will complete the mucosal specimen collection consent and follow collections as indicated in SOE #3.

Those who agree to participate in phase II will stop all activities in phase I and enter the incident infection cohort phase II (SOE #3). Any HIV incident volunteer who refuses to enter phase II will have an exit visit conducted to insure a diagnosis is established accurately and may continue follow-up in phase I of the study for the remainder of their visits. All HIV incident participants are referred to HIV care and treatment programs regardless of their decision to participate in phase I or phase II of the study.

7.8 Phase II Visits

Those volunteers who are determined to be infected (incident) in phase I are given post-test counseling and the opportunity to enroll in phase II. On the first phase II visit they receive a briefing about the procedures and sign the ICF for phase II. Thereafter, they follow the procedures as described on SOE #3.

Figure 7 RV 217 Flow Chart



7.9 Behavior Data Collection

A standardized questionnaire will be administered to each study volunteer using ACASI. If the volunteer is unwilling or unable to take the questionnaire using the ACASI system, a trained interviewer will administer the questionnaire and record the answers in the ACASI system.

Volunteers will have a choice of taking the ACASI interview in English or the local language. Volunteers needing assistance or having questions during the ACASI interview will be aided by a trained staff member. An SOP and interviewer instruction manual will be available on the site and used for reference and training. The volunteer's identifying information will be dissociated from the completed questionnaire and kept separately in the volunteer's file. The questionnaire will have only the volunteer's study number (but no personal identifiers) on each page.

The questionnaire will seek information on basic social demographic information, including occupation and income, knowledge about vaccines including HIV vaccines, willingness to participate in a trial of preventive HIV vaccine, as well as questions on general health, HIV/AIDS knowledge and behavioral risk factors for HIV. A modified questionnaire will be administered at the follow-up visits. The demographic, general health, HIV knowledge and behavioral risk factor questions have been adapted in large part from: the 2000-2001 Uganda Demographic and Health Survey instruments (UBOS 2001), the 2005 Uganda HIV/AIDS Sero-Behavioral Survey (UHSBS) instruments (MoH 2005) and the 1998 Demographic and Health Survey conducted by the Republic of Kenya National Council for Population and Development instruments (Mwidu 2004).

7.10 Mucosal Specimen Collection

For each site, phase I, at Visit B, forty HIV seronegative volunteers, 20 males and 20 females (transgender participants would not be included) are offered the opportunity to provide a mucosal specimen. For this, they will sign a separate consent form and they will receive extra compensation, as the visit will take longer than regular visits.

Thereafter, all acutely infected individuals who have completed phase Ib (not including transgender participants) will be offered the opportunity to participate in the mucosal sample collection arm. Mucosal specimens (CVL or semen, along with a 3 ml serum collection from females) will be collected as indicated on SOEs #1 and 3 with a +/- 10 day window around each visit.

For every incident case that is identified, one of the 40 HIV seronegative volunteers who provided mucosal samples at Visit B, will be matched to the incident case for age and gender, with mucosal specimens collected at the same time points. In addition, they will continue to be followed in their phase I schedule, including SBV. If any of these become infected, they will be replaced with another of the 40.

CVL and semen will be collected according to the procedures detailed in "The Manual for Collection and Processing of Mucosal Specimens; (AVEG Mucosal Immunology Laboratory, University of Alabama at Birmingham, Alabama; Feb 1999). Further details are included in

Section 4.3.4. In addition to the CVL specimen, female participants will be offered a Papanicolaou smear. Abnormal results will be referred for further evaluation and treatment.

8.0 SPECIMEN COLLECTION, LABELING, HANDLING, STORAGE AND SHIPPING

Trained clinic staff using sterile techniques will collect blood specimens. Up to 67 mls of total blood per visit will be collected by venipuncture for testing in vacutainer tubes as defined in the SOE. SBVs will be collected twice weekly for the first year of phase I and then, if the volunteer so chooses, monthly during the 2nd year of phase I.

8.1 Labeling

On blood collection, all blood tubes will be labeled on site using pre-printed barcode labels with only the study identification number of the volunteer, developed by the clinical data and specimen management system. The study identification number will be bar-coded onto tubes to facilitate tracking and processing of the specimen. On arrival at the site laboratory, all specimens will be logged on a reception database.

8.2 Handling

After collection, all specimens will be transported to the site laboratory according to SOPs. Samples will be packaged in isothermal containers for transportation.

For distal sites, blood samples will be transported in sealed boxes on the day of collection with appropriate packaging to control temperature within limits, per SOP. On arrival at the laboratory, all specimens will be logged into a sample reception database before distribution to the different assay benches for processing.

All blood processing will be undertaken using Good Laboratory Practices (GLP), with protective material including lab coats and gloves. Processing will be conducted using sterile techniques and under laminar flow hoods. All specimens will be labeled with a unique barcode, sample type, date and study visit number and will be indexed and cross-referenced in a specimen-tracking database. The processing lab will be blinded to volunteer identifiers except for the study number to ensure confidentiality.

8.3 Storage

EDTA plasma will be separated from the cells by centrifugation of the lavender top tubes after they have been used for the hematology, CD4 counts and HIV screening. The plasma and packed cells will be aliquoted and stored at –70°C. Serum will be separated from coagulated blood in the red top tubes and aliquoted as per visit requirements. One fraction will be kept at 4°C for HIV, syphilis and HSV, and serum chemistry tests while the remainder will be stored at –70°C. PBMC will be isolated from the adrenocortical dysplasia homolog (mouse) (ACD) yellow top tubes and cryopreserved in aliquots of 2 to 10 million cells per vial and stored in the vapor phase of a liquid nitrogen freezer. ACD plasma will be separated, aliquoted, and stored at

–70°C. All specimens will be labeled with bar code of: study numbers, sample type, date and study visit number and will be indexed and cross-referenced in a specimen-tracking database.

Each study volunteer will be asked to separately, and voluntarily consent to their blood samples to be stored for other research studies that may be done after this study is completed. As stated above, the sample will be labeled with bar code of the volunteer study number that can be linked to their study information. In case the volunteer is unwilling to have their blood samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples for which consent has been obtained and for which additional material is available after study specified testing is complete will be stored for future testing at the site. However, WRAIR IRB and local IRB approval will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB.

