

# **PROTOCOL RV 329**

## **African Cohort Study (AFRICOS)**

### **Study Conducted By**

US Military HIV Research Program, Bethesda, MD, U.S.A  
U.S. Army Medical Research Unit – Kenya and Kenya Medical Research Institute, Nairobi,  
Kenya  
Makerere University – Walter Reed Project, Makerere University, Kampala, Uganda  
Mbeya Medical Research Program and Walter Reed Southern Highlands HIV Care Program,  
Mbeya, Tanzania  
Department of Defense Walter Reed Program Nigeria, Abuja, Nigeria

### **Study Funded By**

CA# W81XWH-11-2-0174  
US Military HIV Research Program  
Presidents Emergency Plan for AIDS Relief (PEPFAR)

RV 329/WRAIR #1897  
VERSION # 2.6  
DATE 21 November 2016

Proprietary: The information contained herein is the property of USMHRP and WRAIR; and may not be reproduced, published or disclosed to others without written authorization.

## **INVESTIGATORS AND INSTITUTIONAL AFFILIATIONS**

### **PROTOCOL CHAIR**

Julie Ake, MD, MSc, FACP  
LTC, MC  
U.S. Military HIV Research Program  
Walter Reed Army Institute of Research  
6720-A Rockledge Dr., Bethesda, MD 20817  
Rockville, MD 20850  
Tel: 301-500-3621  
Email: [jake@hivresearch.org](mailto:jake@hivresearch.org)

### **PROTOCOL CO-CHAIR**

Christina Polyak, MD, MPH  
U.S. Military HIV Research Program  
6720A Rockledge Drive, Ste 400  
Bethesda, MD 20817  
Tel: 301-500-3688  
Email: [cpolyak@hivresearch.org](mailto:cpolyak@hivresearch.org)

### **ASSOCIATE INVESTIGATORS**

Merlin Robb, MD  
U.S. Military HIV Research Program  
6720-A Rockledge Dr. Ste 400  
Bethesda, MD 20817  
Tel: 301-500-3603  
Email: [mrobb@hivresearch.org](mailto:mrobb@hivresearch.org)

Tiffany E. Hamm, Ph.D.  
U.S. Military HIV Research Program  
6720-A Rockledge Dr. Ste 400  
Bethesda, MD 20817  
Tel: 301-500-3696  
Email: [thamm@hivresearch.org](mailto:thamm@hivresearch.org)

Trevor Crowell  
U.S. Military HIV Research Program  
6720A Rockledge Drive, Ste 400  
Bethesda, MD 20817  
Email: [tcrowell@hivresearch.org](mailto:tcrowell@hivresearch.org)

Peter Coakley

US Military HIV Research Program  
6720A Rockledge Drive, Ste 400  
Bethesda, MD 20817  
Tel: 301-500-3695  
Email: [pcoakley@hivresearch.org](mailto:pcoakley@hivresearch.org)

Victor Valcour MD, PhD  
Professor of Geriatric Medicine  
Division of Geriatric Medicine and Department of Neurology  
Memory and Aging Center  
Tel: 415-476-3746  
Email: [Vvalcour@memory.ucsf.edu](mailto:Vvalcour@memory.ucsf.edu)

LTC James Mancuso, MD, MPH, DrPh  
Walter Reed Army Institute of Research  
Silver Spring, MD 20910  
Email: [james.mancuso@us.army.mil](mailto:james.mancuso@us.army.mil)

#### **WRAIR LABORATORY INVESTIGATORS**

Leigh Anne Eller, PhD, MS  
Director, International Laboratory Program  
U.S. Military HIV Research Program  
6720-A Rockledge Dr. Ste 400  
Bethesda, MD 20817  
Tel: 301-500-3638  
Email: [leller@hivresearch.org](mailto:leller@hivresearch.org)

Michael Eller, PhD  
Scientist  
U.S. Military HIV Research Program  
503 Rober Grant Ave  
Silver Spring, MD 20910  
Tel: 301-319-2094  
Email: [meller@hivresearch.org](mailto:meller@hivresearch.org)

Ali Taylor  
P.O. Box 606, Village Market  
00621 Nairobi, Kenya  
Email: [taylor.ali@usamkru-k.org](mailto:taylor.ali@usamkru-k.org)

CAPT Jennifer Malia, DrPH  
US Military HIV Research Program  
503 Robert Grant Ave  
Silver Spring, MD 20910  
Tel: 301-319-3510

Email: [jmalia@hivresearch.org](mailto:jmalia@hivresearch.org)

Sheila Peel, Ph.D.  
US Military HIV Research Program  
503 Robert Grant Ave.  
Silver Spring, MD 20910  
Tel: +1 301-319-2297  
Email: [speel@hivresearch.org](mailto:speel@hivresearch.org)

Greg Gromowski, PhD  
Viral Diseases Branch  
[Walter Reed Army Institute of Research](#)  
503 Robert Grant Avenue  
Silver Spring, MD 20910  
Tel: 301-319-3093  
Email: Gregory.d.gromowski.civ@mail.mil

Ben Andagalu, MD, MSc  
KEMRI/WRP  
Walter Reed Project  
PO Box 54-40100  
Kisumu, Kenya  
Tel: +254716004851  
Email: Ben.Andagalu@usamru-k.org

## **MHRP DATA MANAGERS**

Rory Deshano  
Data Coordinating and Analysis Center  
US Military HIV Research Program  
6720-A Rockledge Dr. Ste 400  
Bethesda, MD 20817  
Tel: 301-500-3665  
Email: [rdeshano@hivresearch.org](mailto:rdeshano@hivresearch.org)

Michelle Liu  
Data Coordinating and Analysis Center  
US Military HIV Research Program  
6720-A Rockledge Dr. Ste 400  
Bethesda, MD 20817  
Tel: +1 301-500-3652  
Email: [mliu@hivresearch.org](mailto:mliu@hivresearch.org)

Mark Milazzo

Data Coordinating and Analysis Center  
US Military HIV Research Program  
6720-A Rockledge Dr. Ste 400  
Bethesda, MD 20817  
Tel: +1 301-500-3659  
Email: [mmilazzo@hivresearch.org](mailto:mmilazzo@hivresearch.org)

# TABLE OF CONTENTS

<b>SCHEMA.....</b>	<b>8</b>
<b>LIST OF ABBREVIATIONS AND DEFINITIONS.....</b>	<b>15</b>
<b>1 INTRODUCTION/ BACKGROUND .....</b>	<b>17</b>
1.1 Summary .....	17
1.2 Background.....	17
1.3 Description of Partners.....	27
<b>2 STUDY OBJECTIVES .....</b>	<b>30</b>
2.1 Primary Objective.....	30
2.2 Secondary Objectives.....	30
<b>3 DESIGN AND METHODOLOGY .....</b>	<b>34</b>
3.1 Summary of Methods.....	34
3.2 Overview of Subject Activities .....	34
3.3 Laboratory Methods.....	34
<b>4 STUDY POPULATION.....</b>	<b>34</b>
4.1 Inclusion Criteria .....	35
4.2 Exclusion Criteria .....	36
<b>5 STATISTICAL CONSIDERATIONS.....</b>	<b>37</b>
5.1 Power and Sample Size .....	37
5.2 Analysis of Primary Objective.....	38
5.3 Data Analysis for Secondary / Exploratory Objectives.....	38
<b>6 Study PROCEDURES.....</b>	<b>39</b>
6.1 Volunteer Recruiting.....	39
6.2 Enrollment Study Visit .....	39
6.3 Subsequent Study Visits .....	42
6.4 Hospitalizations and Acute Visits .....	44
6.5 Site Visits.....	45
6.6 Death and Lost to Follow up (LTFU).....	45
<b>7 Sample Disposition .....</b>	<b>45</b>
7.1 Labeling .....	45
7.2 Handling.....	45
7.3 Storage .....	46
7.4 Laboratory Testing/ Methods.....	46
<b>8 MANAGEMENT OF SUBJECTS.....</b>	<b>52</b>
8.1 Test Results.....	52
8.2 Pregnancy .....	53
8.3 Incarceration .....	53
8.4 New HIV infection .....	53
8.5 Unanticipated Events and Social Harms Reporting.....	54
8.6 TERMINATION OF STUDY PARTICIPATION.....	54

<b>9</b>	<b>Compensation of Volunteers</b> .....	<b>55</b>
<b>10</b>	<b>LANGUAGE</b> .....	<b>55</b>
<b>11</b>	<b>DATA MANAGEMENT AND ANALYSIS</b> .....	<b>55</b>
11.1	Data Collection and Monitoring.....	55
11.2	Data Entry .....	56
11.3	Data Analysis .....	56
11.4	Data Storage and Security.....	56
<b>12</b>	<b>Sub-study Use of Data and Samples in AFRICOS Repository</b> .....	<b>57</b>
12.1	Sub-study Procedures.....	57
12.2	Distribution of Study Specimens and Data .....	58
12.3	Protection of Subject Data .....	59
<b>13</b>	<b>ETHICAL CONSIDERATIONS</b> .....	<b>59</b>
13.1	Risks .....	59
13.2	Benefits .....	60
13.3	Informed Consent.....	60
13.4	Volunteer Confidentiality.....	60
13.5	Management of Vulnerable Volunteers .....	61
<b>14</b>	<b>Protocol Deviation Reporting</b> .....	<b>62</b>
<b>15</b>	<b>Protocol Modifications</b> .....	<b>62</b>
<b>16</b>	<b>Continuing Reviews /Closeout Report</b> .....	<b>63</b>
<b>17</b>	<b>STRATEGIES FOR IMPROVING ENROLLMENT AND COHORT RETENTION</b> .....	<b>63</b>
<b>18</b>	<b>RESOURCES AND COORDINATION</b> .....	<b>63</b>
<b>19</b>	<b>Use of InformaTion and Publication</b> .....	<b>64</b>
<b>20</b>	<b>CONDUCT OF THE RESEARCH STUDY</b> .....	<b>64</b>
<b>21</b>	<b>STATEMENT REGARDING POTENTIAL CONFLICT OF FINANCIAL INTEREST</b> .....	<b>64</b>
<b>22</b>	<b>SIGNATURE OF PRINCIPAL INVESTIGATOR(S)</b> .....	<b>65</b>
<b>23</b>	<b>REFERENCES</b> .....	<b>66</b>
<b>Appendix I: Sub-Study Template</b> .....		<b>77</b>
<b>Appendix II: Case Report forms</b> .....		<b>78</b>
<b>Appendix III: External Collaborator’s List</b> .....		<b>79</b>
<b>Attachment I: Site-specific addendum</b> .....		<b>80</b>
<b>Attachment II: Schedule of Events – HIV-infected participants</b> .....		<b>81</b>
<b>Attachment III: Schedule of Events – HIV-uninfected participants</b> .....		<b>82</b>
<b>Attachment IV: Informed Consent FOrM – HIV-infected participants</b> .....		<b>83</b>
<b>Attachment V: informed consent form – hiv-uninfected participants</b> .....		<b>84</b>
<b>Attachment VI: Briefing slides</b> .....		<b>85</b>

## **SCHEMA**

### **Title**

African Cohort Study (AFRICOS)

### **Study Objectives**

Primary objective:

To longitudinally assess the impact of clinical practices, biological factors and socio-behavioral issues on HIV infection and disease progression in an African context.

Secondary objectives:

A. Social and behavioral domain

- 1) Describe, among HIV infected subjects and their families, stigmatizing events and social and economic harms attendant to HIV care and treatment; evaluate their impact on care seeking behaviors, HIV treatment response and disease progression
- 2) Describe adherence to HIV care and treatment and evaluate for predictors/determinants of adherence
- 3) Describe HIV risk behaviors in the study population
- 4) Describe cultural barriers and facilitators of HIV prevention, care and treatment
- 5) Describe the impact of behavioral treatment strategies (including but not limited to status disclosure, treatment partners and support groups) on HIV clinical outcomes
- 6) Describe the impact of substance use on HIV infection and disease outcomes
- 7) Describe the impact of incarceration and/or institutionalization on HIV treatment and outcomes

B. Medical-HIV prevention and management (programmatic)

- 1) Identify attributes of HIV care and treatment programs associated with optimal clinical outcomes (including, but not limited to, organization, location, accessibility, logistic support, compliance with MOH guidelines, and drug distribution)
- 2) Describe clinical features of HIV disease (including, but not limited to, disease progression, response to therapy, regimen durability, development of resistance, change in viral tropism, ART population pharmacokinetics, mortality) as a function of program parameters
- 3) Describe the implementation and uptake of preventive interventions in HIV infected and uninfected subjects
- 4) Describe barriers to subject retention and strategies for its enhancement



### C. Medical-HIV management (subject)

- 1) Describe HIV disease outcomes, including, but not limited to, mortality, progression to AIDS, event-free survival, and prevalence/incidence of HIV related sequelae
- 2) Describe HIV treatment monitoring practices and impact on disease outcomes
- 3) Describe frequency and amplitude of transient viremia (viral load “blips”) and relation to disease outcomes
- 4) Describe HIV disease progression and treatment response for different subtypes of HIV
- 5) Compare accuracy and utility of alternative field expedient HIV diagnostic, viral load and lymphocyte measurement testing platforms to gold standard assays
- 6) Describe frequency and character of HIV resistance mutations and impact on disease outcomes
- 7) Describe frequency and character of HIV resistance mutations as associated with viral subtype and prior exposure to antiretrovirals
- 8) Describe the impact of food insecurity on HIV disease outcomes
- 9) Describe HIV drug related toxicity and interactions

### D. Medical-opportunistic infections and other morbidities

- 1) Describe the type and frequency of other co-morbidities (including, but not limited to, malignancy, cardiovascular events, malnutrition, anemia, renal insufficiency, and cognitive decline), their impact on HIV disease outcomes, and the impact of HIV treatment on their clinical outcomes
- 2) Describe the prevalence and incidence of cardiovascular risk factors (including but not limited to hypertension, lipid abnormalities, systemic inflammation and impaired glucose metabolism), their impact on subject outcomes
- 3) Describe practices of chronic disease management in the PEPFAR setting
- 4) Describe the type and frequency of endemic infections and evaluate their interplay with HIV disease (including, but not limited to tuberculosis, viral hepatitis, malaria and other etiologies of febrile illness, human papillomavirus and other STIs, and stool pathogens)
  - i. Tuberculosis
    1. Estimate the prevalence and incidence of active tuberculosis and rifampicin resistance in HIV infected subjects in the PEPFAR clinical setting
    2. Establish the predictive value of a positive interferon gamma release assay in HIV positive subjects for the development of active TB
  - ii. Human papillomavirus and other STIs
    1. Describe the implementation and outcomes of cervical cancer screening delivered in the PEPFAR setting, to include visual inspection with acetic acid, histopathology based screening (PAP smear), and qualitative rapid HPV tests
    2. Characterize the interplay between HIV, the immune system

- and HPV induced carcinogenesis in the era of HAART
3. Describe the prevalence of ulcerative and nonulcerative STIs and their association with HIV disease
- iii. Viral hepatitis
    1. To investigate the prevalence and incidence of HBsAg, HBeAg, HBcAb and HBV DNA viremia in HIV infected and uninfected subjects
    2. To determine the HBV subtype in HBV viremic participants
    3. Describe the prevalence of increased hepatotoxicity/hepatic flare in participants with acquired HBV viremia/resistance mutations with treatment
    4. Characterization of genotypic (and phenotypic) HBV resistance patterns in HBV viremic participants in relation to the HBV genotypes and clinical characteristics
  - iv. Malaria
    1. Compare incidence of symptomatic malaria infections in HIV-infected and HIV-uninfected individuals
    2. Describe the clinical presentation of acute malaria in HIV-infected persons and compare with presentation in HIV-uninfected individuals
    3. Measure level of parasitemia and gametocytemia in HIV-infected persons versus HIV-uninfected persons
    4. Evaluate the role of antiretroviral therapy and cotrimoxazole on clinical and laboratory aspects of malaria infection in HIV positive subjects
  - v. Stools pathogens – describe the prevalence of helminth and bacterial stool pathogens and their impact on HIV disease outcomes
  - vi. Describe the test characteristics for rapid diagnostic tools for coinfections (including but not limited to Hepatitis B, Hepatitis C, malaria, and tuberculosis) as they apply to the PEPFAR setting
- 5) Describe the type and frequency of opportunistic infections and their impact on disease outcomes
  - 6) Describe opportunistic infection prevention practices (including but not limited to isoniazid preventive therapy, cotrimoxazole and fluconazole prophylaxis) and their impact on disease outcomes
  - 7) Describe the frequency, character and outcome of IRIS and its impact on disease outcomes
- E. Medical-Maternal-child transmission management
- 1) Describe transmission rates by HIV disease severity and presence of other HIV related disease
  - 2) Describe PMTCT program attributes including adherence and relation to transmission rates
  - 3) Describe interaction of prior ART for PMTCT or subject treatment on transmission outcome
  - 4) Describe reproductive health practices and access to family planning

- 5) Evaluate for the determinants of care-seeking behavior in the PMTCT care population
- 6) Describe the impact of coinfections on disease outcomes and HIV transmission

F. Medical-Prevention of horizontal HIV infection

- 1) Describe the impact of HIV preventive interventions on risk behaviors
- 2) Describe prevention with positives interventions and their impact on HIV transmission in serodiscordant partners
- 3) Describe HIV preventive interventions and uptake in individuals undergoing HIV counseling and testing
- 4) Describe the prevalence and incidence of coinfections, chronic diseases and other comorbid health conditions in HIV uninfected individuals as compared to HIV infected individuals

G. Medical-host genetics and pathogenesis

- 1) Describe HLA and other key host genetic markers known to associate with HIV disease acquisition, progression or response to therapy
- 2) Define host genetic markers which relate to HIV acquisition, progression, or response to therapy
- 3) Define immunologic and viral factors which are associated with HIV acquisition, disease progression, or response to therapy
- 4) Investigate markers of systemic inflammation as they relate to HIV disease and its progression

## Study Design

AFRICOS will be an open-ended prospective cohort study, enrolling 3000 HIV infected adults and 600 HIV uninfected adults at MHRP PEPFAR-associated clinical sites in Kenya, Tanzania, Uganda and Nigeria. The study will follow participants every six months and will collect social, demographic, clinical and laboratory data as well as blood and sputum samples for storage in the AFRICOS Repository.

## Participants

The study population will include males and females aged 18 years and older, who receive medical care within the catchment areas of the MHRP DoD PEPFAR program.

## Study Duration

The study duration is fifteen years. Initial study enrollment to meet target enrollment goals is planned over the first three years of the study. Enrollment to replace subjects who are lost to follow-up will continue for the duration of the study.

