CAPRISA 002

VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

CAPRISA: University of KwaZulu-Natal

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PROTOCOL SYNOPSIS

PROTOCOL TITLE: Viral set point and clinical disease progression: the role of immunological, genetic and viral factors over the course of disease and during antiretroviral therapy (ART).

SITE: CAPRISA eThekwini and Vulindlela Clinical Research sites.

PURPOSE: HIV viral load, CD4+ T cell count and CD8+ T cell responses have been proposed as surrogate endpoints in clinical trials to assess HIV vaccines, which are hypothesized to prevent or delay the onset of AIDS. The purpose of this study is to examine the pathogenesis of acute HIV-1 subtype C infection and characterize these potential vaccine endpoints in 2 groups of participants viz participants who seroconvert while in follow up during an observational study of HIV negative women and women who may have had tenofovir pre-exposure prophylaxis prior to seroconversion. Specifically, it investigates the relationship between the magnitude and breadth of humoral and cellular immune responses, host and viral genetics and tenofovir exposure on viral load at 12 months post infection, disease progression and response to treatment in HIV-1 subtype C infected individuals. In this study, a single viral load measurement at 12 months post infection is used as a surrogate marker of viral set point with viral load measurements at 6 and 18 months also analyzed to determine which best predicts disease progression. The relationship between cellular and humoral immune responses, viral genetic changes and viral loads in acute, early and established infection will be characterized and the impact of immune pressure and viral escape on viral load trajectory will be determined. Further, the relationship between tenofovir exposure, immune function, set point and response to ART will be assessed.

PRIMARY OBJECTIVES:

1) To determine whether the magnitude and breadth of HIV-1 specific CD8+ T cell responses at three months post infection correlates with viral load at 12 months post infection.

2) To determine whether viral load at 12 months post infection is related to subsequent progression of HIV disease as defined by: A) CD4+ T cell count below 350 cells/mm³, B) AIDS-defining illness, or C) initiation of ART.

DESIGN: Prospective observational cohort study.

SAMPLE SIZE NUMBER: Approximately 300 HIV infected individuals

DURATION: Participants will be followed up until they initiate ART. After initiating ART, participants will be transitioned to Phase V and will be followed-up for a minimum of 5 years.
POPULATION: Participants with acute HIV infection will be identified from:

- a cohort of female sex workers in KwaZulu-Natal;
- participants in Phase II/IIb microbicide trials in Durban, and
- research cohorts in Vulindlela and Durban (females and males).

EVALUATIONS:

**Phase I: HIV Negative Female Sex Worker cohort** (≤ 24 months).
The following evaluations will be conducted:
physical examination, STI evaluation, genital specimen collection, routine
hematology and chemistry laboratory evaluations, HIV serology, HIV RNA-PCR, HIV risk behavioral assessment, and host genetics. (Completed)

**Phase II: Acute HIV Infection** (≤ 3 months post enrolment into Phase II).
The following evaluations will be conducted:
physical examination, STI evaluation, behavioral risk assessment, HIV
immunology and viral assays, genital specimen collection, routine hematology and chemistry laboratory evaluations, and host genetics.

**Phase III: Early Infection** (> 3 and ≤ 12 months post enrolment into Phase II).
The following evaluations will be conducted:
physical examination, STI evaluation, HIV immunology and viral assays,
genital specimen collection, routine hematology and chemistry laboratory
evaluations, and host genetics.

**Phase IV: Established Infection Phase** (> 12 months post enrolment or post
enrollment into Phase II or until clinical endpoint).
The following evaluations will be conducted:
physical examination, STI evaluation, HIV immunology and viral assays,
genital specimen collection, routine hematology and chemistry laboratory
evaluations.

**Phase V: Post-ART initiation Phase**
The following evaluations will be conducted:
physical examination, STI evaluation with collection of genital specimen, host
and viral genetics, HIV immunology and viral assays, routine hematology and chemistry laboratory evaluations, antiretroviral drug (including tenofovir) resistance testing
List of Abbreviations and Acronyms

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<td>Acquired Immunodeficiency Syndrome</td>
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<td>ANC</td>
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<td>CAPRISA Antiretroviral Treatment Programme</td>
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<tr>
<td>CD</td>
<td>cluster designation</td>
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<tr>
<td>CIOMS</td>
<td>Council for International Organizations of Medical Sciences</td>
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<td>CLP</td>
<td>Community Liaison Person</td>
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<td>CRF</td>
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<td>CTL</td>
<td>cytotoxic T lymphocyte</td>
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<td>Enhancing Care Initiative</td>
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<td>EPT</td>
<td>Expedited Partner Therapy</td>
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<td>FACS</td>
<td>fluorescent activated cell sorter</td>
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<td>FAHI</td>
<td>Functional Assessment of HIV Infection</td>
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<td>Full Blood Count (Complete Blood Count)</td>
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<td>interleukin</td>
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<td>institutional review board</td>
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<td>KEH VIII</td>
<td>King Edward VIIIth Hospital</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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mLFT  modified liver function tests
MRC   Medical Research Council
Nef    Negative Factor
NSI    non-synciti um inducing
OHRP  Office for Human Research Protections
PBMC  peripheral blood mononuclear cells
PBS   phosphate buffered saline
PCR   polymerase chain reaction
PE    phycoerythrin
PI    protease inhibitor
PHC   Public Health Care
pol   polymerase
pro   protease
RNA   ribonucleic acid
RT-PCR reverse transcription-polymerase chain reaction
SOP   standard operating procedure
STI   sexually transmitted infections
TB    tuberculosis
Tat   Trans-activator
TCLA  tissue culture line adapted
U&E   Urine dip stick and serum urea and electrolytes
UCT   University of Cape Town
UN    University of KwaZulu-Natal
UNAIDS Joint United Nations Programme on HIV/AIDS
US    United States
USPHS United States Public Health Service
VCT   Voluntary Counseling and Testing
WHO   World Health Organization
1.0 INTRODUCTION

1.1 Background

Surrogate markers for HIV-1 vaccine trials

HIV viral load along with the CD4+ T cell count are currently the best available predictors of progression to disease and death (Mellors et al., 1996, 1997; Lyles et al., 2000). Reduction of viral load is associated with a delay of disease progression and lower rates of transmission. The primary goal of prophylactic HIV vaccines is to prevent HIV infection. However, it is possible that first generation vaccines may not provide sterilizing immunity. For these vaccines, a secondary endpoint would be to improve prognosis following HIV-1 infection by reducing the incidence of clinical disease and mortality rates. An important marker of vaccine effectiveness could be the impact of vaccines on viral set point. However, this benefit may only be transitory. In the SIV macaque model escape from vaccine induced protection resulted in increased viral load and rapid disease progression (Barouch et al., 2002). It may therefore be important to include clinical endpoints in vaccine trials such as CD4+ T cell count, or time to initiation of therapy (Gilbert et al., 2003). Cellular immune response is a third possible surrogate marker of effectiveness of vaccines. The association between viral load and specific HIV-1 CD8+ T responses remains controversial with some studies showing an inverse correlation (Ogg et al., 1999; Edwards et al., 2002) while others show no correlation (Addo et al., 2003; Cao et al., 2003).

Why focus on subtype C?

There are extensive data on the natural history of subtype B infection in the developed world. However, there is limited information on subtype C infection in Africa to determine if a viral set point is reached; what influences set point; how long it takes to reach set point; and lastly the relationship between set point, decline in CD4+ T cell count and disease progression. Subtype C is responsible for the vast majority of heterosexual infections in South Africa where it has been detected in over 95% of samples analyzed (van Harmelen et al., 1997, 1999; Bredell et al., 1998; Moodley et al., 1998). There is evidence that subtype may impact the natural history of disease. In Uganda, subtype D was associated with faster disease progression compared with subtype A (Kaleebu et al., 2002). Similarly, in Senegal, individuals infected with non-A subtypes were more likely to develop AIDS compared to subtype A - infected individuals (Kanki et al., 1999). In Thailand higher viremia was detected in early, acute infections in subtype E infected individuals compared to subtype B infected individuals (Hu et al., 2001), although this difference decreased over time and viral loads were similar after one year of infection. In subtype C infections in India, although the viral ‘set point’ did not differ from subtype B set point, there were differences in viral load trajectory suggesting more rapid disease progression (Mehendale et al., 2002). However, other studies have found no association between subtype and disease progression (Aleus et al., 1999; Laurent et al., 2002). It is also possible that environmental exposure to an array of co-pathogens weakens exiting host immune protection (Clerici et al., 2000) and this may contribute to the observed different times to development of AIDS in Africa (Grant et al., 1997).

A systematic study to characterize subtype C set point in heterosexual infection in Africa, the role of specific immune responses in the control of replication, and the relationship between viral set point, CD4+ T cell count trajectory and disease progression will make an important contribution to the design and interpretation of vaccine efficacy trials.
Defining viral set point

After infection, viremia reaches a peak followed by control of viral replication to a hypothesized viral set point at which time there is a tenuous balance between production and destruction of virions. The level of this set point has been shown to be inversely correlated with time to disease progression (Schacker et al., 1998; Mellors et al., 1996; O’Brien et al., 1996). There is no standard method for measuring viral set point. Not all individuals reach a set point. Some individuals have slowly declining or controlled viral load, and others have increasing viral load. A natural history study of subtype B HIV viremia after seroconversion revealed that men with different AIDS-free times were characterized by clear variation in initial RNA levels as well as differences in 3-year viral load slopes. However, irrespective of the viral load trajectory, it was shown that a single measurement after 12 to 18 months post HIV infection was the most prognostic of disease progression (Lyles et al., 2000).

Defining acute, early and established HIV infection

Immunological responses in acute infection are thought to be crucial in controlling initial viral replication and may predict subsequent viral set point. Acute infection refers to the period following exposure to, and infection with, HIV. The endpoint of this period is not standardized and ranges from acute retroviral illness to asymptomatic seropositive state with a documented negative HIV test in the previous 6-12 months. For the purpose of this study, we will recruit HIV positive participants into the acute infection phase who have a documented seronegative result within the previous 5 months or participants of other CAPRISA studies and cohorts who have seroconverted recently and will be of particular interest to this study, for example, ex CAPRISA 004 Tenofovir Microbicide participants. We will define acute infection as the three-month period following enrollment into Phase II of the study. The period following the acute infection phase until 12 months post enrollment into Phase II will be referred to as early infection, and after 12 months until endpoint of the study will be referred to as established infection.
Clinical symptoms associated with acute infection

The acute retroviral syndrome is characteristically a systemic febrile illness resembling acute mononucleosis, which occurs approximately 1 to 4 weeks after exposure to HIV, and lasts an average of 2 weeks (Schacker et al., 1996). Some patients with acute HIV infection are asymptomatic while the majority (40-90%) develop generalized flu-like or infectious mononucleosis like disease. Fever is present in >80-90% of patients, fatigue in >70-90% and rash (maculopapular exanthema and usually involving the trunk) in >40-80%, progressing to papulovesicular appearance in 40-80% (Kahn et al., 1998). Other signs and symptoms include myalgias and arthralgias, weight loss, exudative pharyngitis, oral, genital and rectal ulcers, lymphadenopathy, night sweats, nausea, anorexia, headache, aseptic meningitis (fever, headache, photophobia and stiff neck), orthostatic hypotension, lymphopenia, and thrombocytopenia. The differential diagnosis for this syndrome includes mononucleosis (acute Epstein-Barr Virus [EBV] infection), cytomegalovirus (CMV) or herpes simplex virus (HSV) infection, enteroviral meningitis, acute hepatitis B, influenza, rubella, syphilis and acute toxoplasmosis.