8.4 Shipping

All samples for HLA typing and host genetic characterization, viral sequencing, viral isolation, neutralization, and Fiebig staging assays will be shipped to USMHRP laboratories in Rockville, Maryland, United States of America (USA) by dry shipper or on dry ice accompanied with all the required export and import documentation. Alternatively, if technology for the above assays becomes available in one of the USMHRP field laboratories, testing may take place in the local laboratories. It is the intent of the study team to ship only samples as needed to provide uniform scientific data across samples derived from all sites. Some assays will be deployed to the sites for analysis and capacity development of these and other assays will be critical for program sustainability.

All other investigations such as CD4/CD8 count and diagnostic testing will be conducted at the local laboratory.

8.5 Laboratory Testing

Specimens collected for this study will be used for diagnostic testing, exploratory laboratory evaluations corresponding to the study secondary objectives and research assay validation. See the SOE for a breakdown of specific testing to be conducted.

9.0 MANAGEMENT OF SUBJECTS

9.1 HIV Test Results

Prior to the blood draw, pre-test counseling will be provided on an individual basis to each volunteer, as described in section 7.6. Test results will be obtained and recorded and will only be identified by the study number. Written results, only identified by the study number, will be

issued to research study staff. The data linking study number to personal identifiers will be stored on a password-protected database accessible only to the study data management officer.

At the time of specimen collection, the volunteers will be informed of the expected dates for the post-test counseling sessions for giving the HIV results and other clinical results. The goal will always be to provide volunteers with diagnostic data as quickly as feasible. To receive their HIV test results, volunteers must identify themselves to the research staff as outlined in the site-specific addenda. The research study staff will use the volunteer study card or fingerprint to retrieve the results and provide post-test counseling.

All volunteers who test HIV reactive on nucleic acid testing (Aptima) for the first time will enter phase IB (SOE 2) and undergo repeat testing twice weekly for 4 weeks to verify the diagnosis. At the end of phase IB the acutely infected participant will be asked to transition to phase II.

Individuals who test positive for HIV will be counseled about their HIV status and the available options for care before being referred for further medical evaluation. If female and pregnant, volunteers will be referred for services to prevent mother-to-child transmission of HIV. Referral to other health facilities in nearby areas that offer similar services, away from their local community will also be made on request, in order to retain confidentiality and avoid stigma.

9.2 Management of HIV infection

All HIV infected persons identified through the study in Africa will be referred to PEPFAR funded clinics, which are co-located with, or in close proximity of, our research activities. In Thailand, HIV positive participants will be referred for care at the Thai National HIV care and treatment program. More information is provided in the site-specific addenda.

9.3 Positive malaria smear

All smear positive febrile or symptomatic individuals with a history consistent with malaria will be treated with anti-malarial therapy, using appropriate first-line anti-malarials in accordance with the host country's Ministry of Health guidelines; and referred to the health center personnel for follow-up. The clinical study staff will be notified of all volunteers with positive malaria smears to insure that treatment was provided. Malaria smears will be conducted in Africa only.

9.4 Management of STIs

All individuals will be tested for syphilis and if infected, the volunteer will be treated according to the national standards of care in the host country either by the study site or referred for local care in settings where available. All individuals with clinical STIs will be treated according to the national standards of care of their host country (see site-specific protocol for details). Volunteers with chronic and/or severe illness will be referred to the local hospital for further evaluation, treatment and care.

Volunteers testing positive for any STIs, including HIV will be advised and encouraged (through health education) to take their sexual partners to get tested. Such sexual partners of study volunteer who are not participants in this study will be assisted with appropriate referral.

9.5 Other/Opportunistic Infections

Volunteers who are diagnosed with other chronic infections will be referred to a PEPFAR-funded care and treatment facility or to an appropriate medical facility for care.

9.6 Management of Other Medical Conditions

Volunteers will consult study staff for treatment and/or referral to the appropriate health facility during the study period.

9.7 Unanticipated Events and Social Harms Reporting

Unanticipated events and social harms may occur during the course of the study. When such events are related to study participation, the host country and WRAIR IRB will be informed. The study staff, informed of these events, will inform the PI or his/her designee. The PI or designee will then prepare a narrative summary of the event and report to the IRBs and medical monitor.

All unanticipated problems related to the study and involving risk to subjects or others and all subject deaths should be promptly reported by phone (+1 301-319-9940) or by facsimile (+1 301-319-9961) to the WRAIR IRB. A complete written report should follow the initial notification. In addition to the methods above, the complete report will be sent to the Director, Division of Human Subjects Protection (DHSP), Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910-7500.

9.8 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) is seldom employed for monitoring of an observational study. This is because there is no need for an unblinded group to compare safety, toxicity and efficacy data between two or more study arms receiving experimental interventions or controls. It is important to recognize that the IRBs would serve as the logical review point for safety concerns arising in the conduct of RV 217 as there is no blinded data and this group is empowered to review this subject area on behalf of the participants and as an oversight element for the investigative team.

9.9 Follow-Up

Participants enrolled in Phase I will be followed for a period of approximately 24 months after screening. HIV incident volunteers identified at any follow-up visit will be invited to participate in phase II to intensively characterize viral dynamics, host immune response and potential impact of host genetics over a period of at least 50 months beyond the time that they enter phase II. All individuals recruited, whether HIV positive, negative, or a new HIV diagnosis, will be seen and undergo the same study procedures at each visit.

9.10 Termination Of Study Participation

Volunteers are free to withdraw from the study at any point without prejudice. A volunteer's study participation may be terminated for various reasons, to include:

1. Sponsor terminates the study for unforeseen reason. All participants will be informed of this possibility in the consent process.

2. Lost to follow-up (LFU)/non-compliance to visit schedule: every three months, a volunteer's participation is evaluated. If more than two clinic visits have been missed, and greater than 75% of the SBVs have been missed the participant will be considered LFU and no further tracking will be done. Volunteers with a single missed clinic visit and/or more than 50% missed SBV will be counseled and evaluated for consideration of continued participation. The PI may exercise some discretion in assessing compliance as the intensity of surveillance is not consistent with some activities of daily life to include vacations, family and personal medical leaves, etc.

3. Refused further participation:

Refusal of all study activities: volunteer decides not to continue

Partial refusal of study activities, e.g. refuses SBV but willing to continue LBV.

The "lost to follow-up" and "refused further participation" group may be re-contacted to the extent the volunteer permits and may resume follow-up should obstacles or other issues resolve. Participants who refuse SBV will be terminated for non-compliance and will be replaced as noted above.