## Sponsor

US Military HIV Research Program

## **Study Clinical Sites**

Uganda – Kampala, Kayunga and Mukono Districts (Protocol RV 329a)

Kenya – South Rift Valley Province (Protocol RV 329b)

Kenya – Kisumu West, Nyanza Province (Protocol RV 329c)

Tanzania – Southern Highlands (Protocol RV 329d)

Nigeria – DOD WRP-N (Protocol RV 329e)

Kenya – KDF (Protocol RV 329f)

## **Statistical and Data Management Center(s)**

MHRP Data Coordinating and Analysis Center  
6720-A Rockledge Dr., Ste. 400  
Bethesda, MD 20817  
USA

## **Study Laboratories**

US Military HIV Research Program  
Walter Reed Army Institute of Research  
503 Robert Grant Avenue  
Silver Spring, MD 20910

Walter Reed Project/Kenya Medical Research Institute  
Along Kakamega Road  
PO Box 54 - 40100  
Kisumu, Kenya

The Kenya Medical Research Institute/Walter Reed Project  
Kombewa Clinical Research Center Laboratory  
Kombewa, Kisumu, Kenya

Malaria Diagnostics Centre  
Walter Reed Project/Kenya Medical Research Institute  
PO Box 54  
Kisumu, Kenya

Kenya Medical Research Institute/Walter Reed Project  
Clinical Research Center Laboratory  
Kericho, Kenya

P.O Box 1357, Kericho 20200

Makerere University Walter Reed Project (MUWRP) Laboratory  
Faculty of Medicine, Pathology Building A-10  
Old Mulago Hill Road  
Kampala, Uganda

Mbeya Medical Research Centre (MMRC) Laboratory  
P.O. Box 2410  
Mbeya, Tanzania

Mbeya Referral Hospital (MRH) Laboratory  
P.O. Box 419  
Mbeya, Tanzania

Defense Reference Laboratory (DODHPN)  
Mogadishu Cantonment  
Abuja, Nigeria

University of Munich, LMU  
Department for Infectious Diseases & Tropical Medicine  
Leopoldstrasse 5, 80802  
Munich, Germany

#### SERVICE LABORATORIES

Quest Diagnostics Inc.  
1901 Sullphur Spring Road  
Baltimore, Maryland 21227

\*Quest will perform advanced clinical testing for characterization of cormorbidities

#### **Institutional Review Boards**

Institutional Review Boards Walter Reed Army Institute of Research IRB  
[FWA00000015, IRB00000794]  
Human Subjects Protection Branch  
Walter Reed Army Institute of Research  
503 Robert Grant Ave.  
Silver Spring, MD 20910-7500  
Telephone: 1-301-319-9940  
Fax: 1-301-319-9961

Ministry of Defence Health Research Ethics Committee (MODHREC)  
[FWA00011634, IRB00005925]  
Department of Health Planning, Research and Statistics,

Federal Ministry of Defence,  
Ship House, Area 8, Garki  
Abuja  
Tel: +234-8023093714  
Email: joyugwuegede@yahoo.com

Makerere University School of Public Health IRB  
School of Public Health Building, New Mulago Hospital Complex  
Mulago Hospital Complex, Mulago Hill  
P. O. Box 7072  
Kampala, Uganda  
Telephone: +256-312- 112497  
OHRP IRB 00005876

KEMRI Science and Ethics Research Unit  
[FWA00002066; IRB00001625]  
KEMRI, Nairobi, Kenya  
P.O Box 54840, Code 00200  
Tel +254 20 27 22 541

Mbeya Ethical Review Committee  
[FWA# 00002469, IRB# 00002439]  
Mbeya Medical Research and Ethics Committee  
Hospital Hill Road, P. O. Box 419  
Mbeya, Tanzania

University of California, San Francisco IRB  
[FWA# 00000068, IRB# 00000230]  
3333 California Street, Suite 315  
University of California  
San Francisco, CA 94118  
Telephone: +1 415-476-1814  
chr@ucsf.edu

## LIST OF ABBREVIATIONS AND DEFINITIONS

<b>TERM</b>	<b>DEFINITION</b>
3TC	Lamivudine
AFRICOS	African Cohort Study
AFRICOSR	AFRICOS Repository
AI	Associate Investigator
ART	Antiretroviral therapy
ARV	Antiretroviral
CDC	Center for Disease Control
CLADE	Clinic-based ART Diagnostic Evaluation
COP	Country Operating Program
CRF	Case report form
CRR	Continuing review report
DC	Data custodian
DCAC	Data Coordinating and Analysis Center
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DoD	Department of Defense
DOD WRP-N	Department of Defense Walter Reed Program Nigeria
EC	Ethical Committees
FTC	Emtricitabine
GCP	Good clinical practices
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B
HCV	Hepatitis C
HJF	Henry M Jackson Foundation for the Advancement of Military Medicine
HIV	Human immunodeficiency virus
HPTN	HIV Prevention Trials Network
HPV	Human papillomavirus
HSPB	WRAIR Human Subjects Protection Branch
ICF	Informed Consent Form
IGRA	Interferon gamma release assay
IRB	Institutional Review Board
KEMRI	Kenya Medical Research Institute
KDF	Kenya Defense Force
LMU	Department of Infectious Diseases & Tropical Medicine at the University of Munich
LTFU	Loss to follow-up
MHRP	US Military HIV Research Program
MMRC	Mbeya Medical Research Programme

MOH	Ministry of Health
MTA	Material transfer agreement
MUWRP	Makerere University Walter Reed Project
NAT	Nucleic acid test
NIMR	National Institute of Medical Research
NMOD	Nigerian Ministry of Defense
NRTI	Nucleotide reverse transcriptase inhibitors
OGAC	Office of the U.S. Global AIDS Coordinator
OI	Opportunistic infection
OHRP	Office of Human Research Protection
PAVE	Partnership for AIDS Vaccine
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PEPFAR	Presidents Emergency Plan for AIDS Relief
PHI	Public health information
PI	Principal Investigator
PMTCT	Prevention of Mother-to-Child Transmission
POC	Point-of-care
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
RPR	Rapid plasma reagin
SOP	Standard operating procedures
STI	Sexually transmitted infection
TB	tuberculosis
TDF	tenofovir
USAMRMC	US Army Medical Research and Materiel Command
USAMRU-K	US Army Medical Research Unit - Kenya
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
WRP	Walter Reed Project HIV Program
WRSHCP	Walter Reed Southern Highlands HIV Care Program



# **1 INTRODUCTION/ BACKGROUND**

## **1.1 Summary**

The African Cohort Study (AFRICOS) will be an open-ended prospective cohort study to enroll adults with the human immunodeficiency virus (HIV) and HIV uninfected adults at US Military HIV Research Program (MHRP) President's Emergency Plan for AIDS Relief (PEPFAR)-associated clinical sites in Kenya, Tanzania, Uganda, and Nigeria. The overarching goal of this study is to develop and maintain a cohort that will enable ongoing program evaluation and yield a longitudinal repository of data and specimens from enrollees at Walter Reed Army Institute of Research (WRAIR) PEPFAR supported antiretroviral therapy (ART) sites, thereby facilitating PEPFAR evaluative research with implications for HIV clinical care and treatment. This protocol and repository will also evaluate the prevalence and incidence of HIV related coinfections and comorbidities as well as the pathogenesis of these conditions, with particular emphasis on tuberculosis, viral hepatitis, malaria, malignancy and the metabolic and cardiovascular complications of HIV. Another secondary goal of AFRICOS is to facilitate investigation into the pathogenesis of HIV infection and HIV disease progression.

On enrollment, there will be a retrospective data collection, the initiation of prospective data collection, and the initiation of serial sample collection for sputum and blood product archiving and for performance of clinical and research tests. Following enrollment, study visits will be scheduled every six months. Participation in AFRICOS will not preclude enrollment in other research studies and co-enrollment will be encouraged.

## **1.2 Background**

In 2004, the President's Emergency Plan for AIDS Relief (PEPFAR) began funding prevention, care and treatment services through the US Military HIV Research Program (MHRP). These programs engage both civilian and military populations in sub-Saharan Africa, with sites in Kenya, Nigeria, Tanzania, and Uganda. Currently, MHRP PEPFAR programs provide HIV care to over 195,000 subjects, including antiretroviral therapy to over 93,000 individuals. In 2009, these programs provided prevention of mother to child transmission (PMTCT) services to over 218,000 women.

Since the initiation of PEPFAR, there has been a rapid scale-up in provision of services to populations in need: prior to the initiation of PEPFAR, fewer than 50,000 persons in Africa were receiving ART. By FY2009, PEPFAR was supporting ART for more than 2.4 million persons, almost all of whom live in Africa (PEPFAR 6<sup>th</sup> Annual Report). Outcomes data for the program is limited, although a decrease in AIDS-related deaths in PEPFAR countries has been reported (Bendavid and Bhattacharya 2009). Currently, the Office of the U.S. Global AIDS Coordinator (OGAC), which oversees PEPFAR activities, is engaged in a program to evaluate PEPFAR's impact, improve service delivery, and maximize outcomes. MHRP is already actively contributing to this effort through multiple evaluations of its own program and also investigation of broader public health questions to include WRAIR# 1647/ RV 288: A Virological Assessment of Patients on Antiretroviral Therapy in the MHRP/PEPFAR –

Supported Programs in Africa and WRAIR # 1591/ RV 257: Clinic-based ART Diagnostic Evaluation (CLADE). These pioneering protocols evaluate the role of viral load and resistance testing in the PEPFAR programs, with short-term primary outcomes. These studies are building upon the research expertise developed at these sites in the implementation of multiple research cohort studies and vaccine trials.

To better assess treatment strategies, evaluate the impact of social and biologic factors on HIV and its treatment, and to investigate the pathogenesis of HIV, long-term cohort studies have made important contributions to the field. Such studies can adopt primary outcomes such as long-term disease progression and mortality. One example is WRAIR # 1136 /RV 168: A Retrospective and Prospective Observational Study of the Natural History of HIV Infection in Active Duty US Military Personnel and Department of Defense Beneficiaries (DOSE). Clinical data and reposed tissue collected through this study have been used to answer clinical and basic science questions, such as the effect of tenofovir on renal function (Crum-Cianflone, Ganesan et al. 2010), the effect of depression on HIV outcomes (Hartzell, Janke et al. 2008) host genetic determinants of HIV-1 control (Pelak, Goldstein et al. 2010), and the impact of early immunologic and virologic events on disease progression (Ganesan, Chattopadhyay et al. 2010). Other examples include the Multicenter AIDS Cohort Study, the Women's Interagency HIV Study, the Swiss HIV Cohort Study, and EuroSIDA. Most large ongoing HIV cohort studies are conducted in resource rich settings featuring clade B predominant infections.

MHRP's robust PEPFAR programs combined with its history of coordinating large-scale clinical research protocols at co-located research centers provide a significant opportunity for the development of a multinational, multiclade AFRICOS. MHRP has well-developed institutional capacity in these four African countries in terms of laboratory capacity, trained and experienced local investigators and research personnel, as well as active community engagement. Prior vaccine trials and cohort studies, in addition to current PEPFAR evaluations, provide a pool of potential cohort enrollees with significant pre-existing clinical and laboratory databases.

The establishment of a large long term cohort study at multiple African sites would provide the opportunity to evaluate the services provided at MHRP-associated PEPFAR sites, including the long term outcomes (e.g., progression to AIDS, mortality) associated with particular clinical practices. This is a particularly opportune time to follow clinical outcomes as programs transition to the new World Health Organization (WHO) recommendation to initiate ART at a CD4+ T cell count of <350 (Walensky, Wolf et al. 2009). Also, routinely employing viral load monitoring and resistance testing can answer important programmatic and clinical questions, such as the most effective and efficient use of viral load monitoring and resistance testing. A report of genotypic resistance testing in a small South American cohort documented substantial viral resistance among those failing first line therapy, however interpretation of the impact of resistance on outcomes was hindered by the small sample size (Marconi, Sunpath et al. 2008). With the implementation of advance monitoring techniques, PEPFAR clinicians will encounter similar test interpretation dilemmas as their colleagues in resource rich settings. For instance, there is an opportunity to evaluate the long-term clinical

significance of transient elevations in viral load (blips) in this multiclade population with a higher burden of coinfection compared to Western cohorts (Nettles, Kieffer et al. 2005).

Evaluation of social and behavioral aspects of HIV infection and care will also enhance PEPFAR program effectiveness. Increased sample sizes can enable more precise estimates of adherence, with associated impact on outcomes (Stubbs, Micek et al. 2009; Maqutu, Zewotir et al. 2010; Nachega, Leisegang et al. 2010). AFRICOS will evaluate the adherence effects of factors such as age, sex, cultural/social factors, geographic location relative to health care facilities, socioeconomic status, cognitive decline, depressive symptoms, substance use, social stigma, experiences with violence – as well as investigate behaviors such as HIV serostatus disclosure and adoption of treatment partners (Stubbs, Micek et al. 2009; Wouters, van Loon et al. 2009).

This protocol will also attempt to characterize the type and impact of cognitive impairment in the populations studied. The impact of HIV on the brain has been studied in several international settings identifying prevalent cognitive impairment in all settings where evaluated (Valcour et al. 2007; Gupta et al. 2007; Sacktor et al. 2007). Many have speculated that clade specificity may impact the frequency of cognitive disorders, driven, in part, by data from in vitro models suggesting differences that would support altered neuropathogenesis (Ranga et al 2004; Gandhi 2009). Data from Uganda are highly suggestive that individuals with clade D virus are more likely to have dementia than those with clade A; although this study was limited in sample size resulting in only a modest number of dementia cases (Sacktor et al 2009). However, this finding would not be surprising given recent findings differential infectivity with clade D and knowledge that intracellular HIV DNA is linked to dementia (Baalwa et al. 2011; Shiramizu et al. 2005). The proposed work could inform the frequency of cognitive impairment within regions of Africa. Given clade diversity, it is also possible that this work can provide epidemiological evidence for altered cognitive outcomes by clade (Maj et al. 1993; Maj et al.1991).

Collection of data regarding coinfections will provide valuable insight into the impact of coinfections on HIV progression, and also the impact of HIV (and HIV therapy) on coinfections (Karp and Auwaerter 2007). Individual program practices will be evaluated such as the integration of tuberculosis (TB) and HIV care (Harris, Hatwiinda et al. 2008). In particular the impact of program changes on biological outcomes like HIV disease progression and opportunistic infection (OI) incidence will be assessed in an ongoing fashion. This information can guide future care innovations, such as active TB and/or cryptococcal screening at ART initiation, to include the use of rapid diagnostics (Moore, John et al. 2002; Bassett, Wang et al. 2010; Boehme, Nabeta et al. 2010; Meya, Manabe et al. 2010). Given their impact on HIV-related morbidity and mortality, the coinfections of tuberculosis, malaria, viral hepatitis, and human papillomavirus are initial foci for AFRICOS.

### **Tuberculosis:**

Tuberculosis (TB) is one of the most mortal and morbid coinfections complicating HIV disease. In 2006, more than 700,000 people living with HIV were infected with TB, and 200,000 HIV-positive people died from TB. Africa accounted for 85 percent of the estimated global HIV-positive TB cases (The Global Fund). There are very limited data, however, on

the incidence of active TB in HIV infected individuals in Africa. The few available data vary between 1 and 8% (Sonnenberg et al., 2005, Aichelburg et al., 2009, Jonnalagadda et al., 2010, Fielding et. al, 2010, Akolo et al, 2010). In order to design rational intervention and prevention programs more data are urgently needed. With the recommendation of WHO to use Xpert MTB/RIF for the detection of TB, a simple and rapid methodology became available that is almost as sensitive as the gold standard. Furthermore, the minimal difference in sensitivity between a single sputum and 2 or 3 sputum analysis makes it not only an ideal tool to assess TB incidence in a study setting, but also an appropriate screening tool in a care and treatment setting (Boehme et al., 2010). As new tools are deployed in the PEPFAR setting, in addition to traditional methods, AFRICOS will be able to collect improved TB prevalence and incidence data.

Furthermore, there are several lines of evidence that indicate that a positive interferon gamma release assay (IGRA) is associated with an increased risk of developing active TB in HIV positive subjects (HR 4.5 (Kenya)(Jonnalagadda et al., 2010) and OR 4.8 (Austria) (Aichelburg et al., 2009). A recent study suggests that that in subjects with latent MTB infection, MTB specific CD4 T-cells are preferentially depleted after HIV infection (Geldmacher et al., 2010, Geldmacher et al., 2008). As a result, the majority of HIV infected, untreated individuals with latent MTB infection are IGRA negative. However, when these subjects progress to active TB their immune system is exposed to substantial amounts of MTB antigen. This leads to increased antigen-specific stimulation of MTB-specific T cells and causes expansion and maturation of MTB-specific T cells even in the presence of untreated HIV infection (Geldmacher et al., 2010). AFRICOS is positioned to capture relevant clinical data and collect specimens to further describe the utility of IGRAs in the African context.

### **Malaria:**

Malaria coinfection is also a critical comorbidity in HIV infected individuals. Malaria and HIV both account for a significant amount of morbidity and mortality in sub-Saharan Africa with an estimated 22.5 million people living with HIV (UNAIDS 2010) and over 300 to 500 million clinical *Plasmodium falciparum* cases every year. Two geographic areas in which WRAIR PEPFAR programs support HIV care bear a particularly high burden. The Kisumu-West district of Kenya's Nyanza Province has the highest prevalence of malaria and HIV in Kenya with reports of HIV prevalence ranging from 21 to 44 percent and point prevalence of malaria infection reported at 13 to 42 percent (M. Hamel, CDC). The Kayunga district of Uganda is also highly endemic for malaria with malaria accounting for 25 to 40 percent of outpatient visits to health facilities, 15 to 20 percent of all hospital admissions, 9 to 14 percent of all hospital deaths, and nearly half of inpatient pediatric deaths. (PMI: Uganda, November 2009).

Frequent and recurrent infections with *Plasmodium falciparum* in areas with stable malaria transmission result in a semi-immune state in adults which allows for an increasing proportion of infections to stabilize at variable parasite densities without severe disease. These parasitemias can last for months and may either be asymptomatic or mildly symptomatic and tolerated without treatment but likely contribute to transmission. (Schneider et al 2007, Babiker et al 1998, Kraijden et al 1991)

HIV-infected adults with decreased CD4 counts (especially those with CD4 counts less than 200) living in areas of stable malaria transmission have an increased incidence of symptomatic malaria infections compared to HIV-uninfected adults and there is evidence of an inverse relationship between level of parasitemia and CD4 count. (Hewitt et al 2006, French et al 2001, Whitworth et al 2000, Francesconi et al 2001, Patnaik et al 2005, Laufer et al 2006) Rates of malaria treatment failure have been noted to be higher in HIV-infected persons with highest rates of failure noted in individuals with CD4 counts less than 200. (Shah et al 2006, Hewitt et al 2006, van Geertruyden et al 2004). However, Kanya et al used molecular genotyping in a Ugandan cohort and demonstrated that clinical treatment failure of malaria was actually a result of new infections rather than recrudescence of the same infection. (Kanya et al 2006) Malaria infection leads to CD4 activation and the impaired T-cell immunity and loss of antigen-specific memory CD4 cells in HIV-infection (van Geertruyden and D'Alessandro 2007) likely causes some loss of the specific disease protective immune response necessary to prevent infection, protect parasitemic persons from developing severe disease, and clear parasitemia with treatment. Though previous studies have not noted an increase in severe or complicated malaria in regions of stable transmission (Hewitt et al 2006), people living with HIV infection who have no pre-existing immunity to malaria do experience increased disease severity. (Laufer and Plowe 2007)

Malaria infection, even asymptomatic parasitemia, causes a transient increase in HIV viral load (up to 1 log) which persists for as long as 8 weeks.(Kublin et al 2005, Hoffman et al 1999) This increase is seen even if effective anti-malarial treatment is given and is thought to be a result of T-cell activation (specifically CD4+ T-lymphocytes) promoting HIV replication (Xiao et al 1998, Froebel et al 2004), up-regulation of HIV co-receptors, and activation of dendritic cells. (Brentlinger et al 2007) CD4+ lymphocytes also decline during clinical malaria episodes (Van geertruyden and D'Alessandro 2007) and repeated malaria infections are associated with a more rapid decrease over time. (Mermin et al 2006) Successful malaria treatment in HIV-infected individuals has been shown to increase mean CD4 counts. (van Geertruyden et al 2006)

Mathematical modeling has been applied to describe the interaction of HIV and malaria infections in sub-Saharan Africa. Prolonged parasitemia and increased frequency of symptomatic malaria infections in HIV-infected individuals are likely to contribute to malaria transmission. Elevated HIV plasma viral loads associated with malaria infection and malaria-associated immune suppression likely contributes to transmission of HIV. Korenromp et al estimate that an additional 3 million cases of malaria and 65,000 malaria-related deaths annually are due to the impact of HIV. (Korenromp et al 2005) Abu-Raddad applied a mathematical model to the Kisumu district and estimated that since 1980, the disease interaction may have been responsible for 8,500 excess HIV infections and 980,000 excess malaria episodes (Abu-Raddad et al 2006).