In this study, we use the clinical criteria in the Acute Retroviral Syndrome section outlined in Appendix 1 to describe the acute HIV-1 infection syndrome. This tool is based on data from a recent study published by Hecht et al. (2002) which demonstrated that fever and malaise had the highest sensitivity for clinical diagnosis of acute HIV-1 infection, whereas loss of weight and oral ulcers had the highest specificity (Hecht et al., 2002; Daar et al., 2001). It has been shown that the duration and severity of clinical signs and symptoms of acute HIV-1 illness is associated with a more rapid disease progression. Evidence from prospective studies of Swiss and Australian cohorts demonstrated that there are six signs and symptoms contributing to a quantitative scoring system: these included fever, skin rash, lethargy, oral candidiasis, pharyngitis/sore throat, and diarrhea. The authors found a dose-response relationship between the number of symptoms and signs reported at the time of acute HIV-1, subtype B, infection and disease progression and death (Vanhems et al., 1998; Vanhems et al., 2003). In this study, we will correlate the presence and severity of these signs/symptoms with subsequent clinical and virologic progression.

Composite clinically relevant endpoints

Natural history studies of infection with HIV-1 subtype B have characterized the rate of progression to AIDS according to both plasma HIV RNA copy number and CD4+ T cell count (the Multicenter AIDS Cohort Study or MAC study). There has been no comprehensive subtype C natural history study. The MAC Study demonstrated that the 3-year risk for progression to AIDS was 38% among patients with 201-350 cells/mm³ CD4+ T cells, compared with 14.3% for patients with CD4+ T cell counts >350 cells/mm³. However, the short-term risk for progression was also related to the level of plasma HIV RNA, and the risk was relatively low for those with <20,000 copies/ml. An evaluation of 231 persons with CD4+ T cell counts of 201-350 cells/mm³ demonstrated that the 3-year risk of progression to AIDS was 4.1% for the 74 patients with HIV RNA < 20,000 copies/ml; 36.4% for those 53 patients with HIV RNA 20,001-55,000 copies/ml; and 64.4% for those 104 patients with HIV RNA >55,000 copies/ml. (Phair et al., 2002; Mellors et al., 1997). Similar risk gradations by viral load are evident for patients with CD4+ T cell counts >350 cells/mm³.

Although current data provide strong evidence that waiting for CD4+ T cells <200 cells/mm³ to treat patients has been associated with higher mortality rates (Gulick et al., 1997; Kaplan et al., 2000; Hogg et al. 2001), the optimal time to initiate ART is unclear. Most analyses of
observational databases have not demonstrated a clinical benefit from initiation of therapy at CD4+ T cell counts >350 cells/mm³. There is in fact controversy regarding management of patients with CD4 T cell counts in the buffer zone between 200 and 350 cells/mm³, and so CD4+ T cell count of 350 cells/mm³ has been adopted by many as the level to begin consideration of ART in the absence of increased levels of HIV RNA (Department of Health and Human Services (DHHS), the USPHS and IAS-USA panel). At the same time, however, it has been shown that a significant number of individuals with CD4+ T cell count between 201 cells/mm³ and 350 cells/mm³ and plasma RNA levels >20,000 copies/ml, demonstrate a decrease in CD4+ T cell counts to <200 cells/mm³ within 1-3 years. Such individuals require close follow up to prevent development of reduced responsiveness to future treatment and/or progression to clinical AIDS (Phair et al., 2002). This study uses a composite clinically relevant endpoint of CD4+ T cell count of less than 350 cells/mm³, development of AIDS defining illness, or initiation of ART.

Nutritional and metabolic disorders in acute HIV infection

Lipodystrophy syndrome (abnormal lipid/glucose metabolism and fat redistribution) and nutritional deficiencies have long been recognized as a complication of chronic HIV infection. Currently, it is reported that over 60% of patients receiving protease inhibitors (PIs) develop hyperlipidemia, hyperglycemia, central obesity, and fat redistribution (Wanke et al., 2002; Dube et al., 2000; Riddler et al., 2003). Frank diabetes mellitus has also been observed, although in relatively small percentages (1-7%). Anthropometric measurements which consist of body dimension and subcutaneous fat measures, provide a reliable and non-invasive means of monitoring long-term nutritional status, characterizing body fat redistribution, and screening for nutritional risk (Knox et al., 2003; Gerrior et al., 2001; Wanke et al, 2002). These measurements have been shown to correlate well with lean body mass changes measured by dual X-ray and may quite reliably identify persons with HIV-1 infection who are at risk for serious consequences of malnutrition (Paton et al., 1997; Knox et al., 2003).

While the most dramatic metabolic disorders occur after initiation of therapy, patients with chronic HIV infection have abnormal lipid metabolism (low levels of LDL and HDL, followed by rising triglycerides) even in the absence of highly active antiretroviral therapy (HAART); likewise, cross-sectional data suggests that HIV infection itself may be associated with insulin resistance and truncal obesity (Hadigan et al., 1999; Stein 2003). Little is known, however, about the metabolic profiles of patients during acute and early stages of HIV-1 infection. HIV-1 infection is known to have adverse effect on nutritional status. Involuntary weight loss is a feature of HIV disease progression and has been associated with adverse outcomes (Knox et al., 2003; Wheeler et al., 1998). An abrupt decline in Body Mass Index (BMI), for example, predicts progression to AIDS (Maas et al., 1998). The etiology of wasting is thought to be multifactorial, and may include decreased dietary intake, malabsorption or opportunistic infections (Wanke et al., 2003).

Other measures of nutritional status also predict outcome with HIV infection. Cross sectional studies have demonstrated that micronutrient deficiencies are not only common in HIV infection, but deficiencies in serum vitamins (A and B12), selenium and zinc have been associated with progression of disease. In data from asymptomatic HIV-positive individuals, better nutritional status, measured by higher intakes of individual micronutrients and multivitamins, was associated with better outcomes in terms of CD4 T cell counts, clinical progression to AIDS, and mortality (Fawzi, 2003).
The significance of these metabolic disorders is quite clear; it has been shown that the risk for diabetes and cardiovascular disease increases with an increasing waist-to-hip ratio; alteration in glucose and lipid metabolism have similar implications for patients’ cardiovascular risk, and micronutrient deficiencies accompanied by weight loss and wasting may contribute to a weakening of immune status and, thus, a worsening of clinical condition in HIV-infected individuals; this is of particular problem in many developing countries (Fawzi, 2003; Knox et al., 2003; Shevitz et al, 2001).

It has been proposed that a baseline nutritional assessment should include an evaluation of anthropometric, biochemical, clinical, and dietary parameters (Knox et al., 2003). In this study, we will measure patients’ baseline nutritional status, metabolic and morphologic abnormalities in acute HIV-1 infection by evaluating the surrogate markers of BMI, subcutaneous fat stores, waist circumference, and lipid and serum glucose levels; we will plan for future analysis of micronutrient levels from stored samples. This analysis will shed some light on the role of these factors in the natural history of acute and early HIV-1 subtype C infection, and subsequent progression of clinical disease. It is not currently known when, in the course of the early infection, the patient’s vulnerability to subsequent nutritional and metabolic derangements are established. Furthermore, this analysis may aid in instituting early interventions in patients who are at an increased risk for developing insulin resistance, dyslipidemia, fat redistribution, and weight loss with micronutrient deficiencies.

Impact of T cell immunity on HIV replication

Cytotoxic T-lymphocyte (CTL) responses are associated with the initial containment of viral load (Borrow et al., 1994). Their importance in the control of virus replication is illustrated in the SIV-macaque model where CD8+ T cell depletion resulted in increased viral load, which was controlled once CD8+ T cell responses are restored (Schmitz et al., 1999). CTL and T helper responses are also associated with protection as emphasized by their role in long-term non-progressors (Rosenburg et al., 1997) and CTL in highly exposed persistently seronegative individuals (Rowland-Jones et al., 1998; Rowland-Jones et al., 1999). Effective CTL and T helper cell responses elicited at the early stage of infection may be important for determining whether viral burden will be controlled (McMichael 1998). A study of individuals in acute infection (< 3 months) showed that only a few epitopes were recognized at this stage, however a broadening of the immune response occurred over time (Cao et al., 2003). Failure to mount sustained CTL and T helper responses from the early stage of infection may be due to an inability to develop immunodominant CTL responses or that responses may target variable protein regions precipitating viral escape (McMichael & Phillips, 1997; McMichael 1998). Identity of conserved regions in HIV-1 proteins that are recognized by CTL would theoretically be important for control of viral replication (da Silva & Hughes 1998; Goulder et al.1997a). CD8+ T cell phenotype and function may also play a role in determining viral set point.

However, the relationship between specific CTL responses and sustainable control of viral replication remains in question with some investigators reporting an inverse correlation between specific HIV-1 CD8+ T cell responses and viral load (Ogg et al., 1999; Edwards et al., 2002), others have shown no correlation (Addo et al., 2003; Cao et al., 2003). These differences may, in part, be due to the region of the genome analyzed or due to variation in stage of disease in the cohorts studied. It is also possible that responses are being missed due to the mismatch between the peptide reagent and infecting virus sequence. This is illustrated in a study using peptide reagents based on the autologous virus sequence where increased frequency and stronger
responses were detected, especially to variable proteins such as the regulatory and accessory proteins Tat and Vpr (Altfeld et al., 2003).

This study aims to do a comprehensive screen for CD8+ T cell responses to all HIV protein in early infection to determine the association between CD8+ T cell responses and viral set point. Secondly, it aims to determine their role in the maintenance of control on viral replication throughout the study period.

Influence of HLA and other host genetic factors on viral set point

A number of studies have found an association between HLA class I and II allele expression and HIV-1 disease progression and resistance to HIV infection (Kaslow et al., 1996; Mann et al., 1998; Keet et al., 1999; Evans et al., 1999; Migueles et al., 2000). HLA-B27 and HLA-B14 are associated with immunodominance as well as slow progression to AIDS (Goulder et al., 1997a; Kaslow et al., 1996). Conversely, HLA-A29 and HLA-B22 have been associated with rapid disease progression (Hendel et al., 1999). Disease progression is also associated with HLA homozygosity (Carrington et al., 1999), especially within either A and B loci (Tang et al., 1999) which may be a result of limited antigen presentation. DRB1*13-DQB1*06 haplotype has been associated with maintaining viral suppression in a group of HIV-1 patients receiving ART as well as in a group of long-term non-progressors (Malhotra et al., 2001). In the case of the female sex workers (MacDonald et al., 2000), the DRB1*01 allele was independently associated with resistance to infection.

In addition to HLA, a number of human genes with polymorphic variants that influence the outcome of HIV exposure or infection or AIDS restriction genes (ARGs) (O’Brein et al., 1997; O’Brein 1998) have been identified. Among such host factors that have been shown to affect susceptibility to HIV infection and disease progression are chemokine receptor polymorphisms, HIV-specific immune responses, and polymorphisms in molecules that play a key role in antiviral immune responses (Smith et al., 1997; Liu et al., 1996; Huang et al., 1996; Clerici et al., 1992; Rowland-Jones et al., 1995; Mazolli et al., 1997). The frequencies of some of these host factors that influence HIV transmission and pathogenesis differ among populations according to ethnic or racial background (Martinson et al., 1997: Winkler et al., 2004), and alleles associated with susceptibility or resistance to HIV-1 may differ among racially distinct groups (Fowke et al., 1996; Ogg et al., 1999; Fabio et al., 1992).