4. Withdrawal of Consent: volunteer actively withdraws consent, either in writing, in person, or by phone. When a volunteer indicates to the investigator that they are withdrawing consent, the communication should be recorded in the volunteer source documents (documented per SOP). It will be understood that no further interaction by the study team will occur except to provide the volunteer with any IRB-directed information related to participant safety. The "withdrawal of consent" (WDC) group will not resume follow-up. The volunteer's samples and data accrued during participation prior to "withdrawal of consent" will be retained and used only for the analyses approved under the protocol. No samples will be retained for "future use" and no additional data or samples will be collected.

10.0 COMPENSATION OF VOLUNTEERS

In accordance with common practice of the local sites, there may be compensation for lost time, travel expenses and inconvenience, for each scheduled visit, including the mucosal collection visits. The amount of compensation will vary according to visit to account for the differential lost time and inconvenience of longer visits (LBV and initial visit A and B) compared to shorter visits (SBV). For sessions to collect test results, volunteers will be compensated with half the amount compensated for a clinic visit. For those who have moved further away from the site, there may be indirect costs over and above the compensation (e.g. transportation, lodging if necessary) to accommodate study follow-up and retention.

Any applicable guidelines by IRBs/ERCs (ethical review committees) for compensation will be sought and followed and described in the site-specific addenda.

11.0 LANGUAGE

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood. Accordingly, the consent and all other written materials will be translated into the site-specific local language in addition to English.

12.0 DATA MANAGEMENT AND ANALYSIS

12.1 Hardware and Software

Data will be entered on secure computers with access limited by individual usernames and passwords. Data will be stored using a system that is limited by a firewall with access available only to authorized users physically logging on from authorized internet protocol (IP) addresses. Data analysis will be performed using SAS® software (version 8.0 or higher).

The Data Coordinating and Analysis Center (DCAC) is in the process of migrating its data management operations to a new version of a proprietary system called ClinworX that offers the capability for data entry by study sites, if technological requirements for bandwith and security can be met. For this study, CRFs will be maintained and data entry will be conducted at the clinical sites unless technological or other issues preclude this option. If data entry by any site(s) is not feasible, then CRFs (either in hard copy or electronic images) will be sent to DCAC for entry, until such time as those requirements are met.

Study subjects will complete a risk behavior questionnaire on a microcomputer using ACASI software. The questionnaire will be offered in English and in local languages as approved by local IRBs. Each question and response option is visible on the screen as well as read aloud using the audio portion of the software. Subjects use headphones to listen to the questions and response options. Responses are obtained using touch screens. Subjects may decline to use the computer-administered questionnaire. If this happens, a trained interviewer will read the questions and response options to the subject and record the answers in the ACASI software.

ACASI is being offered in this study for several reasons: to reduce site staff and data entry time and potential errors in recording subject responses, and to provide a consistent administration of the questionnaire. For example, skip patterns can be programmed into the software, and individual interviewer biases can be eliminated by using a recorded voice. In addition, there is evidence in the literature that subjects being interviewed about high-risk behavior are more likely to give truthful answers to a computer interview than to a human interviewer. Subjects may feel that the administration of the questionnaire using headphones and computer is more private than a traditional interviewer-based approach. Subjects may also gain experience using computers that gives them a more positive attitude towards study participation.

12.2 Data Entry

Study site staff or data entry personnel at DCAC will perform double data entry of case report forms (CRFs) into a relational database. DCAC will not receive any fingerprints or personal identifiers such as name, national identification number (if applicable), or house number. Identifying information will be kept locally at each site in a secure database. The local database will include a master file containing personal identifiers such as name, national identification number (if applicable), and house number. This file with identifiers will only be accessible by the data management supervisor (DMS) and data management officers (DMO) or designee through password access. During all data entries and subsequent data and laboratory analysis,

only the study number will be used as the identifier. All volunteer folders will be stored in locked filing cabinets and/or rooms accessed only by DMS, DMO or designee.

It is the policy of DCAC and each site data management team to limit availability of personal identifying information to only those individuals with a requirement for the information. All research data and samples are only managed using the coded study number when used for research activities including data or samples analysis. Personal identifying information and links to the study number are limited to those clinical staff who work directly with the participants and must have this information to fulfill their role in the conduct of the study.

12.3 Data Entry of Other Laboratory Assay Results Not Conducted On Site

The results of other specialized laboratory assays not immediately available will be maintained by the individual investigators and merged, as needed, with the analysis file.

12.4 Data Storage and Monitoring

All source documents such as contact information forms and laboratory record sheets will be maintained at each participating site. CRFs will also be maintained at each study site. If CRFs must be transmitted to DCAC for data entry, they will be sent either via hard copy or electronic image on a regular basis. Paper CRFs will be stored for 5 years post study completion (database lock). Data will be stored on dedicated servers (real-time) and tapes/CD-RWs (weekly). Tapes/CD-RW backups will be stored in a secure fireproof cabinet at the on-site storage, and on a duplicate tape/CD-RW set at an off site location (weekly).

All aspects of the study will be carefully monitored by the sponsor or authorized representatives of the sponsor, with respect to current Good Clinical Practice (GCP) and SOPs for compliance with applicable government regulations. These individuals will have access, both during the trial and after trial completion, to review and audit all records necessary to ensure integrity of the data, and will periodically review progress of the study with the principal investigator.

Case report form (CRF) data obtained in the conduct of this study will be housed in a secure database maintained by DCAC. These data will be entered in a secure database, using a double-data entry process, with standardized quality assurance review procedures.

DCAC serves as the central data management facility for USMHRP research protocols. Data managed by DCAC are entered into and maintained in a password-protected database. Data are accessible only to authorized users, including appropriate site study staff and those DCAC and Information Technology staff authorized to work on the protocol. The database is located at the Rockville campus of the USMHRP and is protected by a firewall and a wide range of other security measures.

This data do not contain participant names or Social Security Numbers (or any equivalent national identifiers) but is referenced only by the study number.

Questionnaire data and some other data such as laboratory research results will be reported to DCAC electronically, identified only by the study number.

12.5 Data Analysis

Data analysis will be performed using SAS® version 8.0 or higher. DCAC in Rockville, MD, USA will support the analyses.

All datasets (without the personal identifiers) are stored individually and merged as needed for specific analyses. These datasets will only be made available to personnel performing analyses.

Analysis files are created on a periodic basis and made available to the PI. Data may be made available as a listing, external file, or through a query program.