There are many confounders, however, in determining the epidemiologic interaction between HIV and malaria. The anti-malarial activities of antiretroviral therapy (especially protease inhibitors) have been reported and studies are ongoing. (Skinner-Adams et al 1998, Andrews et al 2006, Parikh et al 2005, Skinner-Adams et al 2007) The symptoms of adverse effects of

medications, acute HIV infection, and other opportunistic infections overlap with those of malaria making definitive diagnosis and treatment difficult in malaria endemic settings (ie potential for asymptomatic parasitemia in an HIV-infected person with other cause of acute fever).

Likely the most important confounding factor is the widespread use of co-trimoxazole (TS) prophylaxis in HIV-infected individuals. As an anti-folate similar to sulfadoxine/pyremethamine (SP/fansidar), TS is also an anti-malarial and has been shown to decrease episodes of malaria in both the individuals on co-trimoxazole and in their HIV-negative household members. (Mermin et al 2005, Gasasira et al 2010) However, Gasasira et al also noted a significant increase in prevalence of the *dhfr* 164L mutation in parasites in a cohort of children in Kampala, Uganda treated with co-trimoxazole over a 3-year period. The *dhfr* 164L mutation confers high-level resistance to anti-folates but also may engender a fitness cost (and hypothetically push parasites to sexual development thereby increasing gametocytemia and transmission). Factors associated with gametocytogenesis include single-species infection, parasite density, anemia/reticulocytosis, duration of infection, stress on the parasite population due to host immunity or anti-malarial treatment, and the stage-specificity of the anti-malarials used. (Price 1999, Trager et al 2005) Treatment of malaria infection with sulphadoxine-pyrimethamine (SP/fansidar) alone results in very high post-treatment gametocyte prevalence that is likely to enhance transmission; treatment with SP against resistant infection is followed by the highest gametocytemia. (Stepniewska et al 2008, Robert et al 2000, Sokhna et al 2001, von Seidlein et al 2001) Gametocyte densities of 1 per  $\mu\text{L}$  are theoretically sufficient to infect mosquitoes (blood meal of approx 2  $\mu\text{L}$ ) (Stepniewska et al 2008) and low densities can best be detected by RT-PCR performed on either whole blood or blood samples collected on filter paper. (Maeno et al 2008, Mlambo et al 2008, Scopel et al 2004, Long et al 2004, Scopel et al 2004, Long et al 2004) It is likely that similarly high gametocytemias exist in the setting of treatment or prophylaxis with co-trimoxazole and the long-term population effects of widespread co-trimoxazole use on incidence of malaria infection in individuals and on transmission are not known.

AFRICOS will be enrolling participants in several areas of high malarial endemicity, to include Kayunga in Uganda and Kombewa in Kenya, as well as endemic areas in Nigeria and parts of the South Rift Valley in Kenya that demonstrate a range of transmission patterns. By evaluating for laboratory and clinical evidence of malaria in the context of ongoing HIV disease monitoring and outcomes recording, the study will shed light on the interaction of malaria with HIV disease and its management. In many malaria endemic areas, non-malaria causes of febrile illness are underdiagnosed and undertreated with common overtreatment for malaria (Jaenisch et al 2014, Onchiri et al 2015, Nadjm et al 2012). AFRICOS will also leverage samples collected for malaria evaluations to investigate non-malaria causes of febrile illness and their implications for HIV disease.

### **Hepatitis B (HBV):**

Chronic hepatitis B is the leading cause of chronic liver disease and a leading cause of death worldwide. In Africa, most HBV transmission appears to occur horizontal during child-to-child spread, with infection beginning in childhood, as opposed to East Asia where vertical

mother-to-infant spread is very common. Comprehensive epidemiologic data is lacking, however the prevalence of chronic hepatitis B in SubSaharan Africa is estimated at 10% (Kew 1996). The low rate of vertical transmission during birth is possibly related to a low prevalence of Hepatitis Be Antigen (HbeAg). HbeAg is association with higher HBV viral loads and both HBe antigenaemia and higher HBV viral loads are independent predictors for vertical transmission and both cirrhosis and hepatocellular carcinoma. HbeAg prevalence is also thought to be associated with the HBV genotype. Different HBV genotypes can influence the clinical course of chronic hepatitis, the development of core and pre-core mutants in the HBV genome, as well as treatment susceptibility to NRTIs (such as lamivudine or tenofovir) or interferon alpha therapy. Currently, there are eight known HBV genotypes (A–H), with Genotype A (subtype a) found in South and East Africa.

Many African countries where HBV prevalence is high are also affected by HIV-1 infection and HIV/HBV co-infections are common. Overall, the prevalence of HBsAg in individuals does not differ between HIV infected and HIV uninfected in Africa, which can be explained by early HBV infections during childhood (in contrast to more often sexual transmitted HBV in the industrialized counties). However, HIV co-infected subjects are twice as likely to have detectable HbeAg levels compared with HIV-uninfected individuals with presumed higher HBV viral loads and clinical implications (Di Bisceglie et al. 2010; Durantel et al. 2005). In the presence of HIV co-infection, HBV liver disease is accelerated, especially when HIV-associated immunodeficiency progresses. Lower CD4 counts seem to be associated with a higher HbeAg prevalence (Bodsworth 1991). Increased hepatotoxicity associated with antiretroviral therapy or TB treatment is more likely to occur in HBV-coinfected HIV subjects. A further aspect concerns occult hepatitis B, which is defined by undetectable serum HbsAg and measurable HBV DNA, with reported prevalence rates in HIV-infected persons up to 22% (Mphahlele 2006). Occult hepatitis B may be associated with progression to cirrhosis and hepatocellular carcinoma, and it might exacerbate with the introduction of ART.

Hepatitis B treatment with interferon alpha or nucleotide/nucleoside inhibitors is currently not routinely conducted in African countries. However, nucleotide reverse transcriptase inhibitors (NRTI) such as Lamivudine (3TC), Emtricitabine (FTC) and Tenofovir (TDF) given as part of antiretroviral therapies are active inhibitors of both HIV RNA- and HBV DNA- dependent DNA polymerase and contain anti-HBV activity with subsequent clinical implications. The latest WHO recommendations on HIV treatment recommend the initiation of ART with HBV active drugs in all HIV/HBV coinfecting individuals. Lamivudine (3TC) is the mostly widely available drug in Africa. HBeAg seroconversion while taking 3TC has been recorded in 22% - 29% of HBV/HIV co-infected adults who receive 3TC. Seroconversion to HBsAg taking 3TC, however, is rarely achieved and is reported to be 1-2% annually. Studies from industrialized countries have shown that during lamivudine therapy HBV resistance occurs at a rate of 25% per year with nearly 100% resistance by 4 years of therapy in HIV co-infected individuals. Resistance to Lamivudine has been associated to a mutation at the YMDD locus but other resistance mutations have been described (e.g. at position 528) and are likely to be differently associated with HBV genotypes. Emtricitabine contains similar resistance properties as Lamivudine. Resistance to Tenofovir has been described, but its incidence in HIV/HBV co-infection is unknown (Dore et al. 1999). At present, there are very few reports

of HBV drug resistance from Africa. In a South African study by Selabe *et al.* (Hoffman CJ et al. 2008), the YMDD motif of the hepatitis reverse transcriptase gene was sequentially evaluated in 17 HBV-monoinfected subjects on lamivudine treatment. A total of 13 subjects had carried YMDD mutations; however, only 5 of these subjects developed clinically significant resistance and treatment failure (Hoffman CJ et al. 2008). Selabe *et al.* (Hoffman CJ et al. 2008) also reported the detection of YMDD mutations in HBV treatment-naive subjects (both HIV-seropositive and -seronegative). Overall, 20% (3/15) of monoinfected HBV subjects and 50% (10/20) of HIV-HBV-coinfected subjects were found to have lamivudine resistance without any known therapy given to the subjects (Puoti 2008).

A Center for Disease Control (CDC) workshop, *Drug-resistant and Vaccine-escape Hepatitis B Virus Mutants: Emergence and Surveillance* in 2010 highlighted the importance of mutant viruses and the challenges they bring in the treatment of Hepatitis B. The significant complication of HIV coinfection along with chronic Hepatitis B in resource-limited countries is concerning as antiretroviral therapy for HIV could become the breeding ground for the development of resistant HBV. There are few data at present on the clinical course, the development of HBV drug resistance or vaccine-escape mutant virus in HIV/HBV coinfecting subjects in resource-limited countries. Prevalence data on HBV treatment efficacy, HBV related drug resistance and especially increased knowledge concerning HBV genotypes related genotypic and phenotypic resistance mechanisms as well as molecular characteristics for immune response or vaccine escape mechanisms from African countries is needed.

### **Human Papillomavirus (HPV)/invasive cervical cancer:**

Cervical cancer is the most common cancer among women in sub-Saharan Africa, where the overall combined prevalence of HPV strains 16 and 18 is approximately 70%. HIV infection significantly increases the incidence of Human Papillomavirus (HPV) associated cervical cancer and other anogenital malignancies even after initiation of highly active antiretroviral therapy (HAART). Cervical cancer screening coverage ranges from 0.4% to 20.2% across the continent (Louie, Sanjose et al 2009), however MHRP associated PEPFAR programs are currently implementing new cervical cancer screening programs in ART clinics, largely based on the practice of visual inspection with acetic acid (VIA), and some sites may also have access to HPV rapid testing methodologies. AFRICOS will study the implementation and outcomes of this new initiative.

Despite the magnitude of the problem, very little is known about the influence of HIV infection and HAART on immune correlates of protection from persistent HPV infection. The increase of HPV associated disease in HIV infected humans implies that defects in the HPV-specific adaptive immune response play an important role in co-pathogenesis. Specimens and data collected in this protocol will be utilized to further elucidate the complicated interplay between HIV, the immune system and HPV induced carcinogenesis in the era of HAART.

### **Stool Pathogens:**

Intestinal helminth infections, schistosomiasis and HIV infection are all highly prevalent in sub-Saharan-Africa and co-infection is common (Brooker 2010; Hotez et al. 2006). Helminth infections are potent inducers of T helper 2 responses with the capacity to modulate the immune response to heterologous antigens (Holland et al. 2000) suggesting that they also



have an influence on the susceptibility to HIV infection and on HIV disease progression (Gopinath et al. 2000; Wolday et al. 2002). Assessment of the gut microbiome has demonstrated that changes in microbiota are associated with HIV disease progression and that dysbiosis contributes to microbial translocation and systemic inflammation in chronic HIV (Vujkovic-Cvijin et al 2013, Dinh et al 2015). Additionally, certain bacterial stool pathogens, such as nontyphoidal Salmonella species, have a disproportionate impact on HIV infected individuals.

### **HIV-diagnostics:**

#### *Point of Care HIV Nucleic Acid Tests*

Once confirmed as HIV-positive, an infected individual moves to the monitoring phase of testing and treatment. Monitoring of HIV infection is based on the amount of plasma viral RNA (viral load) (Nicholson, 1997). Furthermore, HIV-1 nucleic acid tests (NATs) can be used to rule out or confirm true HIV-1 infection in individuals where traditional serologic methodology is no longer a reliable index of HIV-1 infection. HIV-1 NATs are currently used for therapeutic monitoring of HIV-1 infected subjects and for supplemental screening of blood donations. NATs are also used in clinical situations where the sensitivity or specificity of HIV-1 serology is reduced. HIV-1 serology is insensitive in early primary HIV-1 infection. A quantitative plasma HIV-1 ribonucleic acid (RNA) test revealing high viral load can confirm acute HIV infection. When HIV-1 serology is confounded by the prolonged presence of HIV-1 maternal antibody in neonates born to seropositive mothers, qualitative/quantitative plasma HIV-1 RNA or qualitative peripheral blood mononuclear cell (PBMC) proviral DNA tests provide high-specificity confirmatory diagnostic testing.

In support of PEPFAR sites and diagnostics of HIV-1 in acute and chronic infection, this study will evaluate the performance of rapid point-of-care (POC) nucleic acid tests (NAT) described below. A number of assays are in development using different technologies. The desirable attributes for POC NAT assays are rapid, sensitive (>98%), specific (>98%), inexpensive, simple instrumentation, easy execution (minimum training), robust (no refrigeration of reagents) and commercially available.

#### *HIV/HCV/HBV Rapid Diagnostic Tests (RDTs)*

MHRP and PEPFAR have a compelling interest in utilizing the most robust, sensitive and specific screening and diagnostic devices available. Often little published data exists to evaluate performance characteristics of non-FDA approved assays/devices and no data as to the performance in challenging field settings in resource constrained settings. This study will evaluate rapid HCV/ HBV and HIV RDTs (antigen detection tests) as well as 4th generation p24 Ag/HIV antibody RDTs.

#### *Alternative CD4 Technologies*

As antiretroviral therapy (ART) becomes more available in the developing world, there is a growing need to measure CD4+ T-cell counts reliably. A decrease in CD4 T-lymphocytes, the critical immune cell which becomes infected by HIV, is a key factor in determining disease progression and in monitoring treatment (Kovacs & Masur, 2000). The current FDA-approved technologies for monitoring CD4+ T-cell counts are not only complex and time-consuming, requiring highly trained personnel, but expensive as well. These factors severely

limit the ability to monitor HIV disease progression in locations where resources, training, and mobility are limited (Crowe, Turnbull, Oelrichs, & Dunne, 2003). Although the cost of ARVs has fallen, increased access to treatment has raised questions about how best to monitor therapeutic efficacy.

Quality monitoring of HIV-infected individuals is an important policy issue, particularly in countries where health care resources are limited. Ready access to CD4 measurement technologies would have far-reaching public health implications and could provide a less expensive method for measuring CD4+ T-cell counts in resource-poor environments. The Department of Health and Human Services (DHHS) 2009 guidelines recommend CD4+ T-cell measurements every three to four months for subjects on antiretroviral therapy (ART). The CD4+ T-cell count, along with measurement of viral load, is used to monitor the response to antiretroviral (ARV) drugs and to make decisions about the appropriate course of therapy. It is critical that the technology used for immunophenotyping be accurate. To reach many of those in need, cost must also be considered. While ART has been highly effective in reducing the morbidity and mortality rate of HIV/AIDS, the cost of monitoring is prohibitive (Sweat et al., 2000). Current CD4+ T-cell monitoring technologies are complex, time-consuming, and expensive, requiring highly trained personnel. This study will evaluate new alternative easy, cost-effective CD4 methodology – possible examples include the PIMA point of care CD4 analyzer and the Pointcare Now platform.

#### **Cardiovascular and metabolic complications of HIV:**

As the availability of HAART has increased the survival of HIV infected subjects in resource rich settings, chronic diseases such as cardiovascular disease (and its risk factors) have played an increasingly important role in the morbidity and mortality of those with HIV infection. The D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study showed that increased risk of myocardial infarction was associated with traditional cardiovascular risk factors (male sex, family history, smoking, diabetes, hyperlipidemia) but also with previous prolonged treatment with protease inhibitors and abacavir (DAD Study Group, 2008). Other studies have suggested that HAART decreases cardiovascular risk over the short term (El-Sader WM, Lundgren JD et al, 2006) due to improved endothelial function. HIV-related lipodystrophy has also been linked to increased cardiovascular risk as have systemic markers of inflammation in HIV-infected subjects (Triant, Meigs, et al, 2009). The extent to which HIV and its treatment interacts with cardiovascular disease in sub Saharan Africa is largely unknown and AFRICOS presents an opportunity to prospectively evaluate this question.

#### **HIV pathogenesis:**

Data and tissue collected in large long-term cohorts are similarly valuable for conducting basic science research with implications for clinical care and vaccine development. This includes host genetic research: the impact of genetic polymorphisms (such as HLA type) on disease progression, and the effect of host genetic factors on response to therapy (Mann, Carrington et al. 1992; Moore, John et al. 2002). This also includes the immunopathogenesis of HIV (Day, Kaufmann et al. 2006; Douek, Roederer et al 2009). The degree of immune activation for any HIV-1 infected individual is widely variable, but during chronic infection is associated with level of viremia, CD4 T cell counts, and disease progression (Hazenberget al. 2003; Barry 2003; Giorgi et al. 2002; Eggena et al 2005). In addition, striking correlations

between disease progression and immune activation suggest prognostic value of monitoring activation markers for people on antiretroviral therapy (Giorgi et al. 1999; Ondo et al 2005; Tilling et al 2002).

While antibodies are known to play an important role in protection in many viral diseases, the importance of antibodies in human immunodeficiency virus type 1 (HIV-1) protection and pathogenesis remains to be further defined (Moir 2009; Shen 2010). As many previous studies have focused on subtype B, characterization of the development and function of HIV antibodies in subjects infected with both B and non-B subtypes will 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 and development of new therapies.

### **1.3 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 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.

The Makerere University Walter Reed Program (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 the Advancement of Military Medicine of the United States (HJF). Clinical studies were initiated early in 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 from evaluation of the region, and clinical evaluation of these products.

MUWRP's main facility includes administration, data, logistics and the clinic. It is located in Kampala. The MUWRP Central laboratory is located at the campus of Makerere University School of Medicine in Kampala, about 1 km from MUWRP's main facility. MUWRP in conjunction with PEPFAR supports the Kayunga district health services in implementing the HIV treatment and care program. MUWRP/PEPFAR supports 5 HIV clinics. In addition, PEPFAR supports laboratory monitoring of HIV treatment through the Kayunga district hospital laboratory and the MUWRP laboratory at the School of Medicine. MUWRP will oversee the study sample repository and coordinate AFRICOS clinics it routinely supports, starting with the Kayunga District Hospital.

U.S. Army Medical Research Unit – Kenya (USAMRU-K) is a Special Foreign Activity of the Walter Reed Army Institute of Research (WRAIR), Washington, DC. USAMRU-K is affiliated through 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 permanent activity in 1973. Since then, research has been conducted on malaria, trypanosomiasis, leishmaniasis, entomology, HIV/AIDS, and arboviruses, and more than 250 manuscripts published.

In addition to laboratories housed in Nairobi, USAMRU-K has other field sites in western Kenya (Kisumu, Kisian, Kowbewa and Kericho) that are mainly concerned with malaria and HIV/AIDS research.

The United States Army Medical Research Unit of Kenya (USAMRU-K)/ Kenya Medical Research Institute/Walter Reed Project HIV Program (KEMRI/WRP) is located in Kericho at the Kenya Medical Research Institute /Walter Reed Project Clinical Research Center. Within Kenya's largest tea plantations in the African Highlands of the southern Rift Valley Providence, 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 on HIV research in early 2000 given the recognized breadth and depth of HIV disease in Kenya.

For AFRICOS in Kenya, the KEMRI/WRP clinical research center will serve as the reference laboratory and repository center for the country. The KEMRI/WRP CRC will additionally coordinate the conduct of the study at 6 PEPFAR sites in the South Rift Valley. The study will also be conducted by USAMRU-K researchers at the Kombewa District Hospital in Nyanza province and by Kenya Department of Defense investigators at the Armed Forces Memorial Hospital in Nairobi.

The Kenya Defense Force (KDF) is comprised of 3 branches: Army, Navy and Air Force. The USAMRU-K has worked with the KDF for more than 4 years conducting joint missions around the treatment and prevention of HIV, sexually transmitted diseases and TB. The purpose of this collaboration is to maintain the fighting strength of Kenyan soldiers and support and improve the capability and capacity of the KDF health care system.