Recently, it has been demonstrated that certain ARGs that alter the overall rate of disease progression act during distinct intervals after HIV infection (Gao et al., 2005). There is a need therefore to investigate the role of novel antiviral factors during acute and early HIV infection versus chronic phase of infection. Detailed analysis of the role of host genes could also elucidate previously unknown interactions in adaptive and innate immunity in the control of viral replication. Indeed, although a numbers of ARGs have been identified, the mechanisms underlying their effects are mostly poorly understood.

We hypothesize that ARGs may have a significant effect on early virus replication in HIV-1 infected individuals and thus affect the viral set point and or long term control of viral replication in case of chronic infection, leading to long-term non-progression. Specifically, we hypothesize that the following are ARGs in HIV-1 subtype C infections in South Africa: the cytosine deaminase enzyme APOBEC3G; the cytoplasmic body associated protein TRIM5αhu; HLA class
II genes; KIR genes (KIR 3DS1); PD-1; IL-10; LEDGF- a cellular factor involved in HIV integration.

**Role of neutralizing antibodies in controlling viral replication**

Neutralizing antibodies are potent inhibitors of HIV replication *in vitro* but their absence during the acute phase of infection has led many to conclude that they play a minor role in immune control. This view has been reinforced by studies in rhesus monkeys showing that depletion of B-cells did not impact the viral levels during acute infection. However, B-cell depleted animals showed higher viral levels during the post-acute phase (Schmitz *et al.*, 2003). In other studies, virus-specific antibodies have been shown to rapidly clear circulating virus particles (Igarashi *et al.*, 1999) and protect macaques from infection (Mascola *et al.*, 1999; Shibata *et al.*, 1999). These protective or disease ameliorating effects of passively administered antibody were even more pronounced when animals were challenged through the vaginal (Mascola *et al.*, 2000) or oral (Baba *et al.*, 2000) route. These data suggest that neutralizing antibodies are able to control viral replication during the post-acute phase of infection and during chronic infection.

Additional evidence that neutralizing antibodies are involved in controlling viral replication *in vivo* comes from studies on persons who remain disease-free for many years, in the absence of anti-retroviral therapy. A number of studies have shown that long-term non-progressors possess broadly cross-reactive neutralizing antibodies that clearly distinguish them from individuals who have progressive disease (Carotenuto *et al.*, 1998; Cecilia *et al.*, 1999; Pilgrim *et al.*, 1997). These individuals are rare, but are important to identify as they create the opportunity to better analyze and understand the role of neutralizing antibodies in HIV infection. Although it is not clear whether the improved quality of neutralizing antibodies in these individuals are a cause or effect of long-term non-progression, such studies have shown that neutralizing antibodies are not genetic subtype--specific and that potent sera can neutralize viruses from a variety of genetic subtypes.

Few neutralization epitopes on primary isolates have been identified and only three broadly cross-reactive monoclonal antibodies have been produced that are able to neutralize the majority of viral isolates. Studies with monoclonal antibodies and polyclonal sera indicate that neutralizing epitopes occur in multiple regions of the envelope protein including V1/V2, CD4 and coreceptor binding sites and even to glycans (Burton & Montefiori, 1997; Pinter *et al.*, 1998; Wu *et al.*, 1995).

Infection with HIV-1 has been shown to elicit an IgG serum antibody response with neutralizing activity. Secretory IgA (sIgA) and IgG antibodies have also been detected in cervicovaginal secretions of infected individuals (Belec *et al.*, 1989), and in some cases these mucosal responses have been shown to have neutralizing activity (Moja *et al.*, 2000). The presence of cross-clade HIV-1-specific neutralizing IgA in mucosal and systemic compartments of HIV-1-exposed persistently seronegative participants have been described and linked to protection (Devito *et al.*, 2002). Therefore, evaluating mucosal humoral immunity will be important for understanding local immunity induced by HIV infection or vaccination and designing prophylactic strategies.

**Dynamics of viral evolution**

One of the keys to the success of HIV as a pathogen is its ability to rapidly mutate in response to selective pressure and thus to continually circumvent immunity. The characterization of recently
transmitted HIV-1 variants is crucial to our understanding of mechanisms of transmission and relevant to the development of interventions such as vaccines and microbicides. Many of the early studies showed that virus populations in recently infected individuals were homogenous (Zhang et al., 1993; Zhu et al., 1993), suggesting either selective transmission, or selective amplification (outgrowth of a particular quasispecies). However, a recent study in homosexually infected men showed transmission of a heterogeneous virus mixture and that virus population homogenization occurred soon after transmission (Learn et al., 2002). In this study, homogenization was found in env but not gag, suggesting selection occurs at the level of cell tropism. However, there may be gender differences in virus transmission. A study comparing viral diversity in acutely infected men and women in Kenya found that recently infected men harbored homogenous virus populations, whereas the diversity in recently infected women was much higher (Long et al., 2000).

To effectively investigate transmission, it is essential to obtain samples as close to infection as possible. This study attempts to identify individuals who are HIV positive prior to seroconversion. As infection of women occurs across the genital tract it is important to obtain samples from this location as some studies have shown that HIV-1 in the female genital tract compartment and blood compartment are phylogenetically and phenotypically distinct (Hughes et al., 1997; Kuiken et al., 1995; Epstein et al., 1991; Itescu et al., 1994; Zhu et al., 1996; Ellerbrock et al., 2001). This was first shown in proviral populations (Panther et al., 2000; Poss et al., 1995, 1998; Overbaugh et al., 1996) and more recently also in cell-free virions (Ellerbrock et al., 2001). Compartmentalization would imply that the female genital tract selection pressures are different to those in blood. Local cytokine, chemokine and HIV-1 specific immunoglobulin production differs between the two compartments could explain the observed discordance (Belec et al., 1995a; 1995b; Artenstein et al., 1997; Iversen et al., 1998; Hladik et al., 1999). In addition, the response in the mucosa may differ from the periphery due to continual exposure to commensals and pathogens. In our studies we will characterize transmitted virus collected from site of transmission (genital specimens), as well as the peripheral blood, and investigate discordant virus evolution between blood and genital compartments in South African women during acute infection.

There have been a number of conflicting reports on the relationship between viral diversity and disease progression with some studies finding an inverse correlation between rate of diversification and disease progression, while others have found the reverse. The most comprehensive analysis to date on the natural history of viral diversity was reported by Shankarappa et al. (1999), who focused on sequence variation in the C2-V5 region of the env gene in nine men from seroconversion to up to 6 to 12 years of infection. Three distinct phases were defined: in the early phase of infection, increased divergence from the source infection was associated with linear increase in diversity at a rate of ~1.0% per year; in the intermediate phase, there was a continued increase in divergence from source but a stabilization or decrease in intraperson viral diversity, along with the appearance and increase in representation of viruses with tropism for the CXCR4 co-receptor (X4-viruses); in the late phase, divergence stabilized, and intraperson diversity and the representation of X4 viruses declined. A study in Senegal, West Africa has shown an association between intrapatient diversity, divergent and diversification and viral set point (Mani et al., 2002). The detection of dual HIV-1 infection, that is infection with more than one strain of HIV-1, is becoming increasingly common. Dual infection can be a consequence of either co-transmission (infection with two strains at or close to seroconversion) or superinfection (subsequent HIV-1 infection of an already infected individual). Dually infected individuals harbor highly divergent viral populations and one study has shown that co-
transmission is associated with increased disease progression (Goettlieb et al., 2003). Superinfection has been reported in only a few recent individual cases involving the same subtype (Kersten et al., 2003; Altfeld et al., 2002). Superinfection is of interest as it provides information on immunity and protective immune responses and raises concern in vaccine development as responses to the first HIV infection cannot protect from re-infection.

Measuring viral diversity prospectively from onset of infection can provide valuable insight into the nature of immune selection pressure and correlates of protection. Participants will also be screened for dual infection to investigate the relationship between infection with multiple strains of HIV and disease progression.

Phenotypic properties of transmitted viruses

HIV transmission is facilitated by the ability of the infecting strain to utilize CD4 receptor and the CCR5 coreceptor efficiently. As disease develops, some viruses are able to use the second major coreceptor, CXCR4, sometimes in addition to CCR5. This coreceptor switch correlates with the syncitium-inducing (SI) phenotype, which shows more rapid replication kinetics in vitro and is associated with a decline in the number of CD4+ T cells in vivo presumably as a consequence of these newly acquired properties (Connor et al., 1997, Richman & Bozzette, 1994). The ligands for CCR5 (RANTES, MIP-1alpha, and MIP-1beta) and CXCR4 (SDF-1) are potent inhibitors of viral entry and, hence, high levels of these chemokines would be expected to control viral levels in vivo. There has been some evidence to suggest this to be the case (Scala et al., 1997). In addition, it has been shown that decreased sensitivity to these entry inhibitors is associated with rapid disease progression in infants (Scarlatti et al., 1997). Thus, the ability of HIV to become less sensitive to the inhibitory effects of these circulating humoral factors is a potential mechanism that the virus uses to escape immune control. An understanding of these processes has been confounded by the realization that HIV-1 subtype C viruses rarely use CXCR4 (Bjorndal et al., 1999, Morris et al., 2001, Peeters et al., 1999, Tscherning et al., 1998). It is unclear whether this is an intrinsic property of the virus. For example, whether subtype C envelope proteins can accommodate the necessary changes needed to use CXCR4. Recent data suggest that this is unlikely, as CXCR4-using subtype C viruses have been described (Cilliers et al., 2003; van Rensburg et al., 2002). However, these still occur at a low frequency even in advanced AIDS patients (Cilliers et al., 2003). The preference for CCR5 coreceptor usage by subtype C viruses may be due to selection pressure by the host, for example by maintaining high levels of CCR5 coreceptor expression. There could also be more subtle effects, such as the reduced sensitivity of subtype C envelope proteins to anti-CCR5 agents in the absence of an overt coreceptor switch. This may be associated with increased viral replication levels and higher viral set point. In this study, we plan to characterize coreceptor usage at transmission through to clinical endpoint and track overt co-receptor switch, as well as investigate more subtle changes in co-receptor usage through inhibitor studies.

Viral escape from immune responses

Due to the ability for the viruses to continually adapt, escape from CTL pressure is common. However, the impact of CTL escape on disease progression remains unresolved. Viral escape from CTL pressure has been demonstrated in SIV-infected and SHIV-challenged macaques (Allen et al., 2000, Evans et al., 1999b) and in HIV-1 infection (Phillips et al., 1991) (McMichael & Phillips, 1997). Accumulation of CTL epitope variants has been associated with increased viral load (Klennerman & Zinkernagel, 1997) and pathogenesis in HIV (Goulder et al., 1997b) leading
to death in macaques (Evans et al., 1999a). Following transmission, the virus replicates exponentially. Thereafter control of replication is usually achieved, presumably due to CTL responses. Studies in macaques have shown that CTL pressure is one of the major forces driving sequence change in acute infections. In this study, they found that the tat gene was the first region to evolve, and was the only region associated with escape from CTL responses (Allen et al., 2000). One of the aims of this project is to assess the relationship between breadth of CTL response across all protein regions and CTL escape, and to identify which regions are evading detection.

Antibody escape is a common feature of HIV infection with later viral isolates becoming increasingly resistant to neutralizing antibodies. Recent studies have shown specific changes in the envelope glycoproteins result in the acquisition and rearrangement of sugar moieties, forging them into an evolving glycan shield that prevents antibody binding (Wei, et al., 2003). Other epitopes such as the receptor binding site, that need to be conserved to maintain viral fitness avoid neutralization by a ‘conformational masking’ mechanism of escape (Kwong, et al., 2002). Other mechanisms of avoiding antibody neutralization include epitope variation, oligomeric exclusion and the immunologically silent face that is covered in glycans and do not elicit antibodies. A third potential mechanism of evasion, outlined in the section above, involves the ability of HIV to become less sensitive to the inhibitory effects of circulating humoral immunity.