13.0 ETHICAL CONSIDERATIONS

13.1 Risks

The proposed study is unique in several respects. It is the only study with a concerted effort to acquire most HIV incident infections in Fiebig stages I/II. Volunteers will need to briefly encounter the research team on a very frequent basis. This raises the specter of stigmatization and interference with work. Aside from monetary compensation, the rewards for this investment by the volunteer are primarily altruistic as they contribute to our understanding of HIV disease and transmission pathogenesis.

Frequent surveillance visits for uninfected participants and the intense, clinic based visits for those who become infected raise a potential risk to stigmatize the participant. To mitigate this risk, we will follow both HIV infected and uninfected participants so the volunteers and their peers will not be able to determine or assess HIV status based upon the schedule of activities an individual or group undertake. To minimize risk in the work place, visits may be conducted using mobile platforms, vehicles or rented establishments, convenient to the participants at their homes and/or work place and all surveillance visits in these locations will be extremely brief to minimize disruption of the volunteer's normal schedule. In some sites, the research clinic is sufficiently convenient and non-stigmatizing an environment to permit all study related visits. Details are provided in the site-specific addendum. Prior to initiating the proposed studies, we will conduct focus group discussions with potential participants, employers, and key opinion leaders (inclusive of CABs) to a) inform them of the studies goals and value to optimization of vaccines and therapies for HIV and b) identify means of minimizing volunteer harms.

Stigma is the greatest risk for participants but also, simple disruption of work and other activities of daily living must be considered. As noted above, every effort will be made to make the surveillance encounters both brief and convenient to minimize this concern without engendering a further risk of stigmatization.

Although the forgoing represent the greatest potential harms, the intense phlebotomy schedule among the AHI participants is also a potential hazard. Blood volumes will be reduced by 50% if a participant's hemoglobin (Hgb) at entry into the AHI phase is under 10.5 and will be suspended if the Hgb is under 9.5. Anemic volunteers will be offered iron supplementation. At no time will the standards of the American Red Cross of 450 mL limit per 56 days be exceeded. Site study physicians will also assess overall health prior to phlebotomy and may reduce blood volumes as clinical circumstances require. All women who become pregnant during participation will be carefully evaluated by the PI to determine if they may continue participation with SBV and LBV per protocol. They will have a reduction of LBV to only samples needed for HIV status determination and SBV may be deleted if Hgb is less than 10.5.

Many consider the advent of an HIV diagnosis a social harm in the context of reactive depression, potential family disruption and work place stigmatization. The field teams employ trained counselors who have experience with post-test counseling of newly infected individuals through other cohort development studies conducted in each of these countries. Although harms may nevertheless occur, every effort will be made to minimize these. A benefit to volunteers

who are diagnosed with HIV through their participation in this study will be ready access to a care and treatment program, funded by the United States Government (USG) through PEPFAR in Africa and the Thai government in Thailand, and empowerment through knowledge of their diagnosis to avail themselves of prevention interventions to reduce risk to sex partners and, if they become pregnant, maternal-child prevention measures. It must be recognized that the era of early HIV infection is one that poses great risk for HIV transmission.

13.2 Post-exposure prophylaxis (PEP), Pre-exposure prophylaxis (PrEP) and early initiation of HAART

The current US recommendation for individuals seeking care within 72 hours of a sexual or needle sharing exposure with a known HIV positive person is to provide HAART for 28 days. (Smith 2005). WHO PEP recommendations do not include chronically exposed individuals (Joint WHO/ILO PEP Guidelines). The high-risk participants in this study may not know the HIV status of their partners, endure multiple high-risk exposures with partners of unknown status and would not be appropriately placed on HAART for the purpose of post-exposure prophylaxis in ordinary circumstances according to either the US or WHO guidelines. Should a volunteer present with a known exposure to an HIV positive person which meets the standard for intervention as defined in these guidelines, PEP will be discussed (as appropriate) with the volunteer and sought via PEPFAR or other resources.

More applicable is the use of PrEP in these high-risk groups. Currently, PrEP is the subject of several clinical trials. Should these interventions prove safe and effective they will be promptly incorporated in the proposed studies clinical management of these volunteers. The study would require re-design and re-consent in this circumstance as one may expect fewer observed infections. It is recognized that highly effective PrEP may well reduce transmission rates in high-risk populations to such an extent that the proposed study is no longer feasible.

Current US guidelines for treatment of HIV infected persons do not recommend routine use of HAART in acutely infected individuals but rather suggest that it be considered optional based upon expert opinion and enrollment in a clinical trial should be considered (Panel on Antiretroviral Guidelines for Adults and Adolescents). The WHO recommendations on HIV treatment have no comment upon this subject (WHO 2006). Nevertheless, many US and European physicians offer HAART in the setting of acute infection. As summarized recently (Hirsch 2008), the clinical data to support this approach is inconclusive, but there is a theoretical basis for this intervention. This is based upon evidence of substantial injury to the immune system occurring within days to weeks of initial infection and the possibility of very early use of HAART could durably influence the long-term course of disease. Thus far, most studies with HAART intervention in acute infection are acquiring participants after the peak of viremia and may therefore have little opportunity to modify the critical early events of acute infection. In addition, many studies are uncontrolled. In general, those studies, which have evaluated either a short course of HAART or repeated treatment interruptions following initial HAART among acutely infected individuals, have not found sustained benefit. (Hecht 2006; Streek 2006; Hoen 2005) Care providers in the developed world who offer HAART in the acute infection setting typically commit patients to sustained therapy.

In the developing world, the current standard of care initiates therapy based upon symptoms and CD4+ T cell count criteria. The limited availability of second line agents to cope with resistance and the costs associated with life long therapy are considerations which thus far have compelled the national bodies responsible for treatment guideline policy to adopt a more conservative approach. Diagnosis of acute infection in the developing world is a challenge due to the limited availability of nucleic acid testing. Consequently, there is virtually no experience with HAART administered during acute infection. Currently, other studies conducted in acutely infected participants in Africa and China are not routinely providing access to HAART (Streek 2006, Myron Cohen, personal communication).