Walter Reed Southern Highlands HIV Care Program (WRSHCP) is a branch of the MHRP and falls under the larger United States Embassy PEPFAR mission in Tanzania. It is located on the grounds of the Mbeya Referral Hospital (MRH) with a mission to develop and implement a comprehensive community approach to HIV care and treatment in the Southern Highlands of Tanzania. The Mbeya Referral Hospital is the primary location for surveillance and vaccine studies conducted by the MHRP and this is the primary proposed site for implementation of the AFRICOS in Tanzania.

The Mbeya Referral Hospital (MRH), is a Zonal Referral Hospital serving the southern highlands of Tanzania. It has a HIV Care and Treatment Center (CTC) that is supported by

the Walter Reed Southern Highlands HIV Care Program (WRSHCP)/PEPFAR. It serves as a Centre of Excellence and referral point for ARV treatment within the whole Southern Highlands with ~5 million inhabitants. At the MRH CTC ~ 10.000 HIV infected patients are on care, ~5000 patients are on ART and annually ~400 HIV patients are initiating ART. The MRH has a capacity of 477 beds. The functions of Mbeya Referral Hospital are the provision of tertiary healthcare services to referred patients in the Southern Highlands Zone, teaching and conduct of health related research. It has a well-equipped testing laboratory that supports tests that are requested by the hospital clinicians.

The Mbeya Regional Hospital, is a Regional Referral Hospital serving the southern highlands of Tanzania. It has a HIV Care and Treatment Center (CTC) that is supported by PEPFAR. The Mbeya Regional Hospital CTC serves as a referral point for 327 health facilities serving an estimated population of 2.7 million people. At Mbeya Regional Hospital CTC, 69 HIV infected patients are on care, 2707 patients are on first line management, and 86 are on second line management. Its functions are the provisions of broad specialty and tertiary healthcare services to referred patients in the Southern Highlands Zone. It has a fully running laboratory for routine hospital clients that support tests that are requested by the hospital clinicians.

The Mbeya Medical Research Centre (MMRC) 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 MMRC collaborating partners to enable MMRC to become a NIMR research centre. With this Memorandum the new NIMR-MMRC research centre has now full legal status. MMRC 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 MMRC is to evaluate new interventions for these diseases utilising vaccines, drugs or diagnostics.

Department of Defense Walter Reed Program Nigeria (DOD WRP-N) is led by a subordinate unit of the Division of Retrovirology forward deployed to the United States Embassy, Abuja, Nigeria, and partnered with the Henry Jackson Foundation Medical Research International, GTE, LTD, to support MHRP (Nigeria) operations. The DOD WRP-N functions as both a US Government Agency, partnered directly with Centers for Disease Control and Prevention, United States Agency for International Development and State Department to formulate, direct and manage the Nigeria PEPFAR Country Operating Plan (COP) as well as implement the Department of Defense portion of the COP directly with the Nigerian Ministry of Defense (NMOD). By implementing the PEPFAR project, the NMOD and MHRP (Nigeria) have constructed an ethical and technical platform on which to launch investigative activities.

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 (HMJF) and sponsored by the United States Army Medical Research and Materiel 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/ HJMF) 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.

## **2 STUDY OBJECTIVES**

### **2.1 Primary Objective**

To longitudinally assess the impact of clinical practices, biological factors and socio-behavioral issues on HIV infection and disease progression in an African context.

### **2.2 Secondary Objectives**

#### **A. Social and behavioral domain**

- 1) Describe, among HIV infected subjects and their families, stigmatizing events and social and economic harms attendant to HIV care and treatment; evaluate their impact on care seeking behaviors, HIV treatment response and disease progression
- 2) Describe adherence to HIV care and treatment and evaluate for predictors/determinants of adherence
- 3) Describe HIV risk behaviors in the study population
- 4) Describe cultural barriers and facilitators of HIV prevention, care and treatment

- 5) Describe the impact of behavioral treatment strategies (including but not limited to status disclosure, treatment partners and support groups) on HIV clinical outcomes
  - 6) Describe the impact of substance use on HIV infection and disease outcomes
  - 7) Describe the impact of incarceration and/or institutionalization on HIV treatment and outcomes
- B. Medical-HIV prevention and management (programmatic)
- 1) Identify attributes of HIV care and treatment programs associated with optimal clinical outcomes (including, but not limited to, organization, location, accessibility, logistic support, compliance with MOH guidelines, and drug distribution)
  - 2) Describe clinical features of HIV disease (including, but not limited to, disease progression, response to therapy, regimen durability, development of resistance, change in viral tropism, ART population pharmacokinetics, mortality) as a function of program parameters
  - 3) Describe the implementation and uptake of preventive interventions in HIV infected and uninfected subjects
  - 4) Describe barriers to subject retention and strategies for its enhancement
- C. Medical-HIV management (subject)
- 1) Describe HIV disease outcomes, including, but not limited to, mortality, progression to AIDS, event-free survival, and prevalence/incidence of HIV related sequelae
  - 2) Describe HIV treatment monitoring practices and impact on disease outcomes
  - 3) Describe frequency and amplitude of transient viremia (viral load “blips”) and relation to disease outcomes
  - 4) Describe HIV disease progression and treatment response for different subtypes of HIV
  - 5) Compare accuracy and utility of alternative field expedient HIV diagnostic, viral load and lymphocyte measurement testing platforms to gold standard assays
  - 6) Describe frequency and character of HIV resistance mutations and impact on disease outcomes
  - 7) Describe frequency and character of HIV resistance mutations as associated with viral subtype and prior exposure to antiretrovirals
  - 8) Describe the impact of food insecurity on HIV disease outcomes
  - 9) Describe HIV drug related toxicity and interactions
- D. Medical-opportunistic infections and other morbidities
- 1) Describe the type and frequency of other co-morbidities (including, but not limited to, malignancy, cardiovascular events, malnutrition, anemia, renal insufficiency, and cognitive decline), their impact on HIV disease outcomes, and the impact of HIV treatment on their clinical outcomes
  - 2) Describe the prevalence and incidence of cardiovascular risk factors (including

but not limited to hypertension, lipid abnormalities, systemic inflammation and impaired glucose metabolism), their impact on subject outcomes

- 3) Describe practices of chronic disease management in the PEPFAR setting
- 4) Describe the type and frequency of endemic infections and evaluate their interplay with HIV disease (including, but not limited to tuberculosis, viral hepatitis, malaria and other etiologies of febrile illness, human papillomavirus and other STIs, and stool pathogens)
  - i. Tuberculosis
    1. Estimate the prevalence and incidence of active tuberculosis and rifampicin resistance in HIV infected subjects in the PEPFAR clinical setting
    2. Establish the predictive value of a positive interferon gamma release assay in HIV positive subjects for the development of active TB
  - ii. Human papillomavirus and other STIs
    1. Describe the implementation and outcomes of cervical cancer screening delivered in the PEPFAR setting, to include visual inspection with acetic acid, histopathology based screening (PAP smear), and qualitative rapid HPV tests
    2. Characterize the interplay between HIV, the immune system and HPV induced carcinogenesis in the era of HAART
    3. Describe the prevalence of ulcerative and nonulcerative STIs and their association with HIV disease
  - iii. Viral hepatitis
    1. To investigate the prevalence and incidence of HBsAg, HBeAg, HBcAb and HBV DNA viremia in HIV infected and uninfected subjects
    2. To determine the HBV subtype in HBV viremic participants
    3. Describe the prevalence of increased hepatotoxicity/hepatic flare in participants with acquired HBV viremia/resistance mutations with treatment
    4. Characterization of genotypic (and phenotypic) HBV resistance patterns in HBV viremic participants in relation to the HBV genotypes and clinical characteristics
  - iv. Malaria
    1. Compare incidence of symptomatic malaria infections in HIV-infected and HIV-uninfected individuals
    2. Describe the clinical presentation of acute malaria in HIV-infected persons and compare with presentation in HIV-uninfected individuals
    3. Measure level of parasitemia and gametocytemia in HIV-infected persons versus HIV-uninfected persons
    4. Evaluate the role of antiretroviral therapy and cotrimoxazole on clinical and laboratory aspects of malaria infection in HIV positive subjects
  - v. Stools pathogens – describe the prevalence of helminth and bacterial



- stool pathogens and their impact on HIV disease outcomes
- vi. Describe the test characteristics for rapid diagnostic tools for coinfections (including but not limited to Hepatitis B, Hepatitis C, malaria, and tuberculosis) as they apply to the PEPFAR setting
- 5) Describe the type and frequency of opportunistic infections and their impact on disease outcomes
  - 6) Describe opportunistic infection prevention practices (including but not limited to isoniazid preventive therapy, cotrimoxazole and fluconazole prophylaxis) and their impact on disease outcomes
  - 7) Describe the frequency, character and outcome of IRIS and its impact on disease outcomes
- E. Medical-Maternal-child transmission management
- 1) Describe transmission rates by HIV disease severity and presence of other HIV related disease
  - 2) Describe PMTCT program attributes including adherence and relation to transmission rates
  - 3) Describe interaction of prior ART for PMTCT or subject treatment on transmission outcome
  - 4) Describe reproductive health practices and access to family planning.
  - 5) Evaluate for the determinants of care-seeking behavior in the PMTCT care population
  - 6) Describe the impact of coinfections on disease outcomes and HIV transmission
- F. Medical-Prevention of horizontal HIV infection
- 1) Describe the impact of HIV preventive interventions on risk behaviors
  - 2) Describe prevention with positives interventions and their impact on HIV transmission in serodiscordant partners
  - 3) Describe HIV preventive interventions and uptake in individuals undergoing HIV counseling and testing
  - 4) Describe the prevalence and incidence of coinfections, chronic diseases and other comorbid health conditions in HIV uninfected individuals as compared to HIV infected individuals
- G. Medical-host genetics and pathogenesis
- 1) Describe HLA and other key host genetic markers known to associate with HIV disease acquisition, progression or response to therapy
  - 2) Define host genetic markers which relate to HIV acquisition, progression, or response to therapy
  - 3) Define immunologic and viral factors which are associated with HIV acquisition, disease progression, or response to therapy
  - 4) Investigate markers of systemic inflammation as they relate to HIV disease and its progression

## **3 DESIGN AND METHODOLOGY**

### **3.1 Summary of Methods**

The design is an open-ended cohort study with retrospective data collection at enrollment and subsequent prospective serial data collection and routine phlebotomy for the life of the study, with study visits occurring every 6 months. Every attempt will be made to collect data and samples in a standardized fashion across the sites. In addition to clinical tests at each visit, phlebotomy samples will be processed into serum, plasma, and peripheral blood monocytes (PBMC) to be archived in the AFRICOS Repository (AFRICOSR). Sputum samples will also be archived in the AFRICOSR. Dried blood spot cards, swab and stool samples will also be stored pending their analysis. The specimen component of AFRICOSR will be comprised of freezer storage capacity managed by KEMRI/WRP in Kenya, MUWRP in Uganda, MMRP in Tanzania, and DODHPN in Nigeria. Use of AFRICOSR specimens by any investigator must be conducted under the AFRICOS protocol or an AFRICOS substudy as approved by both the WRAIR IRB and the local IRB/ERC for country of specimen origin. The data component of AFRICOSR will be comprised of the central AFRICOS database maintained at MHRP headquarters. Analyses of collected data and samples conducted under the main AFRICOS protocol will be largely descriptive and hypotheses will be exploratory.

### **3.2 Overview of Subject Activities**

At each visit, all subjects will receive a medical history and physical, a questionnaire, and provide blood and urine as indicated for clinical tests relating to general health and screening for infections or other co-morbidities. All subjects will also undergo phlebotomy for repository storage of plasma, serum, and PBMC. Known HIV infected participants will receive serial HIV disease monitoring and will provide sputum samples for tuberculosis diagnosis. Optional stool samples will be requested from subjects. HIV infection status will be assessed upon enrollment and those participants who are HIV uninfected at the time of enrollment will receive serial HIV counseling and testing per local Ministry of Health guidelines. All subjects will be requested to notify study personnel if they are acutely ill or hospitalized.

### **3.3 Laboratory Methods**

Specimens collected for this study will be used for diagnostic testing and for exploratory laboratory evaluations corresponding to the study secondary objectives. See Appendix I & II: Schedule of Events for a schedule of study tests and Section 7.4 Laboratory Testing / Methods under Section 7 Sample Disposition for details.

## **4 STUDY POPULATION**

In meeting enrollment targets for HIV infected and HIV uninfected participants, the study sites will aim to enroll 1) Participants in previous vaccine, therapeutic and cohort studies at those locations, and 2) participants drawn from the general PEPFAR client population.

Table 1: AFRICOS Sites	Total enrollment target	HIV infected	HIV uninfected
Kenya: South Rift Valley Province	1200	1000	200
Kenya: Kisumu West, Nyanza Province	600	500	100
Uganda: Kayunga and Mukono Districts	600	500	100
Tanzania: Southern Highlands HIV Care Program	600	500	100
Nigeria: Dept of Defense HIV Program	450	375	75
Kenya: Kenya Defense Force	150	125	25

Participants in previous MHRP / PEPFAR studies are particularly valuable, as often very important information about the history of HIV infection or lack thereof has been captured in those studies. For many, this includes the time and circumstance of their HIV infection, yielding important sero-incidence data that facilitates the generation of accurate data on disease progression. Similarly, blood samples have been collected from these participants earlier in the course of disease, which may be available for comparison to address a variety of scientific questions. Enrolling these individuals in AFRICOS, with subsequent linkage to the products of previous studies, generates important immediate data.

For the portion of enrollment drawn from the general PEPFAR clinic population, the HIV infected participant composition will proportionally reflect the ratio of females to males and the ratio of subjects on ART to those on care only at each of the PEPFAR sites. This can be accomplished through the use of clinic registries. Replacement enrollments for those subjects who die, withdraw or are lost to follow-up will reflect the female to male ratio of losses, however this enrollment will focus on enrolling subjects early in the stage of disease so as to balance the overall trend toward advanced disease that will occur with the disease progression of the original cohort.

Enrollment of members of known serodiscordant couples will also be an aim for sites where such couples are followed. This will facilitate the evaluation of HIV prevention interventions at those locations.

To recruit subjects who are HIV uninfected and not prior study participants, HIV uninfected adult partners of HIV infected AFRICOS enrollees will be offered enrollment. Additional seronegative adults will be recruited from the community and through program counseling and testing activities.

#### 4.1 Inclusion Criteria

To achieve core protocol goals of evaluating factors related to HIV disease outcomes over time and of creating a repository to be used as a resource in future investigations key to advancing HIV related care in the PEPFAR setting, the study aims to include subjects for whom long term outcomes can be collected and who are willing to contribute to this repository resource. Participants enrolled into the HIV infected component of AFRICOS must meet all of the following criteria:

- 1) Known HIV infection
- 2) Adult aged 18 years or older
- 3) Ability and willingness to sign/mark/thumb print the informed consent form.
- 4) Intends to be a long term resident of the area and to undergo long term HIV care at a participating clinic (i.e., for at least 24 months)
- 5) Willing to provide location or contact information and to be contacted by study staff
- 6) Consents to data and specimen collection and storage for future use
- 7) Must understand English or local language as approved by IRB.

Participants enrolled into the HIV uninfected component of AFRICOS must meet all of the following criteria:

- 1) Consents to HIV testing and to pre- and post-test counseling.
- 2) Adult aged 18 years or older
- 3) Ability and willingness to sign/mark/thumb print the informed consent form.
- 4) Intends to be a long term resident of the area (i.e., for at least 24 months)
- 5) Willing to provide location or contact information and to be contacted by study staff
- 6) Consents to data and specimen collection and storage for future use
- 7) Must understand English or local language as approved by IRB.

## **4.2 Exclusion Criteria**

A volunteer will be excluded from enrollment in the HIV infected component of AFRICOS if one or more of the following conditions apply:

- 1) 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
- 2) The subject is known to be pregnant
- 3) The subject is currently a prisoner

A volunteer will be excluded from enrollment in the HIV uninfected component of AFRICOS if one or more of the following conditions apply:

- 1) The subject is known to be HIV infected
- 2) 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
- 3) The subject is known to be pregnant
- 4) The subject is currently a prisoner

## 5 STATISTICAL CONSIDERATIONS

### 5.1 Power and Sample Size

The primary objective of the study is descriptive and the hypotheses tested will be exploratory. Therefore, the enrollment targets for AFRICOS were determined by the relative size of each WRAIR supported PEPFAR Program, research capacity at each site, and available resources. In the future, as additional ART provision sites in the WRAIR PEPFAR catchment areas develop research capacity, this protocol may be amended to expand enrollment at these sites to achieve increased breadth of the evaluation of HIV infection, disease progression, and prevention/treatment practices at WRAIR PEPFAR supported clinics.

Key objectives of the protocol include evaluating the impact of hepatitis B, TB, and malaria on the cohort population. Chronic hepatitis B prevalence has been in the past estimated at 10% in sub-Saharan Africa. As enrollment is currently planned for 3000 HIV infected subjects, there is excellent statistical power to detect clinical diagnoses of public health significance across the entire study HIV infected population. For conditions with true prevalence of 10% there is a 95% probability that the study will observe a prevalence of 8.95% to 11.13% (upper and lower confidence limits). However, to meet the PEPFAR evaluation goals of this protocol it is important to evaluate disease burdens within different programs. To observe a 10% prevalence within an enrollment site, 95% confidence limits will be 8.21%-12.03% for sites with enrollment of 1000, 7.51-12.97% for sites with an enrollment of 500, 7.16%-13.49% for sites with an enrollment of 375, and 5.36%-16.65% for sites with an enrollment of 125. For our anticipated HIV uninfected enrolled population of 600, there is a 95% probability that a condition with a true prevalence of 10% will be observed within the confidence limits of 7.72%-12.68%.

TB incidence has been estimated to be as low as 1% in the HIV-infected African population. In this HIV infected cohort, for conditions with a true incidence of 1%, there is a 95% probability that the study will observe an incidence of 0.68% to 1.42%. In the highly endemic areas where the study will conduct malaria surveillance, prevalence has been estimated in a

wide range of 13 to over 40%. Enrolling 1500 subjects (HIV infected and uninfected) at participating sites, given a true malaria prevalence of 20%, the study will have a 95% probability of estimating the prevalence between the upper and lower confidence limits of 18.0 and 22.12%.

To compare the difference between the prevalence of a condition or occurrence (for example the prevalence of circumcision) between HIV infected and uninfected subjects, give an anticipated enrollment of 600 HIV uninfected participants, the study has 74% power to detect a difference of 15% between the two populations and 93% power to detect a difference of 20%.

In low-income country antiretroviral therapy clinics, annual rates of loss to follow-up (LTFU) can range from zero to 44% (Braitstein, Brinkhof et al. 2006). However, MHRP research sites have had remarkably good retention among enrollees – for instance, since the inception of the WRP/KEMRI clinical research center in Kericho, the cumulative loss to follow-up of protocol participants has been 7.2%. Therefore we anticipate that 10-15% of the initial enrollees in AFRICOS will be LTFU over the course of the study and will plan to enroll a 15% overage after the first year.

## **5.2 Analysis of Primary Objective**

The analysis of the primary objective will be descriptive, with the use of basic descriptive statistics (including, but not limited to frequencies, measures of central tendency, and ranges) and the calculation of prevalence and incidence rates of documented diseases.