Broadly Cross-Neutralising Antibodies

The induction of broadly cross-neutralizing antibodies is central to vaccine development, however, the vaccine field has had very limited success in achieving this to date. This is largely due to substantial antigenic variability and the ability of the HIV-1 envelope glycoprotein to mask itself. While most individuals mount a potent neutralizing antibody response within the first few months of HIV infection-1, targeting the variable regions of the envelope glycoprotein, the response preferentially neutralises autologous virus which rapidly escapes (Doria-Rose, et al., 2014, Moore, et al. 2011). A subset of people (approximately 20-30%), develop broadly cross-neutralising antibodies after 2-3 years of infection, which are effective against diverse viruses (Doria-Rose, et al., 2014, Moore, et al. 2011). Identification of the targets of these antibodies is crucial as these may inform vaccine development more readily than targets currently used in vaccine design.

One of the individuals identified as having broadly cross neutralising antibodies in this cohort is CAP 256 whose antibody activity peaked at 3 years post-infection, neutralizing 32 (76%) of 42 heterologous viruses, with titres of antibodies against some viruses exceeding 1:10000 (Moore, et al. 2011). CAP 256 preferentially neutralized subtype C and A viruses over subtype B viruses (Moore, et al. 2011). The CAP 256 targets have been extensively delineated and comprise a quaternary epitope including the variable region 1 and 2 (V1V2) of the HIV-1 envelope. Subsequent non-human primate studies have demonstrated potency and protective efficacy of this antibody (Julg, et al., 2017). Phase 1 human trials testing the safety and tolerability of the CAP256 antibody are scheduled to take place in 2018 by the CAPRISA team.

T cell function during ART

HIV induces a litany of systemic immune defects. In addition to CD4+ T cell loss, a range of functional aspects of the immune system are affected. Whilst CD4+ T cell numbers may increase with successful ART, we do not have a good understanding of what functional aspects of the
immune system recover when viral load is suppressed, or why some immune abnormalities may persist despite the absence of virus.

Generalised T cell activation that occurs during chronic infection is thought to be a major determinant in disease progression (Hazenberg et al., 2003). HIV plasma viral load is not the only driver of this generalised cellular activation. High levels of viral replication and depletion of important immune subsets in the gastrointestinal tract, and the resultant damage to the epithelial lining of the gut, causes translocation of microbial products such as lipopolysaccharides (LPS) into the bloodstream (Brenchley et al., 2006), resulting in increased immune activation and cell turnover. Whilst ART suppresses HIV load, other immune damage may persist and influence CD4+ T cell recovery. Indeed, baseline CD4+ and CD8+ T cell activation associated positively with an increase in CD4+ cells in chronically-infected individuals on therapy (Goicoechea et al. 2006). Thus, activation status prior to therapy initiation, or immune causes unrelated to viral load during therapy, may have adverse effects on the level of CD4+ reconstitution. The rate of “deactivation” of the immune system may contribute to the rate of CD4+ recovery.

High levels of immune activation can drive differentiation of memory T cells in a non-specific manner (Papagno et al., 2004). Chronic activation caused by HIV may deplete central memory cells by driving them to differentiate into effector cells. The preservation of total CD4+ central memory cells has been correlated with lower viral loads (Potter et al., 2007) and longer AIDS-free survival times (Letvin et al., 2006). It is not known whether memory subsets that have been skewed towards late differentiation in chronic HIV infection, or depleted substantially, may recover fully during antiretroviral treatment. There is some evidence that HIV-specific central memory CD4+ T cells are not restored by therapy (Elrefaei et al., 2004), but little is known about CD8+ memory subsets. Furthermore, the ability of T cells to secrete multiple cytokines simultaneously has been associated with control of virus replication (Betts et al., 2006; Kannanganat et al., 2007). Only a few published studies have addressed the emergence of polyfunctional T cell responses to HIV during ART (López et al., 2008; Rehr et al., 2008), and conflicting results have been found. There is clearly a need to further investigate the functional nature of HIV-specific T cells during ART.

**Tenofovir gel pre-exposure prophylaxis and its impact on immunity and viral resistance**

Tenofovir is a novel nucleotide analog with potent activity against retroviruses. Tenofovir acts by inhibiting viral post fusion replication thus terminating the growing DNA chain within its target cells, the mononuclear cells. Rapid intracellular phosphorylation of tenofovir into its active metabolites, tenofovir diphosphate, occurs in both active and resting cells. The long half-life of these active metabolites, is thought to be responsible for disrupting the replication cycle of virus (Emau, Jiang et al. 2006).

The oral form of the drug, known as tenofovir disoproxil fumarate (TDF), has been in use for more than seven years for the indication of treatment of HIV infected individuals, as part of the HAART treatment strategies. It is currently being tested for use in pre-exposure prophylaxis (PrEP). This PrEP strategy involves the treatment of uninfected individuals, at high risk of HIV infection, with antiretroviral drugs in advance of exposure to HIV (Subbarao, Otten et al. 2006). Additionally, its gel formulation 9-[(R)-2-(phosphonomethoxy) propyl] adenine monohydrate (PMPA) is currently being studied as a microbicide agent. Much data that informs its potential positive effect as a microbicide agent comes from animal models.
Tenofovir has been shown to be of low risk in selecting for resistance when used in combination with other antiretroviral drugs in HIV-infected individuals. As such it is being increasingly recommended and used in first-line regimens. Over 30 studies in animals have investigated the use of tenofovir for prevention of HIV-1 infection. A single vaginal dose of tenofovir gel given 15 minutes before challenge has been shown to be highly efficacious in macaques (Ambrose et al., 2008). Furthermore, tenofovir used within 24 hours after exposure showed good efficacy although this effect dropped off by 48 and 72 hours (Garcia-Lerma et al., 2008; Parikh et al., in press).

At present the optimal exposure of tenofovir in the genital tract to protect against HIV infection is not known. Studies in animals and humans have shown that tenofovir applied vaginally can be detected in plasma, though at low levels. Given that there may be sub-therapeutic levels for an extended period of time in participants enrolled in the tenofovir gel trial it will be important to monitor for tenofovir resistance once women are found to be HIV infected. Furthermore, oral tenofovir disoproxyl fumarate has recently become available in South Africa and its use in HAART for treatment of AIDS patients is on the increase. While tenofovir resistance is uncommon in South Africa, this may change over the course of the next few years, with concomitant increases in the prevalence of tenofovir resistant viruses being transmitted and possibly compromising use of this drug for HIV prevention.

Resistance to tenofovir is associated with the K65R mutation in the HIV-1 reverse transcriptase gene. A separate pattern of tenofovir resistance is conferred by 3 or more thymidine analogue resistance mutations (TAMS), particularly M41L or L210W. The T69S insertion mutations, associated with resistance to multiple nucleoside analogues, are also associated with resistance to tenofovir. An in vitro study showed that subtype C viruses have a higher propensity to develop the K65R mutation compared to subtype B viruses, when cultured in the presence of tenofovir alone (Brenner et al., AIDS, 2006). While, there is no prospective clinical data on subtype C infected patients to support this laboratory finding it is important to bear this in mind (particularly given that women in the tenofovir gel study are likely to be infected with HIV-1 subtype C viruses and will continue to use tenofovir mono-therapy until their HIV infection is diagnosed).

Thus, it would be important to determine whether the use of tenofovir to prevent HIV-1 infection is associated with an identifiable drug level that correlates with protection. Moreover, it would be important to determine whether tenofovir has the propensity to mediate and or alter immune responses to HIV-1 and to select for drug resistant viruses (including minority populations) that might impact on the utility of tenofovir disoproxil fumarate as treatment in these individuals.

Latent Reservoir (Cure) Studies

Although ART has greatly impacted the life expectancy of HIV-infected individuals, the economic feasibility of drug treatment for all those infected remains a major obstacle. Interest in achieving a lasting cure for HIV has grown with the identification of rare cases of individuals who have either eliminated all infected cells (sterilizing cure) (Hutter et al., 2009) or have sustained control of HIV after the cessation of therapy (functional cure) (Saenz-Cirion et al., 2013). The major challenge for cure research is eliminating the reservoir of latently infected cells in an individual, and many aspects of the reservoir are only partially understood.

In general, the latent reservoir has been under-researched in women and in Africans in particular, and it is imperative that studies are done in different populations where differences in host
genetics, environment, socio-economics, infecting subtype, treatment regimes, and gender may affect the reservoir. In addition, an effective method for sizing the reservoir is needed since recent studies have reported the number of intact integrated viral genomes in latently infected resting memory CD4+ T cells (representing the major viral reservoir) to be far greater than the number of viruses induced from the reservoir following activation of these cells (Ho et al., 2013).

Furthermore, the timing of establishment and the dynamics of entry and decay of viruses in the reservoir is not completely understood. In most countries, especially in Africa, people only initiate treatment in chronic infection resulting in a longer reservoir half-life. The rapid diversification of HIV during infection, together with very limited viral replication in the reservoir, provides the opportunity to track when virus is archived in the latent reservoir in individuals who initiated therapy during chronic infection.

Finally, little is known about the relative contribution that acute infection, and factors such as levels of immune activation and viral load, make to the latent reservoir and the timing of deposition of viruses over the first several years of infection for individuals initiating treatment in chronic infection. Immune activation has long been recognized to play a role in HIV pathogenesis and correlates with clinical progression more robustly than viral load. Early immune activation may be critical in establishing the size of the reservoir.

These studies will measure the size, composition, and change of the latent HIV reservoir in participants on ART, will define evolving viral populations from acute infection to ART initiation and determine how immune activation influences the timing of the establishment, size and change of the reservoir.

1.2 Study Rationale

It is hypothesized that the breadth and magnitude of the HIV-specific CD8+ T cell responses in acute infection (< three months post infection) are inversely proportional to viral load at 12 months post infection; and that viral load is associated with rate of CD4+ T cell decline and progression to disease. Consequently, intervention strategies such as vaccines that do not protect from infection could use these immunological and virological variables as surrogate endpoints and thus as measurements of their efficacy. Following acute HIV subtype B infection, viral load reaches a near steady state that is referred to as viral set point. While not all individuals reach this steady state, it has been shown that a single viral load measurement at 12 to 18 months post infection is the most reliable predictor of clinical progression (Lyles et al., 2000). For our study, a single viral load measurement at 12 months post infection will be used as a surrogate marker of viral set point, with alternative viral load measurements at 6 and 18 months analyzed to determine which best predicts disease progression. While the dynamics of viral replication, set point, and time to clinical endpoint, have been well documented in subtype B infections, there is little data on subtype C infection. The course of disease may differ in the southern African context due to differences in the quality of the immune response, HLA alleles, co-receptor polymorphisms, inter-current infections, or unique characteristics of subtype C viruses. Consequently, the influence of cellular and humoral immune responses on evolving viral populations and viral set point will be characterized. In addition, the restoration of immune function during ART will be characterized. This project will provide essential information on subtype C viral set point, prognostic viral load measurements and disease outcome that will influence the design and analysis of future chemotherapeutic and vaccine efficacy trials in southern Africa.
This prospective observational cohort study will follow seropositive individuals until they are initiated on ART and thereafter will be transitioned to phase V and will be followed-up for a minimum of 5 years. Participants will be diagnosed with acute HIV-1 infection based on the detection of HIV-1 replication in the absence of HIV-1 antibodies. Individuals with a reactive HIV antibody test within 5 months of previously negative antibody result will also be recruited. Time of exposure or infection will be defined as the mid-point between the last HIV seronegative test and the first HIV seropositive test; or if a positive RNA is available on the same date as a negative HIV EIA, the date of HIV infection is estimated at 14 days prior to the negative EIA test date. As time of infection is estimated and will vary for each participant, the follow-up schedule is defined according to the number of months post-enrollment into Phase II and participants will follow the same follow-up schedule. Analysis of data, however, will use the estimated months post infection. Clinical symptoms associated with acute HIV infection will be quantitatively scored and correlated with subsequent disease progression. In addition, the role of nutritional and metabolic factors in disease progression will be described. Overall, this study will describe immune responses in acute and early infection and relate them to the course of viremia in HIV-1 subtype C heterosexually acquired infection. It will ascertain the most prognostic viral load measurement as a surrogate marker of viral set point and the latent reservoir for HIV subtype C infection, as well as determine the relationship between 'set point' and time to a composite clinically relevant endpoint of either CD4+ T cell count below 350 cells/mm³, AIDS-defining illness, or initiation of ART.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

- To determine whether the magnitude and breadth of HIV-1 specific CD8+ T cell responses at 3 months post infection correlates with viral load at 12 months post infection.
- To determine whether viral load at 12 months post infection is related to subsequent progression of HIV disease as defined by: 1) CD4+ T cell count below 350 cells/mm³, 2) AIDS-defining illness, or 3) initiation of ART.