Ongoing research in this arena may provide compelling data to alter the treatment practice at the sites of the proposed study. The RV 217 team would welcome such an advance and facilitate provision the new paradigm to our participant population as quickly as possible and accordingly alter the study design and goals. Although some study objectives might become infeasible, those most novel questions focusing upon early peak viremia and/or eclipse phase provirus and outcome would be preserved. At this point, the feasibility of capturing individuals in the earliest stages of acute infection is not known. Should RV 217 prove that an appreciable number of volunteers in Fiebig stages 1-3 can be acquired, the opportunity to evaluate either drug or immune based interventions in these populations would be pursued.

The current observational study design is ethically defensible only in the circumstance that the available clinical data fail to provide a convincing basis for adopting early highly active antiretroviral treatment in the setting of acute HIV infection. Similarly, pre-exposure prophylaxis (PrEP), which is under active clinical investigation, may impact on the ethical considerations of conducting RV 217 as currently designed as the population enrolled in RV 217 would be candidates for PrEP. In the settings contemplated for RV 217, a paradigm shift to initiating either PrEP or early HAART in acute infection may be difficult to implement. Nevertheless, it is anticipated that research activities would be suspended to permit integration of these interventions into the study design with a likely alteration in objectives.

The well being of participants is the foremost priority of the RV 217 investigators. There is no extant clinical evidence to inform the routine use of HAART in acutely infected individuals, nor to use PEP or PrEP, in MARPs who will participate in RV 217. Ongoing dialogue with community advisory boards (CABs), national regulatory authorities, ethical review boards and key opinion leaders will guide the study team.

13.3 Benefits

Although study volunteers may benefit from clinical testing and physical examination, management of STIs and malaria, health education, HIV counseling and reproductive health counseling, they may receive no direct benefit from participation.

13.4 Community Advisory Board

CABs at each site will serve as a link between the research team and the community, and therefore be able to provide a feedback to the research team on issues about the study that might be of concern to the volunteers or the community.

13.5 Informed Consent

Informed consent will be obtained from each volunteer before enrollment in the study. The Consent Form will be used as the informed consent document for adult volunteers. Consent forms will be available in both English and site-specific local languages, and can be found as attachments to the site-specific protocols. Volunteers may take as much time as needed to decide if he/she wants to participate. A copy of the protocol, proposed informed consent form, other written participant information, and any proposed advertising material will be submitted to the appropriate ethical and scientific review committees in each country where enrollment will occur. In addition, the protocol will undergo review and approval by the country-specific IRBs and the WRAIR IRB as part of the U.S. Medical Research and Materiel Command.

The investigator must submit and, where necessary, obtain approval from the country-specific IRBs and the WRAIR IRB for all subsequent protocol amendments and changes to the informed consent document.

Volunteers may withdraw from the study at any time point. In most cases, volunteers simply disappear (loss to follow-up) or express a desire to discontinue participation. Rarely, a volunteer will not only stop participation but also explicitly withdraw consent. We intend to work cooperatively with volunteers and support their participation constructively with a view to their well-being and respectful of their autonomy. We do not expect withdrawal of consent will occur or will occur only rarely. We use the term "withdrawal of consent" to indicate a declaration by the volunteer that no further interaction with the study team is permitted. Only data and samples already obtained will be analyzed according to protocol but no additional data or samples will be collected. The study team will engage in no further communication with the volunteer except as directed by an IRB on behalf of participant safety. The study team will not utilize samples or data from this volunteer for any future use and will discard residual samples when the study is completed. When a volunteer indicates to the investigator that they are withdrawing consent, the investigator or staff member will insure that the communication should be recorded in the volunteer source documents.

13.6 Volunteer Confidentiality

Volunteers will be assigned a study number that will be used as a personal identifier for volunteer identification. For further identification each volunteer will be issued a Study-ID-card that contains either a photo, or a number that links the study ID card to his or her photo ID card. No card will be prepared if the site adopts the finger print identification system, and no name will appear on it. Further details of volunteer identification can be found in the site-specific addenda.

A database of the volunteer name and study number will be only accessible to the study coordinator and the local PI. The data management personnel will prepare barcode labels that will be fixed on data record/collection forms with only the contact information form bearing identifying information for use by the field staff in data and blood collection. Contact information may be collected from the volunteer at either study visit A or B. Other than the contact information form, the rest of the data record/collection forms will not bear personal identifiers but only the study number. HIV testing will be performed on samples that are identified only by study number. Every effort will be made to maintain confidentiality of records within the limits of the law. All data and medical information obtained about volunteers as individuals will be considered privileged and held in confidence. Research and clinical information relating to volunteers will be shared with other investigators and the scientific community through presentation or publication; however, volunteers will NOT be identified by name or other personal identifying information. Further, all study personnel will undergo training on various aspects of the study, including ethics in human research studies, and the need and importance of protecting the confidentiality of the participants.

Representatives of the USMHRP, DoD, USAMRMC, local IRBs/and or Ethical Committees (ECs), NIAID and the Office for Human Research Protections (OHRP) are eligible to review records from this study as part of their responsibility to protect human subjects in research.

13.7 Participation of Children

Healthy children may be involved in research when the study is determined to be "no greater than minimal risk" (45 CFR 46, subpart D, 401-409). No site plans to enroll minors.

13.8 Management of Vulnerable Volunteers

In the event that the status of an enrolled volunteer changes during the course of their enrollment in the study and that the volunteer's ability to exercise free choice could be limited in some way, the volunteer is recognized as a vulnerable participant. A vulnerable volunteer is any individual whose willingness to volunteer in a clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation; or of a retaliatory response from senior members of a hierarchy in case of refusal to participate.

The volunteer that is likely to be vulnerable to coercion or undue influence might include individuals such as minors, pregnant women, prisoners, soldiers, the physically handicapped, or mentally incompetent persons. Other vulnerable volunteers could include persons in an emergency situation like refugees, persons living on streets, and very sick persons who are incapable of giving consent or providing continuing consent.

If a change in status of a volunteer already enrolled in the study should occur, it is the responsibility of the investigators to assure that appropriate safeguards are in place to protect the rights, safety and welfare of all study subjects. The principal investigator shall notify all IRBs and/or ECs associated with this study in the continuing review report (CRR). The IRB/EC must decide what types of special protections are required and provide direction to the investigator.

Participation of prisoners is not planned and any volunteer will be suspended from study visits while incarcerated. The IRB will be notified of the period of incarceration.

14.0 POLICY REGARDING RESEARCH-RELATED INJURIES

Short-term medical care for any injury resulting from participation in this research will be provided by the project. The U.S. Federal Government will not provide long-term (over 6 months) medical care or financial compensation for research-related injuries.