## **5.3 Data Analysis for Secondary / Exploratory Objectives**

The analysis of secondary objectives will be exploratory and employ the descriptive methods described in 5.2 as well as standard statistical tests for correlations and comparisons (to include survival analysis) and adjustment for confounding variables. The precise statistical plan can only be formulated after the receipt of data. However the initial anticipated approach to exploring the secondary objectives involves multiple statistical tests and methods. Univariate analysis will typically utilize Pearson chi-squared and Fisher's exact test for categorical variables and Wilcoxon or independent samples T-test or for continuous variables. To compare incidence rates, incidence rate ratios will be calculated to describe relative risk and, where appropriate, population attributable risk will be calculated. To adjust for confounding impacts on outcomes, multivariate logistic regression analysis may be employed to generate adjusted odds ratios. Also, survival analysis, including the Cox proportional hazards method, may be used to calculate hazard ratios and the log rank test also used in the comparison of outcomes between groups. Statistical tests will be two-sided and p-values less than 0.05 will be considered statistically significant.

Current analysis plans for secondary objectives include:

For those HIV infected enrollees on HAART, we will evaluate the role of demographic factors, visit adherence, pill adherence, and regimen choice on attainment of viral suppression. This would initially include the comparison of the categorical variables in

univariate chi-squared based testing and then subsequent development of a multivariate logistic regression model to generate adjusted odds ratios. A similar methodology would be used to evaluate the impact of adherence, prior nevirapine exposure, and prior transient viremia on the development of ARV resistance mutations.

For HIV infected and uninfected individuals, we will compare the incidence of coinfections and comorbidites. For example, in comparing the incidence of symptomatic malaria episodes over time in HIV infected and HIV uninfected subjects, incidence will be expressed in number of episodes per person-year and incidence rate ratios calculated to describe relative risk. Adjusted odds ratios will be calculated by using multivariate logistic regression to adjust for potential confounders such as ongoing co-trimoxazole use. As the study progresses, we plan undertake comparative survival analysis using the Cox proportional hazards model to investigate the role of demographic factors, duration of antiretroviral therapy, and type of antiretroviral therapy on the development of comorbidities such as cognitive impairment and cardiovascular disease.

## **6 STUDY PROCEDURES**

### **6.1 Volunteer Recruiting**

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.

Potential participants will be identified from databases of prior MHRP studies conducted at that site. They will be contacted directly and offered enrollment. Potential participants will also be identified among the PEPFAR clinic population. This includes those receiving care and/or treatment at the HIV clinic and those participating in counseling and testing programs. Enrollees will be encouraged to bring their partners in for HIV testing and those partners may also be recruited for the study. HIV negative subjects may also be recruited from the community served by the enrolling PEPFAR clinic.

### **6.2 Enrollment Study Visit**

The enrollment visit will commence with a detailed briefing provided by the site principal investigator (PI), an associate investigator (AI) or their designee (qualified staff such as, a study medical officer, study nurse, or counselor). The briefing will review general information about HIV, HIV research, as well as the purpose of the study, the study design, risks, benefits, compensation, and volunteer rights. A question and answer period will follow the briefing.

Interested participants will then undergo a detailed review of the informed consent form by research study staff and will be asked to provide consent to participate in the study by signing or marking the Informed Consent Form (ICF). The consent to participate will include consent

to store their samples for future use, consent to ship their samples internationally, and consent to access data and specimens from prior MHRP studies in which they have participated. Participants will also have the option to provide or decline consent for their samples to be used in genetic testing.

Following the completion of informed consent, contact information will also be collected from the volunteer at this visit. The volunteer will be asked to provide their contact information using a contact information form. This form will 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 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 used for contacting volunteers who do not return for their scheduled visits, or those who may move away from their places of residence.

Subsequently, the study staff will collect medical history and medication information, socioeconomic data, functional and cognitive ability data, quality of life measurements, and behavioral information with the use of a medical history review with the subject, an interviewer-administered questionnaire and medical record abstraction (medical record abstraction may also be performed in the days surrounding the study visit). Clinic providers may be queried for clarification. The questionnaire will include a screen for depression (the Center for Epidemiologic Studies Depression Scale, CES-D) and brief evaluation of neurocognitive capacity will be conducted. For female volunteers, a women's health and obstetric history forms capturing data regarding childbearing and vertical HIV transmission will be completed on enrollment, and this data will be updated during subsequent study visits. The HIV infected participant will undergo laboratory and other clinical assessments consistent with routine clinical HIV care at the study site, as well as study-related research assessments and phlebotomy for repository. The HIV uninfected participant will undergo HIV counseling and testing as well as study-related research assessments and phlebotomy for repository.

Laboratory testing will vary according to site capacity and participation in relevant evaluations (e.g., malaria evaluations are planned to be performed only at sites with known high malaria endemicity). The following procedures will be conducted at the enrollment visit.

- Briefing about the study
- Administration of informed consent in a private / designated space
- Demographic, biometric, and contact information
- Medical history review (including medications)
- Enrollment volunteer questionnaire (with women's health form for females)
- Retrospective medical record abstraction
- Vital signs (height, weight, temperature, blood pressure, heart rate)
- Physical exam
- Neurocognitive evaluation
- Cervical cancer screening (female subjects only) – visual inspection of the cervix with acetic acid or lugol's iodine will be performed at all sites.



Additionally, expanded cervical cancer screening with PAP smear and/or HPV testing will be performed at participating sites.

- Pregnancy test (female subjects only)
- HIV counseling and testing for HIV uninfected subjects only, according to local guidelines - rapid diagnostic test algorithm with pre and post-test counseling. HIV infected subjects will undergo testing as well to confirm HIV infection on enrollment.
- Routine laboratory and clinical assessments as per routine site practice to include
  - CD4 count or lymphocyte profile (HIV infected subjects only)
  - Serum creatinine or chemistry panel
  - ALT or liver enzyme panel
  - Hemoglobin or complete blood count
- Study-related laboratory and clinical assessments
  - All subjects:
    - Diagnostic testing for syphilis, hepatitis B and hepatitis C
    - Dried blood spot collection for malaria PCR and antibody testing, obtained by fingerstick or venipuncture (participating sites only)
    - Malaria thick and thin film slide (immediately read for febrile subjects only, at participating sites)
    - Optional stool specimen for evaluation of helminths and other stool pathogens
    - Fasting glucose and lipid profile
  - HIV infected subjects:
    - rapid cryptococcal antigen assay
    - TB interferon gamma release assay (ART naïve subjects only)
    - HIV viral load measurement and, for those subjects with VL  $\geq 1000$  copies/mL, HIV drug resistance genotype with *pol* subtype
    - Sputum sample for detection of active tuberculosis (all sites)
- Phlebotomy for repository

Total phlebotomy will be up to 90 ml for the enrollment visit. If there is clinical concern for anemia (e.g., conjunctival pallor), blood will not be drawn for repository storage unless hemoglobin testing demonstrates a normal value (utilizing locally established reference ranges). If co-enrollment in AFRICOS and other studies require greater than 450 mL of blood in a 56-day period, a lower phlebotomy target will be set for participants such that this limit is not exceeded. This will not preclude recording results of clinically indicated tests as ordered per the subject's healthcare provider.

If a subject is unable to complete the processes of the enrollment visit, they may be asked to return to the enrolling site to finish visit activities. This is particularly relevant to instances when the subject presents to the site late in the workday, when phlebotomy cannot be completed prior to courier departure.

Surrounding the enrollment visit, the study nurse/coordinator will use available volunteer and clinic medical records to perform retrospective medical record abstraction, the results of which will be recorded in the Case Report Forms.

### 6.3 Subsequent Study Visits

At subsequent study visits, medical and socio-demographic information will be updated with the use of subject interview, volunteer questionnaire, and medical record inspection. Clinic providers may be queried for clarification. Subjects will undergo physical exams, lab evaluations, and repository phlebotomy according to the Schedule of Events.

Study visits are aimed to coincide with routine clinical care visits. Generally, routine clinical care and treatment for HIV and acute illnesses will be performed by clinic staff as per usual local standard of care. Study staff record the results of the routine clinical care (e.g., lab results, therapy prescribed) and conduct the remainder of study procedures. Study staff also facilitate the reporting of study-related clinical evaluations that are not part of standard care (e.g., genotype resistance testing) to the clinical staff and are available to assist with interpretation and application of those results.

#### 6.3.1 Six month visits

At subsequent 6-month routine study visits (i.e., 6 months, 18 months, etc) the following procedures will be completed:

- Social and demographic information updated
- Medical history information updated (including medications)
- Subsequent visit volunteer questionnaire (with women's health form for females)
- Vital signs (height, weight, temperature, blood pressure, heart rate)
- Physical exam
- HIV counseling and testing according to local guidelines (for HIV uninfected subjects only)
- Routine laboratory and clinical assessments as per routine site practice, to include
  - CD4 count or lymphocyte profile (HIV infected subjects only)
  - Serum creatinine or chemistry panel
  - ALT or liver enzyme panel
  - Hemoglobin or complete blood count
- Study-related laboratory and clinical assessments
  - All subjects:
    - Dried blood spot collection for malaria PCR testing obtained by fingerstick or venipuncture (participating sites only)
    - Malaria thick and thin film slide (immediately read for febrile subjects only, at participating sites)
  - HIV infected subjects:
    - HIV viral load measurement and, for those subjects meeting the threshold for resistance testing, an HIV drug resistance genotype with *pol* subtype

- Sputum sample for detection of active tuberculosis (at sites conducting q6month TB surveillance)

Total phlebotomy for 6-month subsequent visits will be up to 90 ml. If there is clinical concern for anemia (e.g., conjunctival pallor), blood will not be drawn for repository storage unless hemoglobin testing demonstrates a normal value (utilizing locally established reference ranges). If co-enrollment in AFRICOS and other studies require greater than 450 mL of blood in a 56-day period, a lower phlebotomy target will be set for participants such that this limit is not exceeded. This will not preclude recording results of clinically indicated tests as ordered per the subject's healthcare provider.

The threshold for resistance testing will be defined as two viral load measurements  $\geq 1000$  copies/mL at least 4 weeks apart – both drawn while the subject has been on antiretroviral therapy for at least 4 weeks.

### **6.3.2 Annual visits (*every 12 months*)**

At subsequent “annual” routine study visits (i.e., 12 months, 24 months, 36 months, etc) the following evaluations will be completed:

- Social and demographic information updated
- Medical history information updated (including medications)
- Subsequent visit volunteer questionnaire (with women's health form for females)
- Vital signs (height, weight, temperature, blood pressure, heart rate)
- Physical exam
- Neurocognitive evaluation
- HIV counseling and testing according to local guidelines (for HIV uninfected subjects only)
- Cervical cancer screening (female subjects only) – visual inspection of the cervix with acetic acid or lugol's iodine will be performed at all sites. Additionally, expanded cervical cancer screening with PAP smear and/or HPV testing will be performed at participating sites.
- Routine laboratory and clinical assessments as per routine site practice to include
  - CD4 count or lymphocyte profile (HIV infected subjects only)
  - Serum creatinine or chemistry panel
  - ALT or liver enzyme panel
  - Hemoglobin or complete blood count
- Study-related laboratory and clinical assessments
  - All subjects:
    - Diagnostic testing for syphilis and hepatitis B
    - Dried blood spot collection for malaria PCR testing obtained by fingerstick or venipuncture (participating sites only)
    - Malaria thick and thin film slide (immediately read for febrile subjects only, at participating sites)
    - Optional stool specimen for evaluation of helminths and other stool pathogens
    - Fasting glucose and lipid profile

- HIV infected subjects:
  - HIV viral load measurement and, for those subjects meeting the threshold for resistance testing, an HIV drug resistance genotype with *pol* subtype
  - Sputum sample for detection of active tuberculosis (all sites)
- Phlebotomy for repository

Total phlebotomy will be up to 90 ml for the annual subsequent visits. If there is clinical concern for anemia (e.g., conjunctival pallor), blood will only be drawn for indicated clinical tests and not for repository storage unless hemoglobin testing demonstrates a normal value (utilizing locally established reference ranges).

There will be a three-month window on either side of the target dates for subsequent study visits. If a subject misses an annual visit, the annual visit schedule of events will be applied to their next study visit (e.g., if the subject misses the 12 month visit, the 18 month visit will feature the longer evaluation that would have occurred at the 12 month visit). Following this visit, the standard schedule will resume.

If a subject is unable to complete the processes of the subsequent study visit, they may be asked to return to the enrolling site to finish visit activities.

Case Report Forms completed at study visits will also collect results of evaluations that are performed for clinical indications over the course of the volunteer's usual care but that are not performed as part of the study's schedule of events. This includes but is not limited to results from the following evaluations: chest radiography, glycosylated hemoglobin measurement, toxoplasmosis serology, herpes simplex virus I/II serology, cytomegalovirus serology, G6PD (glucose-6-phosphate dehydrogenase) level, erythrocyte sedimentation rate, malaria rapid diagnostic tests and smears, urinalysis, additional pregnancy tests, mycobacterial smear/culture/sensitivity testing, additional HIV viral loads or HIV resistance tests, additional CD4 counts or lymphocyte profiles, stool exams for ova and parasites, stool culture, blood and other body fluid culture, Chlamydia/gonorrhea and other STI diagnostic tests.

## 6.4 Hospitalizations and Acute Visits

Study subjects will be encouraged to notify team members if they seek urgent medical care and/or are hospitalized. When possible, study team members will complete a hospitalization/acute visit case report form recording the clinical aspects of the subject's presentation, the appropriate syndromic diagnosis, and any lab or imaging confirmed diagnosis, as well as subject treatment. If these episodes are not documented at the time they occur, the CRF will be completed at the time of the next scheduled study visit.

At sites evaluating malaria coinfection, additional evaluations will be performed during febrile acute episodes: blood will be collected for performing a malaria smear (thick and thin) – the smear and residual blood sample will be archived, blood spots on filter paper will also be collected for laboratory analysis, and an additional Acute Febrile Illness CRF will be

completed. Anti-malarial treatment will be given as indicated according to local standards of care.

## **6.5 Site Visits**

On an annual basis, the Site PI will visit each study site and complete a site visit case report form. This will collect descriptive information about the site and programmatic data to include site practices for HIV monitoring and treatment, preventive interventions, pharmaceutical distribution, and other pertinent aspects of PEPFAR service delivery. Additionally, analysis of study data will utilize site-related data collected for administrative/reporting purposes by MHRP PEPFAR headquarters.

## **6.6 Death and Lost to Follow up (LTFU)**

LTFU will be defined as being 360 or more days late to a clinic visit despite efforts made by the study team to facilitate return to the site. Site study personnel will conduct LTFU investigations, which will use local vital records, visits to the participant's place of residence, and other resources to determine the circumstances surrounding the LTFU. A Status Change CRF will be completed following this investigation or immediately upon the report of a participant's death. The CRF specifies the reasons for LTFU, including fields for documenting the cause of subject death.

# **7 SAMPLE DISPOSITION**

## **7.1 Labeling**

On blood collection, all research and repository blood tubes will be labeled on site using pre-printed barcode labels 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. In addition to the study number, the labels will also describe the specimen type and the date and study visit obtained. Stool specimens will also be labeled with these barcode labels. On arrival at the site laboratory, all research specimens will be logged on a reception database.

## **7.2 Handling**

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

For distal sites, blood and stool samples will be transported in sealed boxes on the day of collection with appropriate packaging to control temperature within the limits required as per specimen. 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 personal protective equipment, including lab coats and gloves. Processing will be conducted using laboratory SOPs to ensure no cross contamination and/or under laminar flow hoods.

Following processing, 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.

### **7.3 Storage**

Plasma will be separated from the cells by centrifugation of the tubes, aliquoted and stored at  $-70^{\circ}\text{C}$  or in a liquid nitrogen freezer. Serum will be separated from coagulated blood and aliquoted as per visit requirements and stored at  $-70^{\circ}\text{C}$  or in a liquid nitrogen freezer. PBMC will be isolated and cryopreserved in aliquots of 5 to 10 million cells per vial and stored in the vapor phase of a liquid nitrogen freezer. Stool will be homogenized, buffered and frozen at  $-70^{\circ}\text{C}$  or in a liquid nitrogen freezer. All storage 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 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.

All samples for which additional material is available after study specified testing is complete may be stored for future testing in the AFRICOSR. 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.

### **7.4 Laboratory Testing/ Methods**

#### **7.4.1 Clinical diagnostics**

Clinical assays will be performed for general health assessment, HIV diagnosis and monitoring, metabolic assessment, and for coinfection diagnosis. Clinical assays will be performed either at CAP-certified MHRP research labs or at PEPFAR site clinic labs undergoing external quality assurance. Clinical assays will be approved by the FDA, CE, or local Ministry of Health and their results reported to the subject's healthcare provider.

#### **7.4.2 Basic lab evaluation to describe general health**

This evaluation is performed as part of the care of HIV infected individuals at PEPFAR antiretroviral clinics, and this protocol will capture the results of these assays as they are routinely generated at study visits every six months. Depending upon the capacity of the clinical lab at the enrolling site, these parameters may be very basic or may be reported in comprehensive test panels (e.g, small district hospitals may measure only hemoglobin as a hematologic evaluation whereas referral hospital labs may perform a complete blood count with or without differential). This study will collect the specified basic parameters at all sites and when a more complete panel is routinely performed, that will be recorded as well. This basic evaluation includes: hematologic evaluation (hemoglobin, alone or as part of a complete

blood count with or without differential); serum chemistry (creatinine, alone or as part of clinical chemistry panel); and liver function tests (ALT, alone or as part of a liver function panel).

### **7.4.3 HIV Diagnostics**

HIV uninfected subjects will undergo HIV diagnostic testing at every visit. This will be performed in accordance with host country national HIV testing guidelines, which are comprised of an algorithm of rapid diagnostic tests. In rare cases when rapid diagnostic test results are equivocal or conflicting, a blood sample may be referred to the local reference and/or MHRP research lab for HIV EIA, Western Blot, and if indicated, viral load measurement. Samples from subjects who have a previous diagnosis of HIV infection and have a negative HIV test result upon enrollment at the site will be also be referred for further diagnostic evaluation of HIV status.

HIV infected subjects will be routinely evaluated by standard clinical flow cytometric methods for absolute CD4+ T lymphocyte count, alone or as part of a lymphocyte profile, as part of their routine care at the enrolling PEPFAR clinic, and these results will be recorded at every study visit. HIV infected subjects will also provide blood specimens for viral load measurement by nucleic acid amplification technology in standard clinical assays. Blood specimens collected at baseline and then subsequently from subjects experiencing persistently elevated viral loads while on ART will also undergo genotypic resistance testing by viral sequencing in standard clinical assays.

### **7.4.4 Metabolic Assessment**

Subjects will be evaluated annually for lipid abnormalities and impaired fasting glucose/diabetes with lipid (total cholesterol alone or as part of lipid panel including HDL, LDL, and/or triglycerides) and fasting glucose measurements. Elevated fasting glucose measurements may prompt the treating clinician to pursue additional evaluation for diabetes with the measurement of glycosylated hemoglobin, and these results would be extracted onto a laboratory case report form.

### **7.4.5 Coinfection Evaluation**

In addition, co-infecting pathogen screening (syphilis, hepatitis B, hepatitis C, *Cryptococcus*, malaria, stool pathogen and tuberculosis) assays will be performed. Syphilis screening will be conducted annually with nontreponemal rapid plasma reagin (RPR) or venereal disease research laboratory (VDRL) assays. If positive, RPR or VDRL titers will be performed, as well as a confirmatory treponemal syphilis test (such as FTA-ABS or MHA-TP or TP-PA). Cryptococcal infection will be assessed on enrollment with the use of a rapid serum cryptococcal antigen assay. Hepatitis C (HCV) screening will be performed on enrollment by anti-HCV antibody assays (as implemented according to local PEPFAR and/or ministry of health practices), with confirmation of positive testing by EIA, RIBA, or viral load.

Optional stool samples from study participants will be characterized for possible infections with intestinal pathogens, such as helminth infections, parasites and intestinal bacteria.