2.2 Secondary Objectives

- To describe the virological, immunological and clinical course of disease in subtype C infection.
- To determine if viral load at 6 and/or 18 months is a better predictor than viral load at 12 months post infection of progression of HIV disease, defined as CD4+ T cell count below 350 cells/mm³, AIDS-defining illness or initiation of ART.
- To determine whether CD4+ and CD8+ T cell responses are associated with viral load trajectory.
- To determine the association between neutralizing antibody responses and viral load trajectory.
- To describe the dynamics of viral evolution in subtype C infection and to determine if viral genetic changes associated with neutralizing antibody and cellular immune responses are associated with increased viral load.
- To determine if dual infection with two genotypically distinct HIV strains is associated with changes in viral load and disease progression.
- To categorize clinical signs and symptoms during acute HIV-1 infection and to correlate the presence or severity of these symptoms/signs with subsequent virologic, immunologic and clinical progression.
• To assess nutritional and metabolic status in participants with acute and early HIV-1 infection at baseline and during the course of the study and to correlate this with HIV-1 related disease progression.
• To describe the psychosocial and behavioral changes following acute infection.
• To characterize the degree and dynamics of restoration of immune function during antiretroviral therapy.
• To determine whether use of 1% tenofovir gel induces low levels of tenofovir resistant virus that are not detected by conventional genotyping
• To quantify tenofovir concentrations in vaginal secretions, vaginal tissue and blood plasma and tenofovir diphosphate concentrations in cervical cells, vaginal tissue and PBMC’s
• To co-localise the HIV (anti- p24) within target cells in the tenofovir exposed versus tenofovir unexposed across the genital tract mucosa
• To generate and characterize sequences of outgrowth viruses from the latent reservoir in resting CD4+ T cells taken after ART initiation.
• To compare the full-length genome sequence of the viral DNA reservoir after ART initiation.
• To deep sequence a portion of the envelope gene (env) from acute infection until ART initiation.
• Using phylogenetic analysis, to compare viral sequences over the duration of infection to those from latency to determine the dynamics of viral deposition in the establishment of the latent reservoir.
• To determine levels of activation of different memory T cell subsets and soluble inflammation markers during the course of HIV infection.

2.3 Description of Study Endpoints

Primary objectives’ endpoints

Primary Objective #1: To determine whether the magnitude and breadth of HIV-1 specific CD8+ T cell responses at 3 months post infection correlates with viral load at 12 months post infection.

The independent variables for this objective are:
Magnitude of CD8+ T cells enumerated as the number of IFN-g spots in the ELISPOT assay; and Breadth of HIV-specific CD8+ T cell responses assessed by the number of epitopes, which elicit a CD8+ T IFN-g response.

The dependent variable in this objective is viral load at 12 months post infection.

Primary Objective #2: To determine whether viral load at 12 months post infection is related to subsequent progression of HIV disease as defined by: 1) CD4+ T cell count below 350 cells/mm³, 2) AIDS-defining illness, or 3) initiation of ART.

The independent variable for this objective is:
Viral load at 12 months post infection.
The dependent variable for this objective is CD4+ T cell count < 350 cells/μL\(^1\); or AIDS defining illness (Appendix 2); or initiation of ART (Section 5.3).

**Secondary objectives’ endpoints**

**Secondary Objective #1:** To describe the virological, immunological and clinical course of disease in subtype C infection.

The variables for this objective are:
- Viral load trajectory over time;
- CD4+ T cell count trajectory over time;
- Viral coreceptor usages over time;
- Clinical status over time; and
- Hematological and biochemical assessments over time.

The clinical course of infection will be monitored utilizing a clinical assessment tool, which includes criteria for classification of functional status together with Karnofsky score as outlined in Appendix 1. AIDS defining illnesses will be diagnosed according to Appendix 2. Viruses will be cultured to determine if coreceptor switching is associated with changes in CD4+ T cell counts and disease progression. In addition, coreceptor inhibitor studies will be done to investigate subtle changes in coreceptor activity over time in the absence of overt change in coreceptor usage. In selected samples, V3 tracking by heteroduplex tracking assay (HTA) and sequencing will be done to characterize percentage of viral populations utilizing CCR5 and CXCR4 co-receptors at different time points.

**Secondary Objective #2:** To determine if viral load at 6 and/or 18 months is a better predictor than viral load at 12 months post infection of progression of HIV disease defined as CD4+ T cell count below 350 cells/mm\(^3\), AIDS-defining illness or initiation of ART.

The independent variable for this objective is:
- Viral load (at 6, 12 and 18 months).

The dependent variable for this objective is the clinically relevant composite endpoint of the study:
- CD4+ T cell count of below 350 cells/mm\(^3\); or AIDS defining illness; or initiation of ART.

**Secondary Objective #3:** To determine whether CD4+ and CD8+ T cell responses are associated with viral load trajectory.

The independent variables for this objective are:
- Anti-HIV CD4+ and CD8+ T cell numbers;
- CD4+ and CD8+ T cell function; and
- Number of CD4+ and CD8+ T cell epitopes.

The dependent variable for this objective is the viral load trajectory.

The magnitude, breadth and quality of the cellular immune responses which will be assessed utilizing five different methods including \(\gamma\)-interferon ELISPOT assay, intracellular cytokine/antigen staining, chromium release killing assay, lymphoproliferation and cell surface phenotypes.

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\(^1\) Two separate measures, at least one month apart beyond acute infection (i.e. beyond phase 2 visit 7), will be taken to confirm low CD4 count.
**Secondary Objective #4:** To determine the association between neutralizing antibody responses and viral load trajectory.

The independent variables for this objective are:
- Number of isolates neutralized (breadth); and
- HIV-1 neutralizing antibody titers (strength).

The dependent variable for this objective is viral load trajectory.

Neutralizing antibody titers will be measured using a Pseudovirion-based neutralization assay with a luciferase read-out. Viral isolates will be tested against contemporaneous sera as well as sera collected subsequently (autologous neutralization). Sera that show high levels of activity will be tested against other viral isolates both within this cohort and from other cohorts (heterologous neutralization) to determine whether neutralization clusters exist and the extent of cross-clade neutralizing antibody responses.

**Secondary Objective #5:** To describe the dynamics of viral evolution in subtype C infection and to determine if viral genetic changes associated with neutralizing antibody and cellular immune responses are associated with increased viral load.

The independent variables for this objective are:
- DNA distance between viral sequences within an individual;
- Number of viral variants within an individual;
- Phylogenetic relationships between sequences within an individual over time, and between persons;
- Number of non-synonymous changes compared to synonymous changes in regions targeted by the immune system;
- CD4+ and CD8+ epitope responses;
- Neutralization titers against sequential viral isolates; and
- Viral load.

The dependent variable for this objective is viral load (using repeated measurements).

**Secondary Objective #6:** To determine if dual infection with two genotypically distinct HIV strains is associated with changes in viral load and disease progression.

The independent variable for this objective is the presence or absence of HIV-1 dual infections in a subject, where dual infection is defined as infection with two HIV strains. Dual infection can be a consequence of either co-transmission (infection with two strains at or close to seroconversion) or superinfection (subsequent HIV-1 infection of an already infected individual). Dual infections will be identified by HMA and sequencing.

The dependent variables for this objective are:
- Viral load at 12 months post infection; and
- Progression of HIV disease (as defined in Primary Objective #2).
Secondary Objective #7: To categorize clinical signs and symptoms during acute HIV-1 infection and to correlate the presence or severity of these symptoms/signs with subsequent virologic, immunologic and clinical progression.

The independent variables for this objective are:
- Clinical signs and symptoms during acute infection: which will include diarrhea, fever, skin rash, oral candidiasis, lethargy, pharyngitis/sore throat; and
- A quantitative score of the severity of these symptoms will be calculated for each participant.
- The dependent variables for this objective are:
  - Virologic and Immunologic markers over time: Viral load trajectory over time and CD4+ T cell count trajectory over time; and
  - Clinical status over time as measured by development of HIV-related signs and symptoms

Secondary Objective #8: To assess nutritional and metabolic status in participants with acute and early HIV-1 infection at baseline and during the course of the study and to correlate this with HIV-1 related disease progression.

The independent variables for this objective are:
- Morphologic changes over time which will include Body Mass Index (BMI), waist-to-hip ratio, arm circumference, triceps skin fold; and
- Levels of total cholesterol, LDL triglycerides and random glucose will be collected over time and will be related to the disease progression as described by CD4+ T cell counts and viral loads.

The dependent variable for this objective is progression of HIV disease (as defined in Primary Objective #2).

Nutritional status, body composition and morphologic changes in the course of HIV infection are predictive of survival and functional status. Morphologic changes and nutritional assessment over time will include measurements of body mass index, waist-hip ratio, measurement of body composition with arm circumference and triceps skin fold; and

Biochemical assessment of nutritional status will include serum cholesterol, triglyceride and glucose that will be collected over time and related to the disease progression as described by CD4+ cell counts and viral loads.

Secondary Objective #9: To describe the psychosocial and behavioral changes following acute infection.

The impact of HIV infection on psychosocial and behavior of participants will be evaluated using a behavioral questionnaire including risk assessments; as well as Quality of Life Assessments using the Functional Assessment of HIV Infection (FAHI) (Appendix 3). Behavioral risk will also be evaluated by recording the incidence of treatable STI diagnosis. As the incidence of treatable STIs is considerably higher than HIV, these infections provide a reliable biological parameter of risk behavior.
**Secondary Objective #10**: To characterize the degree and dynamics of restoration of cellular immune function during ART.

The independent variables for this objective are:
- Number of CD4+ and CD8+ central memory cells
- CD4+ and CD8+ T cell function
- Number of activated CD4+ and CD8+ T cells and levels of inflammatory cytokines

The dependent variables in this objective are CD4 count recovery and viral load suppression trajectories over time.

The phenotype and function of cellular immune responses which will be assessed utilizing different methods, including γ-interferon ELISPOT assay, intracellular cytokine/antigen staining, tetramer staining, lymphoproliferation, cell surface phenotypes and plasma cytokine assays

**Secondary Objective #11**: To determine whether use of 1% tenofovir gel induces low levels of tenofovir resistant virus that are not detected by conventional genotyping

The independent variables for this objective are:
- population sequencing of *pol* gene
- minority populations of K65R
- thymidine analogue resistance mutations (TAMS), particularly M41L or L210W
- T69S insertion mutations

The dependent variable for this objective is development and persistence of tenofovir viral mutants at varying time-points of follow-up.