15.0 PROTOCOL DEVIATION REPORTING

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

The timeline for reporting protocol deviations to the Division of Human Subjects Protection/ WRAIR IRB is determined by the categorization of the deviation: (1) emergent/significant or (2) non-emergent/minor. Unanticipated problems should be reported in the appropriate timeframe according to the seriousness of the event as a significant deviation or a minor deviation.

Emergent/significant deviations are departures from protocol that have a significant impact on the welfare or safety of a volunteer or on the integrity of the study data and will be reported within 10 working days to the DHSP/WRAIR IRB to the following address wrairdhsp@amedd.army.mil. Example: providing the wrong lab result to a volunteer. Such deviation reports may be initiated without prior IRB/ ethical review committee (ERC) approval, only in cases where the change (s) is /are necessary to eliminate an immediate apparent hazard.

Non-emergent/minor deviations are routine departures that typically involve a volunteer's failure to comply with the protocol. Examples: missing scheduled visits; failing to complete required questionnaire. Minor deviations will be reported to the sponsor and the Division of Human Subjects Protection/IRB in a summary report with the annual continuing review report.

A cumulative deviation report will be submitted to the DHSP/WRAIR IRB with each protocol continuing review report or with the closeout report, whichever comes first.

16.0 PROTOCOL MODIFICATIONS

Any amendments to the protocol, consent form and/or questionnaires, including a change to the principal investigator PI, must be submitted to the WRAIR IRB and local IRB for review and approval. Any change or amendment to the protocol affecting study volunteers, study objectives, study design, study procedures, or significant administrative aspects will require a formal amendment to the protocol. The protocol must be revised to concur with the amendment. Such amendment will be submitted to the WRAIR IRB and local IRBs for review and approval.

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

Administrative changes to the protocol are corrections and/or clarifications that have no effect on the way the study is to be conducted. Such administrative changes will be submitted to both the WRAIR IRB and local IRB/ERCs for review and approval prior to implementation.

17.0 CONTINUING REVIEWS /CLOSEOUT REPORT

A CRR will be submitted to all ERCs/IRBs prior to the anniversary date determined at initial IRB review. If the continuing review is not approved by the local ERC/IRB and WRAIR IRB by the anniversary date, all protocol activities must stop at that site until such time as the approval is obtained. A copy of the approved CRR and local IRB approval notifications will be submitted to the WRAIR DHSP as soon as these documents become available. A copy of the approved closeout report and local IRB approval notifications will be submitted to the WRAIR DHSP as soon as these documents become available.

18.0 STRATEGIES FOR IMPROVING ENROLLMENT AND COHORT RETENTION

A number of strategies are planned in order to enhance recruitment into the cohort and retention of volunteers over time. Recruitment methods and activities are directed at each group separately to minimize stigma risks and for operational efficiency. These are outlined in the site-specific addenda.

In accordance with the protocol objective of defining retention, and to look at issues regarding participation and retention, data will be collected on the outcome of tracking activities. This data will include type of discontinuation (withdrawal or lost to follow-up), and reasons for discontinuation (such as family, job, prison, relocation, invalid contact information, etc).

19.0 RESOURCES AND COORDINATION

This study is funded and sponsored by USMHRP and DAIDS and coordinated locally by MUWRP, USAMRU-K, MMRP and AFRIMS.

Laboratory: Each study site has an established laboratory that is capable of handling most of the tests required. Quality assurance programs will be conducted consistent with the College of American Pathology Standards prior to study initiation. Every site has established a quality assurance (QA) program, which will allow its laboratory to comply with GLP standards of the U.S. FDA.

Staff: The research team to implement this study at each of the participating countries will include the country-specific Principal Investigator who will be responsible for implementation of this study protocol in their country and timely fulfillment of all study activities. The site-specific PI will work together with the co-investigators listed in this protocol to oversee the successful implementation of this study in his/her country.

20.0 USE OF INFORMATION AND PUBLICATION

It is expected that data from this study will be reported in both scientific journals and international scientific meetings. Confidentiality of subjects will be maintained by the fact that no individual results will be reported or published, only group/aggregate results. All research data will be identified by the study number. The linkage between personal identifiers and study number will only be available in a confidential database at the respective sites. The local health authorities will be informed of all scientific outcomes of the study and general prevalence and incidence data however, confidentiality will be maintained, and participant identities will not be released. Only aggregate information will be released. All publications resulting from this study will be cleared through the collaborating partners to this study.

WRAIR recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. Any results of medical investigations and or publication/lecture/manuscripts based thereon, shall be exchanged and discussed by the investigator, the sponsor representative(s) and the U.S. Army Medical Research and Materiel Command 60 days prior to submission for publication or presentation.

Results from investigations shall not be made available to any third party by the investigating team outside the publication procedure as outlined previously. WRAIR will not quote from publications by investigators in its scientific information and/or promotional material without full acknowledgment of the source (i.e., author and reference). All publications written by WRAIR investigators must be reviewed and approved by WRAIR Office of Research Technology and Applications (ORTA).

21.0 CONDUCT OF THE RESEARCH STUDY

This research study will be conducted in accordance with GCP and the revised CFR. Additionally, it will follow the local site guidelines for the conduct of health research involving human subjects. Copies of all the above documents and any other information and/or guidelines that are applicable for the safe and legal conduct of the study will be available at each clinical site.

22.0 STATEMENT REGARDING POTENTIAL CONFLICT OF FINANCIAL INTEREST

The Principal Investigators and the Co-Investigators have no financial interest in any component of this study.

23.0 SIGNATURE OF PRINCIPAL INVESTIGATORS

I will perform the foregoing protocol as written in the above-described Study Protocol

29/MARCH/2009

Overall Study Director, RV 217
Site Principal Investigator, Rockville

Date (dd/mm/yyyy)

30 MARCH 2009

Site Principal Investigator, Kampala, RV 217a

Date (dd/mm/yyyy)

26 MARCH 2009

Site Principal Investigator, Kericho, RV 217b

Date (dd/mm/yyyy)

30/MARCH/2009.