Collected specimens will be stored for molecular diagnosis, including characterization of the gut microbiome. The microbiome will be characterized by methods including whole genome sequencing, targeted 16S ribosomal RNA sequencing and cell culture. Stool specimens may also be processed using the direct technique (saline and iodine mounts) to identify trophozoite and cyst of protozoan parasites and using formol-ether concentration technique to detect eggs and larva of helminths. If deemed necessary, other methods for detection of intestinal pathogens, including bacterial culture, Kato Katz microscopy, among others, will be applied.

Study subjects will also undergo evaluation for hepatitis B, malaria, tuberculosis, and human papillomavirus (cervical carcinoma) in which routine testing is combined with subsequent advanced clinical and/or research assays.

#### **7.4.6 Research Lab Methods**

##### **7.4.6.1 Diagnostics**

At participating sites, residual whole blood and/or plasma remaining from routine clinical testing (as described in Appendix I & II) will be used to evaluate point of care (POC) HIV nucleic acid tests (NATs), rapid diagnostic tests (RDTs) for HIV/HCV/HBV, and alternative CD4 measurement technologies (see Section 1.2). The evaluation of these tests would correspond to the time when their comparative gold standard tests are drawn, – for example, residual specimen from a subject’s clinical assays on enrollment may be utilized in a point of care HIV NAT and that result compared with the viral load performed at that visit by traditional PCR based methods. With regard to evaluations of HIV/HCV/HBV RDTs, each test will be separately compared to its respective “gold standard” screening and supplemental confirmatory test. Sub-study documents will be submitted to obtain approval by WRAIR and Local IRBs for each specific evaluation.

##### **7.4.6.2 Co-infection investigations**

###### **7.4.6.2.1 Hepatitis B**

Hepatitis B screening will be performed annually across the cohort with a hepatitis B surface antigen (HBsAg) screen (as implemented according to local PEPFAR and/or ministry of health practices), with positive tests followed by a confirmatory HBsAg ELISA. These tests will be performed on blood specimens collected during the schedule of events for the study and their results reported to the subject’s healthcare provider.

As resources allow, additional advanced clinical analysis will be performed prospectively on specimens collected for HBV serologies or if needed on stored plasma or serum. HBsAg positive samples will undergo HBV DNA quantification by polymerase chain reaction and also hepatitis B e antigen (HBeAg) testing by ELISA. HBsAg negative samples will undergo HBV PCR to evaluate for occult hepatitis B. Those samples with detectable HBV DNA by PCR will undergo HBV subtyping and HBV genotype resistance testing and/or HBV phenotype resistance testing. All samples in this subset, HBsAg positive and negative, will be



tested for hepatitis B core antibody by ELISA. These evaluations will require up to 10 mL of stored plasma per subject. These test results will be made available to the Site PI to be shared with the subject's healthcare provider at the discretion of the site PI.

#### 7.4.6.2.2 Malaria

At sites with high malaria endemicity, malaria parasitemia will be evaluated on a routine basis (every 6 months) and during acute febrile episodes by standard giemsa stain smear microscopy. Malaria smears will be reviewed at the study site if the subject is ill. Smears (thick and thin films on a single slide) will either be sent to the Malaria Diagnostic Center in Kisumu for storage and quality assurance reading or will have quality assurance and storage performed at the study site, in accordance with the Site Specific Addenda. Quality assurance procedures will be developed with the assistance of the Malaria Diagnostic Center in Kisumu in order to minimize inter-laboratory variability. If sufficient quantity remains, residual blood collected for the performance of malaria smears will be stored in the AFRICOSR for further evaluation of etiologies of febrile illness via serologic and nucleic acid amplification methods.

Malaria infection will also be assessed every 6 months and during acute febrile episodes by using real time polymerase chain reaction methods performed on dried blood samples stored on filter paper to measure parasitemia (DNA) and gametocytemia (RNA). Anti-malarial antibodies will be measured using enzyme-linked immunosorbent assay (ELISA) or Luminex technology performed on stored serum (up to 1 mL) or on samples eluted from dried blood spots. These tests are not intended for clinical application and the results will be reported in aggregate form unlinked to personally identifying information. Dried blood spots not used for malaria detection will be stored for further evaluation of etiologies of febrile illness by nucleic acid amplification methods.

#### 7.4.6.2.3 Tuberculosis

At enrolling sites in Tanzania, all HIV infected subjects will be requested to produce a sputum sample at all study visits (every 6 months). These will be evaluated for active tuberculosis and for rifampicin resistance using rapid TB diagnostic methods such as the WHO-recommended PCR based Cepheid Gene Xpert MTB/RIF platform. Additional standard clinical diagnostics including mycobacterial smear, culture, and molecular or culture-based drug resistance testing, may be performed on select samples. These results will be reported to the subject's healthcare provider.

At all other enrolling sites, all HIV infected subjects will be requested to produce a sputum sample on an annual basis. These evaluations will be conducted in the host country when feasible according to local capacity. These sputum samples will be transported to the country-level AFRICOS Repository and stored. They will be evaluated for active tuberculosis as resources allow. These test results will be made available to the Site PI to be shared with the subject's healthcare provider at the discretion of the site PI. If Gene Xpert and other TB diagnostic capabilities and resources are enhanced at these sites, the samples will be evaluated at the time they are produced and the results reported the subject's healthcare provider.

In selected subjects, in particular those who progress to active tuberculosis, cryopreserved PBMC (up to 30 million) will be accessed to characterize MTB-specific T cell responses. This will include, but not be limited to, evaluations such as quantification of MTB-specific T cell frequencies, flow cytometric characterization of the phenotype and functionality of MTB-specific T cells and multiparametric functional analysis of cytokine and chemokine production. In addition, cellular HIV infection of MTB-specific CD4 T cells and other CD4 T cell subsets might be determined in selected samples.

Furthermore, enrollment screening with a tuberculosis interferon gamma release assay (IGRA) will be performed in those HIV infected subjects who have not yet begun antiretroviral therapy. This assay is currently of research utility only, as its clinical implications are unclear. To investigate the clinical implications of a positive interferon gamma release assay in this subset of HIV infected individuals, and also the prognostic significance of T cell based biomarkers such as CD27, stored PBMC (up to 10 million) from up to 400 volunteers undergoing IGRA testing will be evaluated for such markers using standard flow cytometric techniques and those results compared to the development (or lack thereof) of active TB infection in the study subjects. If this investigation subsequently reveals a clear clinical utility of the IGRA, those test results will be made available to the Site PI to be shared with the subject's healthcare provider at the discretion of the site PI, otherwise the results will be reported in aggregate form unlinked to personal identifying information.

#### 7.4.6.2.4 Human Papillomavirus (cervical carcinoma)

According to the Schedule of Events, female subjects may undergo HPV screening. Female subjects across the cohort will be receiving annual cervical care screening, according to local guidelines, which include visual inspection of the cervix with acetic acid (VIA), with local therapy (e.g., cryotherapy) or referral for visualized lesions. Where capacity allows, colposcopy, cervical biopsy, and Papanicolaou (PAP) smears will also be performed as clinically indicated. Biopsies and PAP smears will be interpreted by licensed pathologists. Additionally, female subjects may undergo HPV screening on vaginal or cervical cells obtained at the time of VIA. These results will be reported to the subject's healthcare provider.

Cervical specimens from a subset of these subjects (up to 300) will undergo further research testing to include HPV subtyping, as well as immune histopathology using immunohistochemical and fluorescent in-situ hybridization techniques, and further characterization of HPV-directed immune responses. These test results will be made available to the Site PI to be shared with the subject's healthcare provider at the discretion of the site PI.

For this subset, stored PBMC may also be employed to evaluate HPV-specific cellular immune responses in peripheral blood, with comparison to the aforementioned findings in mucosal cervical specimens. These tests are not intended for clinical application and the results will be reported in aggregate form unlinked to personally identifying information.

#### 7.4.6.3 HIV pathogenesis investigations

AFRICOS laboratory investigators will conduct immunologic and genetic investigations into the pathogenesis of HIV infection and disease progression.

#### 7.4.6.3.1 Immunopathogenesis

To characterize the role of cellular responses in the immunopathogenesis of HIV, reposed PBMC and plasma samples from up to 400 HIV infected study subjects at the extremes of disease progression and/or severity, and up to 200 HIV uninfected control subjects, will undergo immunologic evaluation, including but not limited to the following: Lymphocyte subsets will be characterized. Polychromatic flow cytometry assays allowing for simultaneous detection of several costimulatory molecules will be used to investigate both positive and negative regulators and other cellular markers of interest, to include markers of immune activation. Distinct T cells subsets of significance will be sorted and undergo quantification of cell associated viral load and other reservoir measurements. Cells will be used for *in vitro* functional interrogation. In plasma samples, cytokine levels and other markers of immune activation and microbial translocation will be assessed using ELISA-based and other multiplexed technologies; these will be compared to viral load measurements, utilizing low copy-number assays in virologically suppressed samples. Innate immune system responses and gene expression will also be investigated. As resources allow, this analysis will be expanded beyond subjects with extreme progression phenotypes to encompass the remainder of the cohort to enable comparison across other host and viral characteristics such as subtype, viral reservoir, and the presence of other comorbidities such as coinfection, metabolic disorders, and malignancy. These evaluations would require up to 20 million PBMC and 7 mL of plasma per subject.

To characterize the role of antibodies in the immunopathogenesis of HIV and in protection from infection, PBMC and serum or plasma samples from HIV negatives and HIV positive study subjects who are early in infection or are at the extremes of disease progression and/or severity will be studied. These samples will undergo humoral immunologic evaluation, including but not limited to the following: 1) assessment of binding antibodies to multiple HIV antigens during all stages of infection; 2) characterization of the evolution of functional antibody responses to HIV-1, including but not limited to ADCC, ADCVI and both autologous and heterologous neutralization, 3) dissection of the biotypes and neutralization phenotypes of envelopes cloned from peripheral blood and other available samples at time points prior to, during, and after the development of immune selection pressures, through use of pseudoviruses, infectious molecular clones, or infectious HIV-1 isolates. Several previous reports highlight the notion that clade-specific differences may indeed have an impact on Env immunogenicity or sensitivity to neutralization (Li et al. 2006; Brown et al. 2008; Derdeyn et al. 2004; Frost 2005). A significant effort has been expended by both cellular and humoral HIV immunologists to obtain *env* clones or sequences of very early, CCR5 coreceptor-utilizing isolates from acutely infected individuals at all Feibig stages (Fiebig 2003; Li 2005). 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 little published data to support this hypothesis. For the studies proposed here, *envs* would be cloned from both peripheral blood and plasma at the earliest timepoints available. This will allow assessment of humoral responses to viruses that are from multiple Feibig stages and from the major clades prevalent. These studies will require 5-10

million PBMC (if available) and 2 mL of stored serum (preferred) or plasma per subject per timepoint.

#### 7.4.6.3.2 Host genetics

MHRP investigators will perform targeted analyses of genetic polymorphisms within host genes reported to influence the rates of HIV acquisition and/or disease progression. These genes function in HIV host restriction, and innate and adaptive immune system responses. Host restriction genes of interest include but are not limited to HIV co-receptors, cognate chemokine ligands, and post-entry factors. Targeted genes of the innate immune system will include, but be limited to, killer immunoglobulin receptors (KIR) and cognate Class 1 ligands; dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN); and toll-like receptors (TLR). Those involved in humoral and cell-mediated immune responses will include Fc $\gamma$  receptors, capable of binding diverse immunoglobulin isotypes; and the alleles comprising Class I HLA-A, -B, and -C loci, respectively.

Whole genome sequencing (WGS)- and/or genome-wide association study (GWAS)-derived associations and cognate viral load measures will be explored in the context of disease progression outcomes. These technologies will facilitate comparative analyses of frequencies of the targeted polymorphisms both between exposed HIV-uninfected vs. HIV-infected individuals, and rapid vs. slow and/or long-term non-progressors (LTNP). Confirmatory follow-on procedures including real-time qPCR will be performed as necessary with either commercial reagents (preferably) or those adapted from the literature. This series of evaluations will require up to 10 million PBMC per subject.

The HLA typing procedures that will be used in this study have yet to be validated for clinical use but represent essential research tools for the analyses of adaptive cellular immune responses in HIV-1 infection. HLA typing data will be unlinked from personal identifiers, and reported in aggregate for the populations studied.

#### 7.4.6.3.3 Viral genetics

MHRP investigators will use molecular biology techniques to characterize viral strains of stored samples of the cohort and to evaluate disease associations with viral genetic diversity. This will typically involve the use of stored plasma for the amplification of RNA, however in certain circumstances amplification of DNA from stored PBMC is required. Viral sequence and subtyping data will be unlinked from personal identifiers and reported in aggregate.

## **8 MANAGEMENT OF SUBJECTS**

### **8.1 Test Results**

Results of routine HIV care and treatment labs will be reported to the clinicians as is done per routine clinic practice.

Clinical tests performed for research purposes (to include viral load results) will be made available to the Site PI and to the participant's treating clinicians at the site of enrollment. For

HIV positive subjects, the results will be shared with the HIV treatment facility's clinical staff and that information incorporated into the ongoing management of the subject according to local standards of care. For HIV negative subjects, the study staff will review clinically significant lab results with the subjects and refer them for appropriate local care, either at the hospital that is the site of subject enrollment or at other local facilities with appropriate capacity.

Nonclinical research tests (those performed in a research setting to address basic pathogenesis questions) are unlikely to produce results with clinical utility, however, should this occur, clinically relevant results will be made available to the Site PI and shared with the participant's treating clinicians at the discretion of the Site PI.

As the study visits will be occurring at the site of medical care provision and will involve collaboration with site clinicians, referral for appropriate care can be readily facilitated. Local guidelines for public health reporting will be followed by site clinical staff.

## **8.2 Pregnancy**

Known pregnancy is an exclusion criteria for enrollment, however female volunteers who become pregnant over the course of the study will continued to be followed through regular study visits. Subjects who become pregnant during the course of the study will not undergo phlebotomy for repository while pregnant unless hemoglobin testing demonstrates a normal value (utilizing locally established reference ranges). In this case, phlebotomy for repository will be performed but the amount will be decreased to 20 mL. Pregnancy outcome will be documented in terms of infant mortality/morbidity and infant HIV status.

## **8.3 Incarceration**

Volunteers will not undergo any AFRICOS study visits while incarcerated. If incarcerated, the volunteer will transition to inactive status for the study, which will be documented in the Status Change CRF. No study visits, data or sample collection can proceed while a subject is on inactive status. At the end of the period of incarceration, the volunteer will be eligible to return to active status and resume study visits, with this reactivation documented in the Status Change CRF. Any volunteer on inactive status for over 365 days will repeat the informed consent process prior to resuming active status. For PEPFAR evaluation purposes, the continuity of care during incarceration is important to document. Therefore, upon resuming active status, the participant will be queried about treatment interruptions, other treatment changes, and major health events occurring during the inactive period. If the study team learns of a subject's death during incarceration, an additional Status Change CRF will be completed to document cause of death.

## **8.4 New HIV infection**

Volunteers in the HIV uninfected component of AFRICOS who acquire HIV infection over the course of the study will undergo HIV counseling and will also be advised and encouraged to refer their sexual partners for HIV counseling and testing. The newly infected volunteer will be offered enrollment into the HIV infected component of AFRICOS, which would

require updating informed consent to reflect the volunteer's participation in this different component of the cohort.

## **8.5 Unanticipated Events and Social Harms Reporting**

This is an observational study of volunteers with a life-threatening illness. Events such as death and hospitalization are an expected part of the natural history of HIV infection, are not a result of participation in the study, and as such, do not represent unanticipated events. Adverse events that are clearly unrelated to the research will not be reported unless, in the Site PI's judgment, the adverse event might have a bearing on the research in which case it will be reported. All events that result in withdrawal from the study will be reported, even if the PI determines that it has no bearing on the research. All deaths will be reported in the annual continuing review report.

Unanticipated events and social harms may occur during the course of the study. These may include physical assault and suicidal attempts and may or may not be related to the study. However, should unanticipated events or social harms involving risks to subjects or others occur, the host country and WRAIR IRBs will be informed. The study staff, informed of these events, will inform the Site-Principal Investigator, or his/her designees. The Site-Principal Investigator will then prepare a narrative summary of the event and report to the local IRB and Protocol Chair. The Protocol Chair will report any significant events to WRAIR HSPB within 48 hours of becoming aware of the event by phone (011-301-319-9940), by email (usarmy.detrack.medcom-wrair.mbx.hspb@mail.mil) or by facsimile (011-301-319-9961), and within 10 business days an official follow-up report should be submitted.

In addition to the methods above, the complete report will be sent to the Director, Human Subjects Protection Branch (HSPB), Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500. Serious and non-serious unanticipated problems, social harms and deaths will be summarized in the annual continuing review report. The WRAIR HSPB will report unanticipated events, social harms and deaths to USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636 due to the funding mechanism.

## **8.6 TERMINATION OF STUDY PARTICIPATION**

Volunteers will be free to withdraw from the study at any point without prejudice. Volunteers who withdraw from the study will be considered "lost to follow-up." If a volunteer misses a study visit, attempts will be made to contact the subject (using all previously agreed methods of contact) to determine if the subject is still willing to continue participation in the study, and to schedule a new study visit. If contact is not achieved within 360 days of a missed study visit, the subject will be considered lost-to-follow up and no further data will be collected, except to document the circumstances surrounding a subject's death.

Volunteers who withdraw consent are indicating a termination of interaction with the study team entirely and will not be contacted further unless, as approved by the WRAIR and local IRBs, an issue pertaining to their safety and well-being requires communication.

## **9 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. Unscheduled visits for acute illness may be compensated according to the discretion of the site investigators. The amount of compensation may vary according to visit to account for the differential lost time and inconvenience. 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.

## **10 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.

## **11 DATA MANAGEMENT AND ANALYSIS**

Data Coordinating and Analysis Center (DCAC), MHRP, HJF in US serves as the central data management facility for MHRP research protocols. DCAC PEPFAR study team is responsible for RV329 data management and analysis activities. The study site data management teams will work closely with DCAC PEPFAR study team with data related activities. The study principal investigator(s) and the study team will provide DCAC study information, and DCAC will coordinate activities from CRFs and annotated CRFs creations, database structure design, data entry screen set-up, data collection, data entry, data clean, and data transfer to analysis with study sites.

Detailed procedure will be outlined in the Data Management Plan (DMP) according to DCAC SOP.

### **11.1 Data Collection and Monitoring**

The primary source document for this study will be the subject's medical records and information from subjects' previous study data if any. The information collected by the study team will be the research records. Both medical records and the research records will be considered the source documents for the purposes of auditing the study.

Data recorded on source documents will be transcribed onto Case Report Forms (CRFs) provided by DCAC. The CRFs are created and designed by the joint efforts of the principal investigators, clinical staff and DCAC study team. The study sites use the CRFs as a tool to collect data at the sites. They will be reviewed and retained by the site investigator. The data

will be entered into a central AFRICOS database, which will serve as the data component of the AFRICOS Repository (AFRICOSR).

The CRF templates for study usage will be maintained and updated by the DCAC team as needed at the principal investigator's or study team's request. The database will be monitored by DCAC on a regular basis throughout the study. 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.

## **11.2 Data Entry**

The ClinPlus® Data Management (CPDM) system will be used as the platform for database modeling and data entry. The collected data from the CRFs will be double entered, at the sites, into the AFRICOS database by the site data management teams through ClinPlus. Should 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 requirements are met.

Personal identifiers such as name, national identification number (if applicable), or house number will be kept locally at each site in a secure database. The 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 site data manager 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 file folders will be stored in locked filing cabinets and/or rooms accessed only by the data manager or designee.

## **11.3 Data Analysis**

Data analysis will be performed using SAS® version 9.0 or higher or equivalent statistical programs. DCAC in Bethesda, MD, USA, in collaboration with the protocol investigators, will conduct and support the data analyses. It includes data cleaning, data queries, listings, statistical tables and graphs. In addition, data reports in various forms will also be made available to the PI and the study team on a regular basis (monthly/periodically or at request).