Genotyping will be used for resistance testing but can only detect mutant viruses when they are present in at least 20% of the population. Recently real-time PCR assays have been developed that allow for accurate quantification of individual mutations at less than 1% and will thus be adopted in this study. These assays amplify specific populations of resistant virus based on the sequence at codons known to confer resistance. Lastly, ultra-deep pyrosequencing (UDPS) a significant methodological advance in the field that has allowed for high-throughput sequencing of hundreds of DNA molecules in a complex mixture will be utilised.

**Secondary Objective #12**: To quantify tenofovir concentrations in vaginal secretions, vaginal tissue and blood plasma and tenofovir diphosphate concentrations in cervical cells, vaginal tissue and PBMC’s

The variables for this objective are:
- Tenofovir concentrations in vaginal secretions, vaginal tissue and blood plasma
- Tenofovir diphosphate concentrations in cervical cells, vaginal tissue and PBMC’s:

The specific aims of this analysis are to compare tenofovir and tenofovir diphosphate concentrations between different compartments. Samples will be analyzed for tenofovir and tenofovir diphosphate using HPLC with tandem mass spectrometry.
Secondary Objective #13: To co-localise the HIV (anti-p24) within target cells in the tenofovir exposed versus tenofovir unexposed across the genital tract mucosa:
The specific aims of this analysis are to compare and co-localise the:

- Presence of HIV across genital tract mucosa (cervix and vagina)
- Different target cells across the mucosa: Langerhans cells, dendritic cells, mucosal epithelial cells, activated T cells, macrophages, and natural killer cells
- Presence of Tenofovir in different target cells

Immuno-fluorescence (IMF) staining and viewing with confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) will be utilized to analyse these specimens.

Secondary Objective #14: To generate and characterize sequences of outgrowth viruses from the latent reservoir in resting CD4+ T cells taken after ART initiation.

We will use approximately 15 million CD4+ T cells for the outgrowth assay with a typical titre of one latently infected cell per million, giving on average 15 outgrowth viruses per sample/subject. The virus-positive wells in the viral outgrowth assay will be used to extract viral RNA which will be reverse transcribed to cDNA using an oligo dT primer followed by PCR of an env amplicon. This full-length env amplicon will be sequenced using Sanger sequencing to compare the inducible reservoir sequence to that obtained by analysis of integrated DNA and to the evolving virus population present prior to the initiation of ART.

Secondary Objective #15: To compare the full-length genome sequence of the viral DNA reservoir after ART initiation.

We will extract whole cell DNA from approximately 5 million purified, resting CD4+ T cells to analyze the sequence composition of the DNA reservoir. Single HIV templates will be identified by end-point dilution of total DNA and nested PCR. Thereafter, three amplicons of approximately 3 kbp each, representing the overlapping segments of the single provirus, will be generated for sequencing using the PacBio platform (produces approximately 150,000 reads per run, with an estimated 50,000 reads reaching a length of 3 kb). A primer ID approach will be used, allowing for derivation of a consensus of all sequences obtained from a given RNA template, thereby correcting for errors introduced by PCR and sequencing, as well as allelic skewing.

Secondary Objective #16: To deep sequence a portion of the envelope gene (env) from acute infection until ART initiation.

The Illumina MiSeq 300 next generation sequencing platform will be used to generate V1-V3 env sequences. Viral RNA (5,000 to 10,000 copies) will be extracted from plasma and reverse-transcribed using a specifically designed cDNA primer containing a unique primer ID. This approach will allow about 500 nucleotides for viral sequence, spanning all of V1-V2 linked to part of C2 and all of V3. These sequences will be trimmed to have common ends and joined to give one continuous sequence for analysis. Sequences from the reservoir will be evaluated for
their positioning within a phylogenetic tree of the longitudinal samples taken prior to ART to estimate when they entered the latent pool.

In a subset of viruses, entry phenotype will be compared in an Affinofile cell entry assay. We will generate functional env clones representing replication competent viruses generated, and compare to clones (5-10) representative of contemporary viruses circulating when the reservoir was deposited. Full-length env single genome-derived amplicons will be generated based on well-established methodologies in the laboratory. Amplicons will be cloned into the pcDNA3.1 mammalian expression vector. Env pseudotyped viruses will be generated following cotransfection of env clones along with the pNL4-3.Luc.R-E- HIV-1 backbone into HEK293T cells. Affinofile cells will be stimulated and receptor expression levels quantified using flow cytometry as described. Cell entry of env pseudoviruses will be measured by luciferase production.

**Secondary Objective #17:** Using phylogenetic analysis, to compare viral sequences over the duration of infection to those from latency to determine the dynamics of viral deposition in the establishment of the latent reservoir.

Replication competent viruses from the latent cellular reservoir will be isolated using a quantitative viral outgrowth assay (QVOA) following purification of resting CD4+ T cells from a 200ml blood draw at ≥ 4 years post-ART initiation. The complete genome of the outgrowth viruses will be sequenced in two halves using the Pacific Biosciences (PacBio) next-generation sequencing technology. In addition, the DNA/mRNA reservoir will be sized by quantification of intracellular multiply-spliced tat/rev transcripts (mRNA) and/or gag and pol copy numbers (DNA) from stored PBMCs at approximately 2 and 4 years post-ART initiation. Viral sequences from the DNA reservoir will be generated using Illumina MiSeq 2x300 paired-end sequencing on regions of the env gene or by limiting dilution near full-length genome PCR and Sanger sequencing.

Longitudinal sequencing of up to 14 genome regions of viruses isolated from blood plasma prior to ART initiation will be carried out using Illumina MiSeq 2x300 paired-end sequencing.

Sequences derived from pre-ART time points will be compared to those from the latent reservoir using Maximum-Likelihood trees.

**Secondary Objective #18:** To determine levels of activation of different memory T cell subsets and soluble inflammation markers during the course of HIV infection, in order to investigate the association of immune activation with HIV reservoir size.

A range of inflammatory markers, acute phase proteins and cytokines of interest will be measured by ELISA and multiplex assays, including IL-6, TNF-α, IFN-α, sCD14, sCD163; as well as markers of microbial translocation. The activation status of memory and naïve CD4+ and CD8+ T cells (defined by a range of memory markers, including CCR7, CD27, CD45RA/RO and CD95) will be determined by measurement of CD38 and HLA-DR expression by flow cytometry. Innate activation (including monocyte activation) may also be measured. The memory and activation status of potential markers of the latent HIV reservoir will also be characterised. A minimum of 200,000 gated events will be acquired. Flow cytometry data will be analyzed in FlowJo, with established gating strategies based on fluorescence minus one controls.
Secondary Objective #19: To validate and characterise candidate markers of the latent HIV reservoir.

Putative markers of CD4+ T cells that harbour latent HIV have recently been identified. These will be further investigated by cell sorting and HIV DNA PCR to determine whether there is an enrichment of the HIV reservoir. The viral population present in infected cells expressing the putative reservoir markers will be characterised, and the memory and activation phenotype of these cells will be characterised by flow cytometry as described in Objective 18.

3.0 STUDY DESIGN
3.1 Description

This is a prospective observational cohort study conducted at the CAPRISA eThekwini and Vulindlela Clinical Research sites. Study participants with acute HIV infection will be identified among female sex workers in KwaZulu-Natal; from participants in Phase II/IIb microbicide trials in Durban; and from research cohorts in and around Durban and in Vulindlela. Other sources may include Acute Infections identified by other HIV prevention trials in and around Durban, or the blood bank in Durban.

Seropositive individuals will be followed-up until they are initiated on ART and thereafter will be transitioned to Phase V and will be followed-up for a minimum of 5 years. The screening, recruitment and enrollment process is diagrammatically illustrated in Figure 1: Study Design. Consistent with the SA DOH adult treatment guidelines participants who are recently diagnosed with HIV may also initiate ART immediately, i.e. get directly enrolled into Phase V of this study.

3.2 Sample Size
Approximately 300 participants with acute HIV infection will be enrolled into Phase II or Phase V of the study.

3.3 Duration of Subject Participation
The screening and recruitment of FSWs in Phase I has already been completed. Follow-up of this cohort continued until seroconversion or for a maximum of 24 months and the last HIV negative participants were terminated in May 2007. Participants in Phase II-IV will be followed-up until they are initiated on ART and thereafter will be transitioned to Phase V and will be followed-up for a minimum of 5 years, or they will be offered to enrol immediately into Phase V if they fit the criteria for ART initiation. Optional annual follow-up for up to 15 additional years will be offered to participants enrolled in Phase V after 5 years of follow-up have been completed. This long-term extension of the protocol will allow for extended follow-up of participants who have demonstrated unique characteristics, such as CAP 256, who are of special interest to the research team. Furthermore, this cohort provides the opportunity to assess longterm disease progression during ART. The screening, recruitment and enrollment processes are diagrammatically illustrated in Figure 1: Study Design.
**Screening and Recruitment**

- Screen 600 female sex workers over 6 m (completed)

**Enrollment into Phase I**

**Phase I: HIV Negative Cohort**
- 200 HIV negative female sex workers.
- Follow-up monthly for maximum of 24 m (completed)

**Enrollment into Phase II**

**Phase II: Acute Infection (≤ 3 m*)**
- Enroll approximately 300 participants:
  - Female sex workers
  - Microbicide Cohorts
  - Vulindlela and Other cohorts
- Follow-up fortnightly for three months post-enrolment

**Phase III: Early Infection (> 3 m, ≤ 12m*)**
- Follow-up monthly until 12 months post-enrollment.

**Phase IV: Chronic Infection (> 12 m*)**
- Follow-up quarterly until end of study or a clinical endpoint is reached. Maximum duration until initiating ART.

**Phase V: Post-ART initiation**
- Enrolled on initiation of ART and followed up at month 1, month 3, month 6 and 6 monthly thereafter.
- Optional annual follow-up after 5 years

* Indicates months post enrollment into Phase II.

**Figure 1: Study Design**
4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Description of Cohorts, Serostatus, and Study Phases

Table 1 is a summary of the Phases and follow-up of the study, indicating both serostatus at Enrollment into each phase and their follow-up schedule, as well as time from enrollment (into Phase II). Phase I, the HIV negative phase, has been completed.