Site Principal Investigator, Mbeya, RV 217c

Date (dd/mm/yyyy)

29 March 2009

Site Principal Investigator, Thailand, RV 217d

Date (dd/mm/yyyy)

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APPENDIX I: SITE SPECIFIC ADDENDUM

APPENDIX II: TEST OF UNDERSTANDING

US MILITARY HIV RESEARCH PROGRAM

RV217 TEST OF UNDERSTANDING FOR STUDY VOLUNTEER- ENGLISH VERSION

Volunteer Study #: | | | | | | |

Date: ____/ _____/ _____
dd mon yyyy

Answer the following with "True" or "False"

(Please check √)

1.	Taking part in the study is voluntary.	**True**		**False**	
2.	Once you have consented to take part in this study, you are not free to withdraw at any time.	**True**		**False**	
3.	With your permission, a portion of your blood sample will be stored for future HIV related studies	**True**		**False**	
4.	The study will provide counseling, information and support if you are found to be HIV infected.	**True**		**False**	
5.	The expected length of participation in this study is approximately 24 months.	**True**		**False**	
6.	Your information and test results will be provided to your family and friends	**True**		**False**	
7.	You will be asked questions about your sex life and risk factors for HIV infection	**True**		**False**	
8.	You will be expected to pay for all laboratory tests in this study	**True**		**False**	
9.	Some of your specimens will be sent to a laboratory in the United States for testing	**True**		**False**	
10.	Volunteers who become HIV infected during the study will be offered enrollment in another part of the study that will better characterize the virus and infection	**True**		**False**	

Volunteer Study #:						

RESULTS

Test number	No. correct out of 10
# 1	
# 2	
# 3	

REMARKS:

_____

_____

_____

_____

_____

_____

INTERVIEWER NAME: _____

DATE: _____/ _____/ _____
dd mon yyyy

APPENDIX III: TEST OF UNDERSTANDING ANSWERS (INTERNAL USE)

US MILITARY HIV RESEARCH PROGRAM

ANSWERS TO RV217 TEST OF UNDERSTANDING FOR STUDY VOLUNTEERS

About this study

1.	Taking part in the study is voluntary.	**True**	☒	**False**	☐
2.	Once you have consented to take part in this study, you are not free to withdraw at any time.	**True**	☐	**False**	☒
3.	With your permission, a portion of your blood sample will be stored for future HIV related studies	**True**	☒	**False**	☐
4.	The study will provide counseling, information and support if you are found to be HIV infected.	**True**	☒	**False**	☐
5.	The expected duration of participation in this study is approximately 24 months.	**True**	☒	**False**	☐
6.	Your information and test results will be provided to your family and friends	**True**	☐	**False**	☒
7.	I will be asked questions about my sex life and risk factors for HIV infection	**True**	☒	**False**	☐
8.	You will be expected to pay for all laboratory tests in this study	**True**	☐	**False**	☒
9.	Some of the specimens will be sent to a laboratory in the United States for testing	**True**	☒	**False**	☐
10.	Volunteers who become HIV infected during the study will be offered enrollment in another part of the study that will better characterize the HIV virus and infection	**True**	☒	**False**	☐

APPENDIX IV: SCHEDULE OF EVALUATIONS #1, PHASE I

SOE #1: Basic Surveillance (Phase I)

VISIT		A[1]	B[2]	C	D	E	F	G	H	I	J	Exit Visit[3]
WEEK OF STUDY		0	4	12	24	36	48	60	72	84	96	
DAY OF STUDY		0	28	86	168	252	336	420	504	588	672	
Clinical												
Informed Consent		X										
Test of Understanding		X										
Photo ID or Fingerprint ID		check	check	check	check	check	check	check	check	check	check	check
Questionnaire		X	X[4]		X		X		X		X	X
HIV Counseling		X	X[5]	X	X	X	X	X	X	X	X[6]	X
Capillary Blood Tube Collection (bi-weekly)			X[7]									
Medical and Physical Exam[8]		X										X
Laboratory[8]												
HIV Diagnostics	Anti-Coagulant	10 mL	10 mL		10 ml		10 ml		10 ml		10 ml	10 mL
CBC, differential, platelets	Anti-Coagulant	2.7 mL	NB[9]		NB		NB		NB		NB	NB
Serum Chemistry (Creatinine, ALT)	Coagulant	2.7 mL										
RNA PCR	Anti-Coagulant	6 mL	6 mL		6 mL		6 mL		6 mL		6 mL	
Research												
Syphilis(RPR/TPPA)/Malaria/Eosinophils/HSV	Anti-Coagulant	NB	2.7 mL		2.7 mL		2.7 mL		2.7 mL		2.7 mL	NB
Hepatitis B	Anti-Coagulant	NB										
LPS	Anti-Coagulant	NB	NB		NB		NB		NB		NB	NB
Mucosal Collections[9]			X									
Hormone levels[10]	Coagulant		2.7 mL									
Plasma/PBMC for Immunoassays	Anti-Coagulant	45 mL										
Host/Viral Genetics and GWAS	Anti-Coagulant	NB	NB		NB							
Viral Isolation			7.5 mL									NB
PBMC for Gene Expression	Anti-Coagulant	NB										
T Cell Immunophenotyping		NB			NB				NB			
Vector Antibody Testing		NB										
Pre-Infection Control-Reposed	Anti-Coagulant		7.5 mL		7.5 mL		7.5 mL		7.5 mL		7.5 mL	
Daily Volume (mL)		**66.4**	**36.4**	**NB**	**26.2**	**NB**	**26.2**	**NB**	**26.2**	**NB**	**26.2**	**10**
Microvette Blood Volume (mL)		0	0	9.6	14.4	14.4	14.4	14.4	14.4	14.4	14.4	
Cumulative Volume Total (mL)		66.4	102.8	112.4	153	167.4	208	222.4	263	277.4	318	328
Specimen Type												
EDTA plasma (1 mL/vial)		7	11		11		11		11		11	5
EDTA cells (5M/vial)		3	5		5		5		5		5	1
ACD plasma (1 mL/vial)		23	5									
ACD cells (10M/vial)		4	1									
Serum (0.5 ml/vial)		2										
CVL/Semen (0.4 mL/0.2 mL)			5/5									
EDTA whole blood (1 mL/vial)		2	2		2		2		2		2	

[1] Visit A may be conducted over two days within a 7-day window.
[2] Visit B may occur within 7 – 21 days of Visit A.
[3] Exit visit is for individuals who have had an LBV in the last six weeks, are not able to continue the study, but who do not want to withdraw consent,
[4] Visit B only the vaccine knowledge and interest portion of the ACASI questionnaire is collected, if not collected at Visit A.
[5] At Visit B volunteers who may be infected receive counseling and the complete Visit B blood draw, and are asked to return for final results.
[6] HIV surveillance is halted for those volunteers who remain HIV negative at visit J (final visit).
[7] Capillary blood tube collection will be collected via capillary puncture or venipuncture on a bi-weekly basis for one year.
[8] Visit A collect a full medical history and physical exam; at all other visits update medical history and directed physical exam.
[9] NB = no extra blood required.
[8]Blood volumes are actual volumes drawn into tubes.
[9]Mucosal collections done on 40 volunteers at each site at day 28 for assay quality control evaluation. After that, mucosal collections will vary by site and incident infection number.
[10] Only females participating as negative controls for mucosal component.