It is the policy of 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 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.

## **11.4 Data Storage and Security**

All source documents such as contact information forms, laboratory record sheets and CRFs will be maintained at each participating site. If CRFs must be transmitted to DCAC for data



entry, they will be sent either via hard copy or electronic. Paper CRFs will be stored at the site for 2 years post entry into the electronic database. Data will be stored on dedicated servers (real-time) and tapes/CD-RWs. 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.

All the computers at DCAC and the sites are securely protected. Only personnel with study accounts can login to the data entry system. Data is managed 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 Maryland campus of the MHRP and is protected by a firewall and a wide range of other security measures. This central AFRICOS database, the data component of the AFRICOS Repository (AFRICOSR), is stored under the supervision of the Data Custodian (DC), a programmer analyst at DCAC. The AFRICOS database will contain only coded data with no link to identifying information (this link will be maintained separately at the sites). The DC (or designee) will be responsible for distributing data as indicated to AFRICOS investigators. All investigators external to the site, including external collaborators, who receive data and/or specimens, will receive them in coded form only without a link to participant identifiers.

## **12 SUB-STUDY USE OF DATA AND SAMPLES IN AFRICOS REPOSITORY**

It is anticipated that much of the research conducted on data and samples collected by AFRICOS will occur under the auspices of approved sub-study protocols. To pursue research objectives not listed in the main RV329 AFRICOS protocol, investigators will need to undertake AFRICOS sub-studies (numbered RV329-## starting with RV329-01) by submitting specific research proposals for the use of data and samples stored in the AFRICOS Repository (AFRICOSR). Sub-study investigations may occur at WRAIR or at outside collaborating research institutions, and all sub-studies require coordination with the AFRICOS Protocol Chair (or designee) and approval by the WRAIR IRB and local IRB.

AFRICOS will only enroll volunteers explicitly consenting to future use of samples and data; therefore the AFRICOSR Repository will contain samples and data only from volunteers consenting to their future use.

### **12.1 Sub-study Procedures**

Investigators and/or laboratory collaborators will be required to complete the information listed below using the RV 329 Sub-Study Template (Appendix III):

- Objectives of analysis
- Names and contact information of investigators and/or collaborating investigators.
- Description of the data and/or samples requested
  - Number of samples
  - Type of samples /data
- A summary of laboratory testing being performed.

- A description of the anticipated data analysis.
- Materials transfer agreement (MTA) if required.
- Collaborating institutions IRB determination to receive samples and/or data

No laboratory or data analysis will be performed on contents of the AFRICOSR without prior approval of the WRAIR IRB and local IRBs. Laboratories outside of the MHRP will be outlined within each sub-study memorandum, if applicable.

Specimens may be obtained from the following MHRP laboratories, which together comprise the AFRICOSR:

US Military HIV Research Program (MHRP)  
Walter Reed Army Institute of Research  
503 Robert Grant Avenue  
Silver Spring, MD 20910 USA

Kenya Medical Research Institute/Walter Reed Project (KEMRI/WRP)  
Clinical Research Center Laboratory  
P.O Box 1357, Kericho 20200  
Kericho, Kenya

Makerere University-Walter Reed Project (MUWRP)  
P.O. Box 16524  
Kampala, Uganda

Mbeya Medical Research Centre (MMRC) Laboratory  
P.O. Box 2410  
Mbeya, Tanzania

Defense Reference Laboratory (DODHPN)  
Mogadishu Cantonment  
Abuja, Nigeria

Data will be obtained from the central AFRICOS database, to be distributed by the DC (or designee). Upon completion of sub-studies, data from any analyses or experiments will be returned to the DC in a coded fashion and added to the AFRICOSR database.

## **12.2 Distribution of Study Specimens and Data**

The Protocol Chair (or designee) will submit an approved sub-study memorandum to the Data Collection and Analysis Center (DCAC) detailing the requested specimens and data. It is the responsibility of the sub-study PI to ensure that all approvals, including all approved and finalized Materials Transfer Agreements (MTAs), where applicable, have been submitted/made available to DCAC along with the approved sub-study memoranda. Sub-study data and specimens will retain their original study ID numbers and timepoints when distributed.

The MHRP International Laboratory Program (INLAP) will coordinate with DCAC to contact the individual MHRP laboratories in the AFRICOSR (as outlined above) to request shipment of coded specimens to the Rockville SPL or collaborating laboratory according to the sample size, inclusion/exclusion criteria, and protocol outlined in the sub-study memorandum.

Upon receipt of the specimen request from INLAP, the MHRP laboratories will document, collect and ship the requested specimen to the SPL or to a collaborating laboratory listed in the protocol or sub-study memorandum. MHRP Laboratories will provide INLAP/DCAC with the list of PINs. No protected health information (PHI) will be distributed with the samples.

Upon receipt of the specimen, SPL or collaborating lab personnel will inspect, document, and verify receipt of each specimen. For specimens shipped to the SPL, SPL personnel will contact the appropriate WRAIR laboratory to arrange for specimen pick-up from the SPL.

### **12.3 Protection of Subject Data**

The sub-study investigators, laboratory personnel and other sub-study staff members will not have access to any original and identifiable volunteer information. The Data Custodian assigned from the Data Coordinating and Analysis Center in Bethesda, MD will not have access to the identifiable information and any requests for additional information will be in a coded manner. Should there be a need to match data contained in the AFRICOSR with coded samples, the DC (or designee) will be responsible for linking the data to the requested specimens and distributing the data requested in the sub-study memorandum from the AFRICOSR to sub-study investigators.

## **13 ETHICAL CONSIDERATIONS**

### **13.1 Risks**

The primary risks to study participants are potential social harm from study participation, very minimal risks of physical harm from additional phlebotomy for study participation, and a small risk of breach of confidentiality. Efforts are in place to mitigate any study related risks. All sites have identified HIV clinics, and stigma and potential for social harm while real are far less than pre-ART or in the earlier days of opening HIV clinics. The potential risks of the needle stick for blood drawing include pain, bruising, hematoma, fainting, and very rarely, infection. Breaching of confidentiality is greatest during enrollment and the interviewing stages of the study, and steps have been taken to mitigate that risk. In addition to procedures put in place to assure participant confidentiality, participants will not be identified in any reports on this study. Mitigating steps also include that, by design, no subject identifying information will be extracted from clinical records. Any copies of case report forms & data forms bearing any identifiers will be kept in locked, secured areas. Finally, security measures are in place at the country-level data management centers and at DCAC to assure data safety and confidentiality.

## **13.2 Benefits**

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

## **13.3 Informed Consent**

Informed consent will be obtained from each volunteer before enrollment in the study. There will be separate consent forms for HIV-infected and HIV-uninfected subjects. Consent forms will be available in both English and site-specific local languages, and can be found as attachments to the site-specific protocols. Informed consent will be administered by research study staff in a private space. For illiterate subjects, a thumbprint will be substituted for signature, and their informed consent will be witnessed by an adult who is not a member of the research study staff. Volunteers may take as much time as needed to decide if he/she wants to participate and may share the consent form with family members or friends prior to agreeing 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 local 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 local 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.

## **13.4 Volunteer Confidentiality**

Volunteers will be assigned a study number that will be used as a personal identifier for volunteer identification. This study number will be linked to subject identifying data, contact information and a fingerprint biometric identifier in a database maintained locally at each enrollment site and separate from the study database. The database of the volunteer

identifying data, fingerprint, and study number will be only accessible to the study coordinators and the local PI. The data management personnel will prepare 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 the enrollment visit and updated at subsequent visits. 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. The database containing identifying information on subjects will only be accessible to the study coordinators and local site PI.

Further details of volunteer identification can be found in the site-specific addenda (Appendix I).

Representatives of the MHRP, DoD, USAMRMC, local IRBs/and or Ethical Committees (ECs) 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.

### **13.5 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 research study 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 site principal investigator shall notify all IRBs and/or Ethical Committees (EC) 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.

## **14 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 WRAIR HSPB/ IRB is determined by the categorization of the deviation: (1) emergent/major or (2) non-emergent/minor. Deviations should be reported in the appropriate timeframe according to the seriousness of the event as a major deviation or a minor deviation.

Emergent/major 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. Examples: providing the wrong lab result to a volunteer or failure to obtain a scheduled blood draw for multiple participants. Such deviation reports may be initiated without prior IRB/ERC approval, only in cases where the change (s) is /are necessary to eliminate an immediate apparent hazard.

Notification of major deviations to the local IRB and WRAIR HSPB/IRB shall occur promptly, within 48 hours, and they should be reported in writing within 10 business days. The WRAIR HSPB will report events to the US Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP) Human Research Protection Office (HRPO) as per UWZ-C-636 due to the funding mechanism.

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 WRAIR HSPB/IRB and local IRB in a summary report with the annual continuing review report.

A summary of the deviations, to include both major and minor deviations, should be submitted for the current reporting period with the continuing review report and closeout report, as appropriate.

## **15 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. 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 HSPB/IRB and local IRB/ECs for review and approval prior to implementation.

Such amendment will be submitted to the WRAIR HSPB / IRB and local IRBs for review and approval. The WRAIR HSPB will submit protocol amendments to USAMRMC ORP HRPO

as per UWZ-C-636.

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.

## **16 CONTINUING REVIEWS /CLOSEOUT REPORT**

A continuing review report (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 HSPB/ IRB 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 HSPB/IRB as soon as these documents become available. WRAIR HSPB will forward the continuing review reports and closeout reports to the USAMRMC ORP HRPO as per UWZ-C-636.

## **17 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. These are outlined in the site-specific addenda.

To enhance the execution of study activities and cohort integrity 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).

## **18 RESOURCES AND COORDINATION**

This study is funded and sponsored by MHRP and PEPFAR and coordinated locally by MUWRP, USAMRU-K, MMRP, DODHPN and KDOD.

Laboratory: Each study site has an established laboratory that is capable of handling most of the tests required or has access to such a reference laboratory. Every site has established a quality assurance (QA) program.

Staff: The research team to implement this study at each of the participating countries will include the Principal Investigator who will be responsible for implementation of this study protocol 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.

## **19 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 relevant scientific outcomes of the study and general prevalence and incidence data, however confidentiality will be maintained, and participant identities will not 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 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).

## **20 CONDUCT OF THE RESEARCH STUDY**

This research study will be conducted in accordance with 32 CFR 219, the DoD regulations and all corresponding local regulations and requirements. 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 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 SIGNATURE OF PRINCIPAL INVESTIGATOR(S)

1. I agree to follow this protocol version as approved by the IRBs/ERCs.
2. I will conduct the study in accordance with applicable IRB/ERC requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I will not modify the protocol without first obtaining an IRB/ERC approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants.
5. I 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.
6. I will promptly report changes to the research or unanticipated problems to the WRAIR IRB immediately via the WRAIR Human Subjects Protection Branch at (301) 319-9940 (during duty hours) or to the [usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil](mailto:usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil) and submit a written report within 10 working days of knowledge of the event.
7. 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.
8. I will immediately report to the WRAIR Human Subjects Protection Branch knowledge of any pending compliance inspection by any outside governmental agency.
9. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

Principal Investigator(s):

\_\_\_\_\_  
Name,

\_\_\_\_\_  
Date (mm/dd/yyyy)

## 23 REFERENCES

Aichleburg MC, Rieger A, Breiteneker F, et al. (2009) “Detection and prediction of active tuberculosis disease by a whole-blood interferon-gamma release assay in HIV-1-infected individuals”. *Clin Infect Dis*, 48, 954-62.

Andrews KT, Fairlie DP, Madala PK, et al (2006). [Potencies of human immunodeficiency virus protease inhibitors in vitro against Plasmodium falciparum and in vivo against murine malaria](#). *Antimicrob Agents Chemother*. 50(2):639-648.

Baalwa J, Wang S, Yue L, Kaleebu P, Cormier E, Gilmour J, et al. (2011) “Enhanced Fusogenicity, Replication Kinetics, and Macrophage Tropism of Subtype D Transmitted/Founder Viruses in Acute HIV-1 Infection”. Conference on Retroviruses and Opportunistic Infections; Boston, MA.

Babiker HA, Abdel-Muhsin AA, Ranford-Cartwright L, Satti G, Walliker D.(1998) “Characteristics of *Plasmodium falciparum* parasites that survive the lengthy dry season in eastern Sudan where malaria transmission is markedly seasonal”. *Am J Trop Med Hyg*. 59:582–90.

Babiker HA, Abdel-Wahab A, Ahmed S, Suleiman S, et al.(1999) “Detection of low level *Plasmodium falciparum* gametocytes using reverse transcriptase polymerase chain reaction”. *Mol Biochem Parasitol*. 99:143–148.

Barry, SM, Johnson, MA & Janossy, G. (2003) “Increased proportions of activated and proliferating memory CD8+ T lymphocytes in both blood and lung are associated with blood HIV viral load”. *J Acquir Immune Defic Syndr* **34**, 351-357.

Bartholomew MM, Jnasen RW, Jeffers J, et al. (1997) “Hepatitis B virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation”. *Lancet*. 349:20-2.

Bassett, IV, B Wang, et al. (2010) “Intensive tuberculosis screening for HIV-infected patients starting antiretroviral therapy in Durban, South Africa.” *Clin Infect Dis* 51(7):823-829.

Bendavid, E and J Bhattacharya (2009). “The President’s Emergency Plan for AIDS Relief in Africa: an evaluation of outcomes.” *Ann Intern Med* 150(10): 688-695.

Bodsworth NJ, Cooper D, Donovan B. (1991) “The influence of human immunodeficiency virus type 1 infection on the development of hepatitis B virus carrier state”. *J Infect Dis* 163:1138-40.

Boehme, CC, P Nabeta, et al. (2010) “Rapid molecular detection of tuberculosis and rifampin resistance.” *N Engl J Med* 363(11): 1005-1015.

Braitstein, P, MW Brinkhof, et al. (2006) “Morality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries.” *Lancet* 367(9513): 817-824.

Brentlinger PE, Behrens CB, Kublin JG. (2007) “Challenges in the prevention, diagnosis, and treatment of malaria in Human Immunodeficiency Virus–infected adults in sub-Saharan Africa”. *Arch Intern Med* 167(17):1827-1836.

Brooker S, Hotez PJ, Bundy DA. (2010) “The global atlas of helminth infection: mapping the way forward in neglected tropical disease control”. *PLoS Negl Trop Dis*. Jul 27;4(7):e779.

Brown BK, Wiczorek L, Bautista C, Michael NL, Robb ML, et al. (2008) “Cross-clade neutralization patterns among HIV-1 strains from the six major clades of the pandemic evaluated and compared in two different models”. *Virology* 375:529-538.

Burnett RJ, Ngobeni JM, Francois G, et al. (2007) “Increased exposure to hepatitis B infection HIV-positive South African antenatal women”. *Int J STD AIDS* 18:152-156.

Chi, BH, RA Cantrell, et al. (2010). “An empirical approach to defining loss to follow-up among patients enrolled in antiretroviral treatment programs.” *Am J Epidemiol* 171(8): 924-931.

Crowe S, Turnbull S, Oelrichs R, Dunne A (2003) Monitoring of human immunodeficiency virus infection in resource-constrained countries. *Clin Infect Dis* 37(Suppl 1):S25-S35.

Crum-Cianfione, N., A. Ganesan, et al. (2010). “Prevalence and factors associated with renal dysfunction among HIV-infected patients.” *AIDS Patient Care STDS* 24(6): 353-360.

D:A:D Study Groups. (2008) “Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients”. *AIDS*. 22:F17-F24.

Derdeyn CA, Decker JM, Bibollet-Ruche F, et al. (2004). “Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission”. *Science* 303(5666), 2019-22.

DHHS guideline for the use of antiretroviral agents in HIV-infected adults and adolescents. [http://aidsinfo.nih.gov/guidelines/adult/AA\\_032304.html](http://aidsinfo.nih.gov/guidelines/adult/AA_032304.html)

Di Bisceglie A, Maskew M, Schulze D, et al. (2010) “HIV-HBV coinfection among South African patients receiving antiretroviral therapy”. *Antiviral Therapy* 15:499-503.

Dinh DM, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane AV, Wanke CA, Ward HD. “Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection.” *J Infect Dis* 211(1): 19-27.

Dore G, Cooper D, Barret C, et al. (1999) “Dual efficacy of lamivudine treatment in human immunodeficiency virus/ hepatitis B virus coinfection persons in a randomized, controlled study (CAESAR)”. The CAESAR Coordinating Committee. *J Infect Dis* 180:607-13.

Durantel D, Brunelle MN, Gros E, et al. (2005) “Resistance of human hepatitis B virus to reverse transcriptase inhibitors: from genotypic to phenotypic testing”. *J Clin Virol* 34:S34-S43.

Eggena, M.P., et al. (2005) “T cell activation in HIV-seropositive Ugandans: differential associations with viral load, CD4+ T cell depletion, and coinfection”. *J Infect Dis* 191, 694-701.

El-Sadr WM, Lundgren JD, et al. (2006) “CD4+ count-guided interruption of antiretroviral treatment”. *N Engl J Med*. 355:2283-2296.

Fiebig, E.W., et al. (2003) “Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection”. *AIDS* 17(13): p. 1871-9.

Firnhaber C, Reyneke A, Schulze D, et al. (2008) “The prevalence of hepatitis B coinfection in a South African urban government HIV clinic”. *S Afr Med J* 98:541-544.

Francesconi P, Fabiani M, Dente M, Lukwiya M, Okwey R, Ouma J, et al. (2001) “HIV, malaria parasites, and acute febrile episodes in Ugandan adults: a case–control study”. *AIDS* 15:2445–2450.

French N, Nakiyingi J, Lugada E, Watera C, Whitworth J, Gilks C. (2001) “Increasing rates of malarial fever with deteriorating immune status in HIV-1 infected Ugandan adults”. *AIDS* 15:899–906.

Froebel J, Howard W, Schafer JR, Howie F, Whitworth J, Kaleebu P, et al. (2004) “Activation by malaria antigens renders mononuclear cells susceptible to HIV infection and re-activates replication of endogenous HIV in cells from HIV-infected adults”. *Parasite Immunol* 26:213–217

Frost, S. D., Liu, Y., Pond, S. L., Chappey, C., Wrin, T., Petropoulos, C. J., Little, S. J., and Richman, D. D. (2005). “Characterization of human immunodeficiency virus type 1 (HIV-1) envelope variation and neutralizing antibody responses during transmission of HIV-1 subtype B”. *J Virol* 79(10), 6523-7.

Gandhi N, Saiyed Z, Thangavel S, Rodriguez J, Rao KV, Nair MP. (2009) “Differential effects of HIV type 1 clade B and clade C Tat protein on expression of proinflammatory and antiinflammatory cytokines by primary monocytes”. *AIDS Res Hum Retroviruses*. 25(7): 691-9.

Ganesan, A., PK Chattopadhyay, et al. (2010) “Immunologic and virologic events in early HIV infection predict subsequent rate of progression.” *J Infect Dis* 201(2): 272-284.

Geldmacher C, Ngwenyama N, Schuetz A, et al. (2010) “Preferential infection and depletion of Mycobacterium tuberculosis-specific CD4 T cells after HIV-1 infection”. *J Exp Med*, 207, 2869-81.

Geldmacher C, Schuetz A, Ngwenyama N, et al. (2008). “Early depletion of Mycobacterium tuberculosis-specific T helper 1 cell responses after HIV-1 infection”. *J Infect Dis*, 198, 1590-8

Giorgi JV, et al. (2002) “Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *J Acquir Immune Defic Syndr* 29, 346-355.

Giorgi JV, et al. (1999) “Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage”. *J Infect Dis* 179, 859-870.