Table 1: Serostatus at Enrollment, and Phase Enrollment

<table>
<thead>
<tr>
<th>Study Phase</th>
<th>Cohort</th>
<th>HIV Status</th>
<th>Months from Enrollment</th>
<th>Visit Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: HIV negative</td>
<td>FSW</td>
<td>HIV Antibody negative and HIV RNA negative</td>
<td>N/A</td>
<td>Monthly (completed)</td>
</tr>
<tr>
<td>II: Acute Infection</td>
<td>FSW, Microbicide Cohorts, Vulindlela, Other</td>
<td>HIV antibody negative and HIV RNA positive; OR HIV antibody positive with a documented HIV negative within the previous 5 months or recently seroconverted as part of another CAPRISA study or CAPRISA related cohort</td>
<td>0 - 3</td>
<td>Fortnightly until 3 months post enrollment</td>
</tr>
<tr>
<td>III: Early Infection</td>
<td>FSW, Microbicide Cohorts and Vulindlela, Other</td>
<td>HIV seropositive</td>
<td>3 - 12</td>
<td>Monthly, post diagnosis</td>
</tr>
<tr>
<td>IV: Established Infection</td>
<td>FSW, Microbicide Cohorts and Vulindlela, Other</td>
<td>HIV seropositive</td>
<td>&gt; 12</td>
<td>Quarterly, post enrollment</td>
</tr>
<tr>
<td>V: Post-ART initiation phase</td>
<td>FSW, Microbicide Cohorts and Vulindlela, Other</td>
<td>HIV seropositive on ART</td>
<td>At ART initiation</td>
<td>Month 1, month 3, month 6 and 6 monthly thereafter. Optional annual follow-up after 5 years</td>
</tr>
</tbody>
</table>

Subjects will be enrolled into Phase II based on the following:

**Acute Infection (Phase II):** HIV seropositive with a documented seronegative in the previous 5 months or recently seroconverted as part of another CAPRISA study or CAPRISA related cohort

To qualify for Phase II, participants must meet one of the following criteria:

- Subjects who are HIV antibody negative with HIV infection demonstrated by HIV-1 RNA testing; OR
- Participants with a reactive HIV antibody test within 5 months of previously negative antibody result; OR
- HIV antibody negative within another CAPRISA HIV prevention study or cohort with a recent seroconversion date and of particular interest to this study, e.g the CAPRISA 004 Microbicide Trial participants
Date of HIV infection will be estimated using the following algorithm:

- If no previous HIV serology result available and a positive RNA is available on the same date as a negative HIV EIA, the HIV infection is estimated at 14 days prior to the negative EIA test date; OR
- HIV infection is estimated as the midpoint between the last documented HIV negative EIA and any of the first positive EIA. HIV incidence tests may be utilized to improve the accuracy of the diagnosis date estimate.

Participants will proceed in their follow-up from Phase II into:

- **Early Infection (Phase III):** ≥3-12 months post diagnosis; and **Established Infection (Phase IV):** > 12 months post enrollment into Phase II.

Participants who are initiated on ART as indicated by CD4 count, AIDS defining illness, or readiness to initiate regardless of CD4 count, will be offered enrolment into ART initiation phase (Phase V) for a minimum of 5 years.

4.2 **Description of Population**

**Cohort I:** female sex worker cohort

Recruitment and follow up of the HIV negative female sex worker cohort has been completed.

**Cohort II:** Microbicide cohorts, Vulindlela community research cohorts and other CAPRISA research cohorts

The CAPRISA Acute Infection Study will recruit from a prospective cohort study and a Phase IIb/Proof of concept Microbicide Trial. The CAPRISA 004 Phase IIb trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir gel for the prevention of HIV infection in women in South Africa has also been completed. Further recruitment will take place from CAPRISA 008 (tenofovir gel implementation study) during screening and follow-up.

This study will be conducted at two Clinical Research Sites in KwaZulu-Natal, South Africa and will enrol women at high risk of HIV infection in Durban and Vulindlela.

**CAPRISA Clinical Research Site: Vulindlela:**

The Vulindlela Clinical Research Site is situated in a rural community with approximately 400,000 residents in the KwaZulu-Natal midlands, about 150 km north-west of Durban. Primary Health Care (PHC) services are provided through seven clinics in the district. These nurse-managed services provide antenatal care, family planning, childhood immunization, STI treatment, minor ailment care, tuberculosis treatment and HIV Voluntary Counselling and Testing (VCT). The closest referral hospitals are Grey’s and Edendale. The CAPRISA Clinical Research Site in Vulindlela adjoins the Mafakathini PHC Clinic. Vulindlela is a rural area in the KwaZulu-Natal midlands, forms part of the greater Pietermaritzburg, Lions River and Mooi River District. It is situated about 170 km from Durban, and 70 km from Pietermaritzburg, and has a total population of approximately 400,000. Three anonymous HIV sero-surveys among first visit antenatal clinic attendees have been conducted at 7 Public Health Care clinics (PHC) between 2001-2002. The prevalence of HIV infection was 26% (n=55) in May-June 2001, 33% (n=295) in October-November 2001 and 34% (n= 278) in September- October 2002.
CAPRISA Clinical Research Site: eThekwini

The CAPRISA eThekwini Clinical Research site is located adjacent to the Prince Cyril Zulu Communicable Disease Centre (CDC), a designated PHC of the Durban City Health Department, for the diagnosis and treatment of STIs and tuberculosis. The clinic is conveniently situated in the Warwick triangle in the metropolitan region of Durban which serves as the nucleus of the public transportation with the central bus, “minibus” taxi station and rail station all within a 500 metre radius of the clinic building. This clinic is readily accessible in terms of the transport infrastructure. This clinic provides free STI and tuberculosis treatment. Annually, approximately 40 000 cases of STIs are treated at this clinic, approximately 36 000 of which are new cases. The majority of the STI patients accessing these facilities are self-referred either symptomatic with genital ulceration and/or vaginal discharge syndrome or as contacts of patients with a diagnosis of a STI and include both males and females. Given the high prevalence of HIV infection in South Africa and the strong association between STIs and HIV acquisition, these patients are at an increased risk of acquiring and transmitting HIV through sex.

HIV infection is one of the endpoints for this trial. Participants who seroconvert will be referred to the CAPRISA Acute Infection Study and will be followed up at either the CAPRISA eThekwini or Vulindlea Clinical Research Sites.

Cohort III: Other Cohorts

Through research links and links with other organizations conducting HIV testing, acute HIV infections will be identified and referred to CAPRISA for possible enrolment into the acute infection study. Examples would include other HIV research studies in which acute infections are identified, or the South African blood transfusion service, which has instituted testing for acute infection. All acute infections identified from other cohorts would have to meet the criteria for acute infection as specified in the protocol.

Rationale for Inclusion of Adolescents

The inclusion of adolescents in HIV prevention and treatment research has become a public health imperative due to young women continuing to experience high rates of HIV infection (Abdool Karim, et al., 2014, Singh, et al., 2006). Young women, aged between 15-24 years, account for almost a third of new infections occurring in women in this setting and remain at high risk of infection (Abdool Karim, et al., 2014). In 2013 the HIV prevalence among 15-19 year-old pregnant women, often considered a proxy measure for incident HIV infection in this age group, was 12.7%. Currently, several HIV prevention studies, including vaccine trials in advanced phases of research and PrEP demonstration projects are underway which may provide evidence for preventing HIV infection in women. However, as adolescents are not included in many of the studies already underway, they are unlikely to benefit immediately from any positive outcomes of these studies, despite contributing significantly to the epidemic (Abdool Karim, et al., 2014). The inclusion of adolescents, particularly young women, in this study is crucial for the following reasons:

- to enhance the understanding of HIV-1 subtype-C acquisition, pathogenesis and disease progression in adolescents in order to inform prevention efforts, particularly vaccine development
• to contribute to the latent reservoir study objectives to advance and expand the cure research agenda and include older adolescents
• to provide early ART and care to adolescents who may acquire HIV infection during participation in other CAPRISA studies

Recruitment of adolescents for participation in this study will be done in consultation with the CAPRISA Community Advisory Board.

4.3 Core Inclusion Criteria

- Able and willing to provide adequate locator information for study retention purposes as defined in the SOP;
- Willing to participate in the follow-up phase of the protocol;
- Willing to receive HIV test result; and
- Willing and capable of providing documentation of informed consent.

Inclusion criteria Phase I (FSW cohort only, recruitment completed)
- Age ≥ 18 years;
- HIV antibody negative or indeterminate on screening; and
- Self-reported sex with more than 3 different partners in the 3 months prior to screening\(^2\).

Inclusion criteria for Phase II - IV
- Willing to adhere to the Phase II evaluation schedule;
- Haemoglobin > 9.0 g/dl at enrolment;
- HIV-1 infection with an HIV antibody negative result within the previous 5 months; or recently seroconverted as part of another CAPRISA study or CAPRISA related cohort
- Age ≥ 16 years.

Inclusion criteria for Phase V
- Age ≥ 16 years
- Willing to adhere to the Phase V evaluation schedule;
- Initiated or willing to initiate on ART

4.4 Core Exclusion Criteria

Participants who meet any of the following criteria are NOT eligible for this study:
- Pregnant on screening for Phase I;
- Subjects who plan to travel away from the recruitment area for > 3 months during the 24 months following screening; and
- Subjects who have any other condition that, in the opinion of the Principal Investigator or designee, would preclude provision of informed consent, make participation in the study unsafe.

\(^2\) This high risk criterion is used as it is anticipated that some women may not wish to identify themselves as engaging in sex for compensation, negatively affecting recruitment.
4.5 Recruitment Procedures by Cohort – recruitment completed

Sex worker cohort

A network of community liaison persons (CLPs) has been elected at the site and has been trained to provide information about the study to the sex workers. The CLPs accompany the sex workers to the study clinic for the screening visit, and assist with transport and logistical arrangements in consultation with the study coordinator. (Enrolment into this cohort has been completed)

CAPRISA Vulindlela and eThekwini trial participants

A referral system already exists whereby any seroconversions from other CAPRISA trials are given an opportunity to participate in the Acute Infection Study. On seroconversion, participants will be asked if they would like to enroll into the Acute Infection Study.

Other Research Cohorts

Through links with other institutions and groups, for example other research groups or the South African blood transfusion service, recruitment of additional participants is possible. These sites will be provided with ethics committee approved information to patients leaflets, and their staff will be briefed on the nature of the acute infection study. Any individuals who may meet the criteria for participation will give their consent to be referred to the acute infection study, where study staff will then provide more detailed information and consent patients who are able and eligible for participation and willing to share the necessary HIV testing documentation.

4.6 Enrollment Procedures

Phase I: HIV negative (female sex worker cohort)

Enrolment into this cohort has been completed.

Phase II: Acute Infection (FSW, Microbicide, Vulindlela Cohorts and Other Research Cohorts)

Individuals with acute HIV infection (see Table 1) will be recruited into Phase II. Specimens will be collected and procedures followed as described in Study Evaluations and Appendices 1 and 4.

Phase V: Individuals from Phase II-IV or seroconversions from CAPRISA 004 who are initiated onto ART will be approached for enrolment into Phase V. Specimens will be collected and procedures followed as described in Study Evaluations and Appendices 1 and 4.

4.7 Consent Procedures

The following table summarizes the informed consent procedures for different cohorts:

Table 2: Informed consent requirements by cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Required Informed Consent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex Worker Cohort</td>
<td>This phase of the study has been completed</td>
</tr>
<tr>
<td>Microbicide Cohort</td>
<td>Enrollment into Acute Infection (Phase II) Sample Storage</td>
</tr>
<tr>
<td>Vulindlela Research Cohorts and Other Cohorts</td>
<td>Enrollment into Acute Infection (Phase II) Sample Storage</td>
</tr>
<tr>
<td>All cohorts</td>
<td>Enrollment into ART Initiation (Phase V)</td>
</tr>
</tbody>
</table>

The informed consent documents (Appendix 5) and any subsequent modifications will be reviewed and approved by the Institutional Review Board or Ethics Committees responsible for
oversight of the study. Written informed consent will be obtained from the participant prior to
implementing any study procedures. The informed consent will describe the purpose of the study,
the procedures to be followed, and the risks and benefits of participation. A copy of the consent
form will be offered to the participant.

All STI testing will be performed in the context of pretest, risk reduction and posttest counseling.

4.8 Co-Enrollment Guidelines
Participants in this study may not take part in other concurrent research studies, except for the
following:

- Ancillary studies approved by the CAPRISA Principal Investigator;
- Subjects recruited from Vulindlela may continue to participate in the follow-up evaluations
  for the Vulindlela studies;
- Subjects recruited from microbicide cohorts and other research cohorts may continue to
  participate in the follow-up evaluations for that study; and
- The FSWs participating in the Phase I cohort study will be encouraged to participate in
  other prevention trials (e.g. microbicide) that may enhance risk reduction and improve
  retention.