APPENDIX V: SCHEDULE OF EVALUATIONS #2, PHASE IB

SOE #2: Stutter Phase (Phase IB)

VISIT		1	2	3	4	5	6	7	8	9
WEEK OF STUDY		0	.5	1	1.5	2	2.5	3	3.5	4
DAY OF STUDY		0	3	7	10	14	17	21	24	28
Clinical										
Informed Consent										
Photo ID		check	check	check	check	check	check	check	check	check
Medical and Physical Exam[1]		X	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]
Laboratory										
CBC, differential, platelets/T cell phenotypes	Anti-Coagulant	2.7 mL				NB [2]				NB
Serum Chemistry (Creatinine, ALT)	Coagulant	2.7 mL								
RNA PCR	Anti-Coagulant	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
HIV Diagnostics	Anti-Coagulant	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL
Research										
Hepatitis B	Anti- Coagulant	NB								
Syphilis(RPR/TPPA)/Malaria//Eosinophils/HSV	Anti-Coagulant	NB				2.7 mL				2.7 mL
LPS	Anti-Coagulant	NB	NB	NB	NB	NB	NB	NB	NB	NB
Hormone levels[3]	Coagulant									
Plasma/PBMC for Immunoassays	Anti-Coagulant					60 mL				
Viral Isolation	Anti-Coagulant	7.5 mL								
Viral Genetics	Anti-Coagulant	NB	NB	NB	NB	NB	NB	NB	NB	NB
B cell responses	Anti-Coagulant		7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL
PBMC for Gene Expression	Anti-Coagulant	7.5 mL				NB				NB
T Cell Immunophenotyping		NB NB	NB NB	NB NB	NB NB	NB NB	NB NB	NB NB	NB NB	NB NB
Daily Volume (mL)		36.4	23.5	23.5	23.5	86.2	23.5	23.5	23.5	26.2
Cumulative Volume (mL)		36.4	59.9	83.4	106.9	193.1	216.6	240.1	263.6	289.8
Specimen Type										
EDTA plasma (1 mL/vial)		8	8	8	8	8	8	8	8	8
EDTA cells (5M/vial)		3	3	3	3	3	3	3	3	3
ACD plasma (I mL/vial)		8	4	4	4	34	4	4	4	4
ACD cells (10M/vial)		1				6				
ACD cells (5M/vial)		1	2	2	2	2	2	2	2	2
Serum (1ml/vial)		2		2		2				2
EDTA whole blood (1ml/vial)		2				2				2

[1] Visit 1 collect a full medical history and physical exam; at all other visits update medical history and directed physical exam.

[2] NB = no extra blood required

[3] Only for females participating as negative controls for mucosal component

APPENDIX VI: SCHEDULE OF EVALUATIONS #3, PHASE II

VISIT		10	11	12	13	14	15	16	17	18[1]	Exit Visit
WEEK OF STUDY		5	6	8	12	24	36	48	60		
DAY OF STUDY		35	42	56	84	168	252	336	420		
Clinical											
Informed Consent		X									
Photo ID		check	check	check	check	check	check	check	check	check	check
Medical and Physical Exam[2]		[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	X
Laboratory											
CBC, differential, platelets/T cell phenotypes	Anti-Coagulant		NB[3]		NB		NB				NB
RNA PCR	Anti-Coagulant	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
HIV Diagnostics	Anti-Coagulant	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL
Research											
Syphilis(RPR/TPPA)/Malaria//Eosinophils/HSV	Anti-Coagulant		2.7 mL		2.7 mL		2.7 mL				2.7 mL
LPS	Anti-Coagulant	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
Mucosal Collection		X		X	X	X	X	X	X		X
Hormone levels[4]	Coagulant	2.7 mL		2.7 mL	2.7 mL	2.7 mL	2.7 mL	2.7 mL	2.7 mL		2.7 mL
Plasma/PBMC for Immunoassays	Anti-Coagulant		60 mL		60 mL						
Viral Isolation	Anti-Coagulant										
Viral Genetics	Anti-Coagulant	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
B cell responses	Anti-Coagulant	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL
PBMC for Gene Expression	Anti-Coagulant										
T Cell Immunophenotyping		NB | NB	NB | NB	NB	NB	NB	NB	NB	NB	NB	NB
Daily Volume (mL)		26.2	86.2	26.2	88.9	26.2	28.9	26.2	26.2	23.5	28.9
Cumulative Volume (mL)		326.8	413	439.2	528.1	554.3	583.2	609.4	635.6	659.1	688
Running 56 day total				439.2							
Specimen Type											
EDTA plasma (1 mL/vial)		8	8	8	8	8	8	8	8	8	8
EDTA cells (5M/vial)		3	3	3	3	3	3	3	3	3	3
ACD plasma (I mL/vial)		4	34	4	34	4	4	4	4	4	4
ACD cells (10M/vial)			6		6						
ACD cells (5M/vial)		2	2	2		2	2	2	2	2	2
Serum (1ml/vail)		2		2	2	2	2	2	2	2	2
CVL/Semen (0.4 mL/0.2 mL)		5/5		5/5	5/5	5/5	5/5	5/5	5/5		5/5
EDTA whole blood (1ml/vial)			2	2			2				2

[1] After one year, the volunteer will be seen every 3 months—for at least 50 months
[2] Visit 10 and Exit Visit collect a full medical history and physical exam; at all other visits update medical history and directed physical exam.
[3] NB = no extra blood required
[4] Only for females participating as negative controls for mucosal component

APPENDIX VII: BASELINE QUESTIONNAIRE

APPENDIX VIII: FOLLOW-UP QUESTIONNAIRE

ATTACHMENT I: CASE REPORT FORMS

ATTACHMENT II: BRIEFINGS

ATTACHMENT III: SAMPLE BROCHURE