Gopinath R, Ostrowski M, Justement SJ, Fauci AS, Nutman TB. (2000) “Filarial infections increase susceptibility to human immunodeficiency virus infection in peripheral blood mononuclear cells in vitro”. *J Infect Dis*. Dec;182(6):1804-8. Epub 2000 Nov 8.

Gupta JD, Satishchandra P, Gopukumar K, Wilkie F, Waldrop-Valverde D, Ellis R, et al. (2007) “Neuropsychological deficits in human immunodeficiency virus type 1 clade C-seropositive adults from South India”. *J Neurovirol*. 13(3): 195-202.

Harris, JB, SM Hatwiinda, et al. (2008) “Early lessons from the integration of tuberculosis and HIV services in primary care centers in Lukasa, Zambia.” *Int J Tuberc Lung Dis* 12(7): 773-779.

Hartzell, JD, IE Janke, et al. (2008) “Impact of depression on HIV outcomes in the HAART era.” *J Antimicrob Chemother* 62(2): 246-255.

Hasegawa I, Tanaka Y, Kurbanov F, et al. (2006) “Molecular Epidemiology of Hepatitis B Virus in the United Republic of Tanzania”. *J Med Virol* 78: 1035-1042.

Hazenbergh, MD, et al. (2003) “Persistent immune activation in HIV-1 infection is associated with progression to AIDS”. *AIDS* 17, 1881-1888.

Hewitt K, Steketeeb R, Mwapasac V, Whitworth J, Frenche N. (2006) “Interactions between HIV and malaria in nonpregnant adults: evidence and implications”. *AIDS* 20:1993–2004.

Hochman S and Kim K. (2009) “The impact of HIV and Malaria coinfection: what is known and suggested venues for further study”. *Inter Persp on Infect Dis* 617954.

Hoffman CJ, Charalambous S, Martin DJ, et al. (2008) "Hepatitis B virus infection and response to antiretroviral therapy (ART) in South Africa ART program". *Clin Infect Dis* 47:1479-1485.

Hoffman IF, Jere CS, Taylor TE, et al. (1999) "The effect of *Plasmodium falciparum* malaria on HIV-1 RNA blood plasma concentration". *AIDS* 13(4):487-494.

Holland MJ, Harcus YM, Riches PL, Maizels RM. (2000) "Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses". *Eur J Immunol*. Jul;30(7):1977-87.

Hotez PJ, Bundy DAP, Beegle K, Brooker S, et al. (2006) "Helminth Infections: Soil-transmitted Helminth Infections and Schistosomiasis". Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): World Bank. Chapter 24.

Jacobs B, Mayaud P, Chagalucha, et al. (1997) "Sexual transmission of hepatitis B in Mwanza, Tanzania." *Sex Transm Dis*. Mar;24(3):121-6.

Jaenisch T, Junghanss T, Willis B, Brady OJ, Eckerle I, Farlow A, et al. "Dengue expansion in Africa – not recognized or not happening?" *Emerg Infect Dis* [Internet] 2014 Oct. <http://dx.doi.org/10.3201/eid2010.140487>

Jonnalagadda S, Lohman Payne B, Brown E, et al. (2010) "Latent tuberculosis detection by interferon gamma release assay during pregnancy predicts active tuberculosis and mortality in human immunodeficiency virus type 1-infected women and their children". *J Infect Dis*, 202, 1826-35.

Kanya M, Gasasira AF, Yeka A, Bakyaite N, Nsobya SL, Francis D, et al. (2006) "Effect of HIV-1 infection on antimalarial treatment outcomes in Uganda: a population-based study". *J Infect Dis* 193:9–15.

Karp, CL and PG Auwaerter (2007) "Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens" *Clin Infect Dis* 45(9): 1208-1213.

Karp, CL and PG Auwaerter (2007) "Coinfection with HIV and tropical infectious diseases. II. Helminthic, fungal, bacterial, and viral pathogens." *Clin Infect Dis* 45(9): 1214-1220.

Kew MC. (1996) "Progress towards the comprehensive control of hepatitis in Africa: a view from South Africa". *Gut* 38 Suppl 2:S31-S36.

Korenromp EL, Williams BG, de Vlas SJ, et al. (2005) "Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa". *Emerging Infectious Diseases* 11(9):1410-1419.

Kovacs JA, Masur H (2000) "Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection", *N Engl J Med* 342(19):1416-1429.

Krajden S, Panisko DM, Tobe B, Yang J, Keystone JS. (1991) "Prolonged infection with *Plasmodium falciparum* in a semi-immune patient." *Trans R Soc Trop Med Hyg* 85:731-2.

Kublin JG, Patnaik P, Jere CS, et al. (2005) "Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in blood of adults in rural Malawi: a prospective cohort study." *Lancet* 365 (9455):233-240.

Kuller LH, Tracy R, Belloso W, et al. (2008) "Inflammatory and coagulation biomarkers and mortality in patients with HIV infection." *PLoS Med.* 5:e203.

Laufer MK and Plowe CV. (2007) "The interaction between HIV and Malaria in Africa." *Tropical and Travel Med* 9(1):47-54.

Laufer MK, van Oosterhaut JJG, Thesing PC, Thumba F, Zijlastra EE, Graham SM. (2006) "Impact of HIV- associated immunosuppression on malaria infection and disease in Malawi." *J Infect Dis* 193:872-878.

Li B, Decker JM, Johnson RW, et al. (2006). "Evidence for potent autologous neutralizing antibody titers and compact envelopes in early infection with subtype C human immunodeficiency virus type 1." *J Virol* 80(11), 5211-8.

Li M, Gao F, Mascola JR, Stamatatos L, et al. (2005). "Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies." *J Virol* 79(16), 10108-25.

Lodenyo H, Schoub B, Ally R. (2000) "Hepatitis B and C virus infections and liver function in AIDS patients at Chris Bani Baragwanath Hospital, Johannesburg." *East Afr Med J* 77:13-15.

Louie KS, Sanjose S, Mayaud P.(2009) "Epidemiology and prevention of human papilloma virus and cervical cancer in sub-Saharan Africa: a comprehensive review." *Tropical Medicine and International Health* 14:1287-1302.

Mann, DL, M Carrington, et al. (1992) "HLA phenotype is a factor in determining rate of disease progression and outcome in HIV-1-infected individuals." *AIDS Res Hum Retroviruses* 8(8): 1345-1346.

Maqutu, D, T Zewotir, et al. (2010) "Determination of optimal adherence over time to antiretroviral therapy among HIV positive adults in South Africa: a longitudinal study." *AIDS Behav*

Marconi, VC, H Sunpath, et al. (2008) "Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen I KwaZulu Natal, South Africa." *Clin Infect Dis* 46(10): 1589-1597.

Maj M, D'Elia L, Satz P, Janssen R, Zaudig M, Uchiyama C, et al. (1993) "Evaluation of two new neuropsychological tests designed to minimize cultural bias in the assessment of HIV-1 seropositive persons: a WHO study." *Arch Clin Neuropsychol* **8**(2): 123-35.

Maj M, Janssen R, Satz P, Zaudig M, Starace F, Boor D, et al. (1991) "The World Health Organization's cross-cultural study on neuropsychiatric aspects of infection with the human immunodeficiency virus 1 (HIV-1). Preparation and pilot phase." *Br J Psychiatry*. **159**: 351-6.

Matee MI, Magesa PM, Lyamuya EF. (2006) "Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis infections among blood donors at the Muhimbili National Hospital in Dar es Salaam, Tanzania." *BMC Public Health* Jan 30;6:21.

Menendez C, Sanchez-Tapias JM, Kahigwa E, et al. (1999) "Prevalence and mother-to-infant-transmission of hepatitis viruses B, C, and E in Southern Tanzania." *J Med Virol* Jul;58(3):215-20.

Mermin J, Lule JR, Ekwaru JP. (2006) "Association between malaria and CD4 cell count decline among persons with HIV." *J AIDS* 4(1):129–130.

Mermin J, Lule J, Ekwaru J, Downing R, Hughes P, Bunnell R, et al. (2005) "Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members." *AIDS* 19:1035–1042.

Meya, DB, YC Manabe, et al. (2010) "Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings." *Clin Infect Dis* 51(4): 448-455.

Moir S, and Fauci AS. (2009) "B cells in HIV infection and disease." *Nat Rev Immunol* **9**:235-45.

Moore, CB, M John, et al. (2002) "Evidence of HIV-1 adaptation of HLA-restricted immune responses at a population level." *Science* 296(5572): 1439-1443.

Mphahlele MJ, Lukhwareni A, Burnett R, et al. (2006) "High risk of occult hepatitis B virus infection in HIV-positive patients from South Africa." *J Clin Virol* 35:14-20.

Nachega, JB, R Leisegang, et al. (2010) "Association of antiretroviral therapy adherence and health care costs." *Ann Intern Med* 152(1): 18-25.

Nadjm B, Mtove G, Amos B, Walker NF, Diefendal H, Reyburn H, Whitty CJ. "Severe febrile illness in adult hospital admissions in Tanzania: a prospective study in an area of high malaria transmission." *Trans R Soc Trop Med Hyg* 2012 Nov; 106(11) : 688-95.



Nagu TJ, Bakari M, Matee M. (2008) "Hepatitis A, B, and C viral co-infections among HIV-infected adults presenting for care and treatment at Muhimbili National Hospital in Dar es Salaam, Tanzania." *BMC Public Health* Dec 19;8:416.

Nettles, RE, TL Kieffer, et al. (2005) "Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART." *JAMA* 293(7): 817-829.

Niesters HG, Honkoop P, Haagsma EB, et al. (1998) "Identification of more than one mutation in the hepatitis B virus polymerase gene during prolonged lamivudine treatment." *J Infect Dis* 177:1382-5.

Niesters HGM, Pas S, de Man RA. (2005) "Detection of hepatitis B virus genotypes and mutants: current status." *J Clin Virol* 34:S4-S8.

Onchiri FM, Pavlinac PB, Singa BO, Naulikha JM, Odundo EA, Farquhar C, Richardson BA, John-Stewart G, Walson JL. "Frequency and correlates of malaria over-treatment in areas of differing malaria treatment: cross-sectional study in rural Western Kenya." *Malar J* 2015 Mar 1;14(1): 97.

Ondoa, P., et al. (2005) "Assessment of CD8 T cell immune activation markers to monitor response to antiretroviral therapy among HIV-1 infected patients in Cote d'Ivoire." *Clin Exp Immunol* 140, 138-148.

Palumbo E. (2007) "Hepatitis B Genotypes and Response to Antiviral Therapy: A Review." *American J Therap*, 14:306-309.

Pelak, K, DB Goldstein, et al. (2010) "Host determinants of HIV-1 control in African Americans." *J Infect Dis* 201(8): 1141-1149.

Parikh S, Gut J, Istvan E, Goldberg DE, Havlir DV, Rosenthal PJ. (2005) "[Antimalarial activity of human immunodeficiency virus type 1 protease inhibitors.](#)" *Antimicrob Agents Chemother* 49(7):2983-5.

Patnaik P, Jere C, Miller W, Hoffman I, Wirima J, Pendame R, et al. (2005) "Effects of HIV-1 serostatus, HIV-1 RNA concentration, and CD4 cell count on the incidence of malaria infection in a cohort of adults in rural Malawi." *J Infect Dis* 192:984–991.

President's Malaria Initiative: Uganda, November 2009 available at [http://www.fightingmalaria.gov/countries/mops/fy10/uganda\\_mop-fy10.pdf](http://www.fightingmalaria.gov/countries/mops/fy10/uganda_mop-fy10.pdf) accessed 28 Jan 2011, accessed 28 Jan 2011

Price R, Nosten F, Simpson JA, Luxemburger C, et al. (1999) "Risk factors for gametocyte carriage in uncomplicated falciparum malaria." *Am J Trop Med Hyg* 60:1019-1023.

Puoti M, Manno D, Nasta P, Carosi G. (2008) "Hepatitis B Virus and HIV Coinfection in Low-income Countries." *Clin Infect Dis* 46:367-9.

Ranga U, Shankarappa R, Siddappa NB, Ramakrishna L, Nagendran R, Mahalingam M, et al. (2004) "Tat protein of human immunodeficiency virus type 1 subtype C strains is a defective chemokine." *J Virol.* **78**(5): 2586-90.

Robert V, Awono-Ambene HP, Le Hesran JY, Trape JF. (2000) "Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine." *Am J Trop Med Hyg* 62:210–216.

Sacktor N, Nakasujja N, Robertson K, Clifford DB. (2007) "HIV-associated cognitive impairment in sub-Saharan Africa--the potential effect of clade diversity." *Nat Clin Pract Neurol* **3**(8): 436-43.

Sacktor N, Nakasujja N, Skolasky RL, Rezapour M, Robertson K, Musisi S, et al. (2009) "HIV subtype D is associated with dementia, compared with subtype A, in immunosuppressed individuals at risk of cognitive impairment in Kampala, Uganda." *Clin Infect Dis* **49**(5): 780-6.

Schneider P, Bousema JT, Gouagna LC, Otieno S, et al. (2007) "Submicroscopic *Plasmodium falciparum* gametocyte densities requently result in mosquito infection." *Am J Trop Med Hyg* 76:470-474.

Shah SN, Smith E, Obonyo C, et al. (2006) "HIV immunosuppression and antimalarial efficacy: sulfadoxine-pyrimethamine for the treatment of uncomplicated malaria in HIV-infected adults in Siaya, Kenya." *J Infect Dis* 194(11): 1519-1528.

Shen, X., and GD Tomaras. (2010) "Alterations of the B-Cell response by HIV-1 replication." *Curr HIV/AIDS Rep*, 2.

Shiramizu B, Gartner S, Williams A, Shikuma C, Ratto-Kim S, Watters M, et al. (2005) "Circulating proviral HIV DNA and HIV-associated dementia." *AIDS* **19**(1): 45-52.

Skinner-Adams TS, Andrews KT, Melville L, McCarthy J, Gardiner DL. (2007) "Synergistic interactions of the antiretroviral protease inhibitors saquinavir and ritonavir with chloroquine and mefloquine against *Plasmodium falciparum* in vitro." *Antimicrob Agents Chemother* 51(2):759-62.

Skinner-Adams TS, McCarthy JS, Gardiner DL, Hilton PM, Andrews KT. (2004) "Antiretrovirals as antimalarial agents." *J Infect Dis* 190(11):1998-2000.

Sokhna CS, Trape JF, Robert V. (2001) "Gametocyaemia in Senegalese children with uncomplicated falciparum malaria treated with chloroquine, amodiaquine or sulfadoxine plus pyrimethamine." *Parasite* 8:243–250.

Sonnenberg P, Glynn JR, Fielding K, et al. (2005) “How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners.” *J Infect Dis*, 191, 150-8.

Soriano V, Sheldon J, Ramos B, Nunez M. (2006) “Confronting chronic hepatitis B virus infection in HIV: new diagnostic tools and more weapons.” *AIDS* 20:451-453.

Stepniewska K, Price RN, Sutherland CJ, Drakeley CJ, von Seidlein L, Nosten F, White NJ. (2008) “[Plasmodium falciparum gametocyte dynamics in areas of different malaria endemicity.](#)” *Malar J* 7:249.

Stubbs, BA, MA Micek, et al. (2009) “Treatment partners and adherence to HAART in Central Mozambique.” *AIDS Care* 21(11): 1412-1419.

Sweat M, Gregorich S, Sangiwa G, et al. (2000) “Cost-effectiveness of voluntary HIV-1 counseling and testing in reducing sexual transmission of HIV-1 in Kenya and Tanzania.” *Lancet*. 356(9224):113-121.

The Global Fund to Fight AIDS, TB, and Malaria  
<http://www.theglobalfund.org/en/tuberculosis/?lang=en> (Accessed 2 March 2011)

Tilling, R., et al. (2002) “Parallel decline of CD8+/CD38++ T cells and viraemia in response to quadruple highly active antiretroviral therapy in primary HIV infection.” *AIDS* 16, 589-596.

Trager W. (2005) “What triggers the gametocyte pathway in Plasmodium falciparum?” *TRENDS in Parasitology* 21(6): 262-264.

Triant VA, Meigs JB, Grinspoon SK. (2009) “Association of C-reactive protein and HIV infection with acute myocardial infarction.” *JAIDS* 51:268-273.

[UNAIDS report on the global AIDS epidemic](#) available at  
[http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2010/20101123\\_globalreport\\_en.pdf](http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2010/20101123_globalreport_en.pdf) , accessed on 28 Jan 2011

van Geertruyden JP, Mwananyanda L, Chalwe V, Moerman F, Chilengi R, Kestens L, et al. (2004) “Higher risk of antimalarial treatment failure in HIV positive than in HIV negative individuals with clinical malaria.” *Am J Trop Med Hyg* 71:315.

van Geertruyden JP, Mulenga M, Kasongo W, et al. (2006) “CD4 Tcell count and HIV-1 infection in adults with uncomplicated malaria.” *J AIDS* 43(3):363–367.

von Seidlein L, Jawara M, Coleman R, Doherty T, Walraven G, Targett G. (2001) “Parasitaemia and gametocytaemia after treatment with chloroquine,

pyrimethamine/sulfadoxine, and pyrimethamine/sulfadoxine combined with artesunate in young Gambians with uncomplicated malaria.” *Trop Med Int Health* 6:92–98.

Valcour VG, Sithinamsuwan P, Nidhinandana S, Thitivichianlert S, Ratto-Kim S, Apateerapong W, et al. (2007) “Neuropsychological abnormalities in patients with dementia in CRF 01\_AE HIV-1 infection.” *Neurology* 68(7): 525-7.

Zzzvujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, Hernandez RD, Lederman MM, Huang Y, Somsouk M, Deeks SG, Hunt PW, Lynch SV, McUune JM. “Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism.” *Sci Transl Med* 2013 Jul 10;5(193): 193ra91.

Walensky, RP, LL Wolf, et al. (2009) “When to start antiretroviral therapy in resource-limited settings.” *Ann Intern Med* 151(3): 157-166.

Whitworth J, Morgan D, Quiqley M, et al. (2000) “Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study.” *Lancet* 356(9235):1051-1056.

Wolday D, Mayaan S, Mariam ZG, Berhe N, Seboxa T, Britton S, Galai N, Landay A, Bentwich Z. (2002) “Treatment of intestinal worms is associated with decreased HIV plasma viral load.” *J Acquir Immune Defic Syndr* 1;31(1):56-62.

Wouters, E, F van Loon, et al. (2009) “Community support and disclosure of HV serostatus to family members by public-sector antiretroviral treatment patients in the Free State Providence of South Africa.” *AIDS Patient Care STDS* 23(5): 357-364.

Xiao L, Owen SM, Rudolph DL, Lal RB, Lal AA. (1998) “*Plasmodium falciparum* antigen-induced human immunodeficiency virus type 1 replication is mediated through induction of tumour necrosis factor-alpha.” *J Infect Dis* 177:437–445.

## **APPENDIX I: SUB-STUDY TEMPLATE**

## **APPENDIX II: CASE REPORT FORMS**

## **APPENDIX III: EXTERNAL COLLABORATOR'S LIST**

## **ATTACHMENT I: SITE-SPECIFIC ADDENDUM**



## **ATTACHMENT II: SCHEDULE OF EVENTS – HIV-INFECTED PARTICIPANTS**

## **ATTACHMENT III: SCHEDULE OF EVENTS – HIV-UNINFECTED PARTICIPANTS**

## **ATTACHMENT IV: INFORMED CONSENT FORM – HIV-INFECTED PARTICIPANTS**

**ATTACHMENT V: INFORMED CONSENT FORM – HIV-  
UNINFECTED PARTICIPANTS**

## **ATTACHMENT VI: BRIEFING SLIDES**