5.0 STUDY EVALUATIONS
An overview of procedures is provided below followed by a more detailed description of each
procedure and test conducted.

5.1 Study Procedures Overview
Appendix 4 shows the study schedule and procedures to be carried out at each scheduled clinic
visit.

Screening for enrollment into Phase I (Screening into Phase I has been completed)

Potential study participants will be recruited from the sex worker community as described in
section 5.5.1.

Screening procedures include the following:

- Informed consent for screening;
- HIV pre- and posttest counseling;
- HIV/STI risk reduction counseling, provision of condoms and prevention education
  supplies;
- Blood collection for HIV testing (as described in Appendix 6); and
- Urine collection for pregnancy (only if HIV negative).

Eligible HIV negative participants will be referred for potential Enrollment into Phase I of the
study. Participants who are found to be HIV positive will be provided counseling, and referred to
appropriate public health care clinics (VCT centers) for confirmatory testing and follow-up care
in accordance with the South African Department of Health guidelines.
Phase I: HIV negative cohort (This phase of study follow-up has been completed)

Only participants who are HIV negative and not pregnant have been enrolled into the Phase I. Participants remained in the HIV negative cohort with monthly visits for a maximum of 24 months or until the diagnosis of HIV infection as described in Appendix 6. Multiple visits may be conducted to complete all required procedures.

At Enrollment into Phase I the following procedures and tests will be performed:
- Informed consent for Enrollment into Phase I;
- Informed consent for sample storage;
- HIV pre- and posttest counseling;
- HIV/STI risk reduction counseling, provision of condoms and prevention education supplies;
- Collection of demographic and locator information;
- HIV behavioral risk assessment;
- Routine clinical evaluation (as outlined in Appendix 1);
- Blood collection for routine laboratory assessment (5.4.2), host genetic studies (5.4.4) and HIV status (Appendix 6);
- Urine dipstick for routine laboratory assessment (URICHEK 10 measures leukocytes, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketone, bilirubin, and glucose); and
- Specimen collection, including genital specimens, for STI diagnosis (5.4.1).
- After Enrollment, visits will be monthly. The following tests and procedures will be performed monthly until diagnosis of HIV infection or end of Phase I (maximum of 2 years):
  - HIV infection serology and RNA-PCR (Appendix 7);
  - HIV pre- and posttest counseling;
  - STI risk reduction counseling, provision of condoms and prevention education supplies;
  - Targeted clinical examination and routine laboratory assessment (Appendix 1); and
  - Locator information will be updated.
  - Urine dipstick will be done on clinical suspicion of pregnancy or if requested by the participant.
  - Blood and urine collection for routine laboratory assessment (5.4.2) as well as STI work up for behavioral risk and effectiveness of counseling assessment will be done every 6 months during the Phase 1 period.
  - Participants requiring care not related to this study will be referred to the Infectious Diseases Unit (Family Clinic) within King Edward VIII Hospital (KEH VIII). Participants with diagnosed STI will be treated syndromically, according to national STI treatment guidelines of South Africa. See Section 7.4 for a description of the referral mechanism for HIV related care.

Phase II: Acute Infection (first three months after enrollment into Phase II)

Participants who recently seroconverted will be approached for enrolment into Phase II and, upon consent, will be followed fortnightly until 3 months after date of enrolment into Phase II. Multiple visits may be required to complete all required procedures.

At Enrollment into Phase II the following procedures and tests will be performed:
• Informed consent for Enrollment into Phase II;
• Informed consent for sample storage;
• HIV posttest counselling (where applicable);
• STI risk reduction counseling, provision of condoms and prevention education supplies;
• Collect locator information;
• Baseline clinical assessment, including medical history and physical examination (Appendix 1);
• Hemoglobin for diagnosis of anemia;
• Blood collection for routine laboratory assessment, immunological and virological studies (5.5.2, 5.5.3 and 5.5.6);
• Urine dipstick and pregnancy test for routine laboratory assessment;
• Genital specimen collection for virological and immunological studies;
• Specimen collection for STI diagnosis (5.5.1);
• HIV behavioral risk assessment; and
• Confirmation of HIV infection status (Appendix 6).
• Optional expedited partner therapy for STIs

During subsequent Phase II visits, the following tests and procedures will be performed at the frequency detailed in Appendix 4:

• HIV/STI risk reduction counseling sessions, condoms and other prevention supplies;
• Update of locator information;
• Clinical examination as detailed in Appendix 1;
• Blood collection for routine laboratory assessment, immunological and virological studies;
• Urine dipstick for routine laboratory assessment (if indicated);
• Genital specimen collection for virological and immunological studies;
• Blood collection for serum and PBMC storage;

Participants requiring care not related to this study will be referred to appropriate public health clinics. Participants will be tested for STIs, including *N. gonorrhoeae, C. trachomatis* and *T. vaginalis* during Phase 2 – 5 follow-up using point-of-care assays. Those diagnosed with a STI will be offered appropriate treatment as soon as possible by the research team. A key aspect of STI control is to ensure sexual partners are contacted, tested and treated appropriately. If partners are not adequately treated, patients are reinfected and the transmission cycle continues. A pilot study undertaken in this setting found that EPT uptake was high and re-infection rates were lower among women using EPT and no social harms were reported (Garrett N, *et al.* P4.115 High uptake of effective expedited partner therapy among young women with STIs and their partners in South Africa. STI 2017; 93(Suppl 2): A233-A234). Participants with STIs will therefore be offered expedited partner therapy packs comprising of appropriate antibiotic treatment, condoms and information for the sexual partners. See Section 7.4 for a description of the referral mechanism for access to HIV related care.

Participants who reach a clinical endpoint of Phase II-IV will be approached for enrolment into phase V. If they choose not to participate in Phase V of the study they will be referred to an appropriate facility for AIDS care and ARTs when they qualify.
Phase III and IV: early infection and established infection (> 3 months post enrollment into Phase II)

Participants from Phase II will progress to Phase III. Participants will be screened 2-monthly until 12 months and then every 3 months until they initiate ART.

Participants who reach a clinical endpoint of Phase II-IV will be approached for enrolment into Phase V. If they choose not to participate in Phase V of the study they will be referred to an appropriate facility for AIDS care and ARTs when they qualify.

The following tests and procedures will be performed as detailed in Appendix 4:

- HIV/STI risk reduction counseling, provision of condoms and prevention education supplies;
- Update of locator information;
- Clinical examination as detailed in Appendix 1;
- Blood collection for routine laboratory assessment, host genetic, immunological and virological studies;
- Urine dipstick and pregnancy test (where indicated);
- STI workup for behavioral risk assessment;
- Specimen collection for STI diagnosis;
- Genital specimen collection for virological and immunological studies; and
- Cervicovaginal lavages and blood for storage.
- Optional expedited partner therapy for STIs

Phase V: Initiated onto ART

Participants initiating ART will be approached for enrolment into Phase V. Participants will be assessed at the time of initiation, and at 1 month, 3 months, 6 months, and 6 monthly, thereafter. Consistent with the SA DOH adult treatment guidelines participants who are recently diagnosed with HIV are encouraged to initiate ART immediately, i.e. get directly enrolled into Phase V of this study.

The following tests and procedures will be performed as detailed in Appendix 4:

- Informed consent for enrolment into Phase V
- Informed consent for sample storage
- HIV/STI risk reduction counseling, provision of condoms and prevention education supplies;
- Update of locator information;
- Clinical examination as detailed in Appendix 1;
- Blood collection for routine laboratory assessment, host genetic, immunological and virological studies and storage;
- Urine dipstick and pregnancy test (where indicated);
- STI workup for behavioral risk assessment; and
- Specimen collection for STI diagnosis;
- Optional expedited partner therapy for STIs
5.2 Overview of Behavioral and Clinical Evaluations
Study participants will receive behavioural and clinical evaluations according to the Schedule of Evaluations outlined in Appendix 4. These evaluations include demographic and locator information, medical history, targeted physical exams, as well as HIV behavioural risk assessments.

5.3 Clinical Evaluations

5.3.1 Evaluation of AIDS-defining Illness
AIDS is defined by the occurrence of an opportunistic infection or tumor considered indicative of advanced infection with HIV. The WHO has further developed case definitions of AIDS to include a range of opportunistic infections used in the current classification of AIDS-defining illnesses (See Appendix 2).

Given the availability of ancillary laboratory, radiographic, and invasive tests, the diagnoses of AIDS defining illnesses will be categorized as either confirmed or presumptive on the basis of supporting clinical and laboratory data. Clinical evaluations for the presumptive diagnoses of AIDS defining illness will be performed at scheduled monthly visits. Confirmation diagnostic procedures will be conducted only for presumptive cases. To avoid duplicate efforts, available records from past medical care at the KEH clinic of confirmed AIDS defining conditions will also be retrieved and used. The stage IV of the WHO staging system for HIV infection and disease in adults and adolescents will be used to define presumptive and confirmed AIDS defining illness in this study (See Appendix 2).

5.3.2 CD4+ Cell Count.
Participants will be offered initiation onto ART at any CD4+ cell count and they will be approached for enrolment into Phase V. If they choose not to participate in Phase V of the study, they will be referred to their local clinic for further management and patient care in preparation for initiation of ART.

5.3.3 Initiation of ART
Participants who meet the criteria for ART initiation according to the World Health Organization Antiretroviral Treatment Guidelines, future guidelines, or those who are keen to start at any CD4 count will be approached for enrolment into Phase V of the CAPRISA 002 study. Treatment in Phase V is usually initiated at the CAPRISA clinic and can then either be continued in the participant’s local health care facility of choice or at CAPRISA clinics. The WHO eligibility criteria are shown in Table 3 below.

**Table 3: Criteria for ARV Initiation in Adults and Adolescents (WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach Second edition, 2016)**
Adults and Adolescents

- ART should be initiated in all adults (>19 years old) and adolescents (10-19 years of age) living with HIV, regardless of WHO clinical stage and at any CD4 cell count.
- As a priority, ART should be initiated in all adults and adolescents with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and adults or adolescents with a CD4 count ≤350 cells/mm³.
- ART should be initiated in all pregnant and breastfeeding women living with HIV, regardless of WHO clinical stage and at any CD4 cell count and continued lifelong.
- ART should be started in all TB patients living with HIV regardless of CD4 count.
  - TB treatment should be initiated first, followed by ART as soon as possible within the first 8 weeks of treatment.
  - HIV-positive TB patients with profound immunosuppression (e.g. CD4 counts less than 50 cells/mm³) should receive ART within the first two weeks of initiating TB treatment.

5.4 Behavioral Evaluations
Each participant will undergo a structured interview to ascertain HIV risk behaviors, and the FAHI on the initial Phase I and Phase II visits and thereafter as indicated in Appendix 4. The behavioral risk questionnaire has been developed from existing instruments in use and in consultation with the CAPRISA Community Core (Appendix 3).

5.5 Laboratory Assays and Procedures
Laboratory evaluation will be performed for STI diagnosis, clinical parameters, as well as virological and immunological investigations.

STI and BV diagnosis

STI will be used as a biological marker for behavioral risk. Participants will be tested for STIs, including *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* during Phase 2 – 5 follow up using point-of-care assays. Those diagnosed with a STI or BV will be offered appropriate treatment as soon as possible by the research team. Participants will provide urine samples and a vaginal swab to diagnose STIs using point-of-care assays and a vaginal swab to diagnose bacterial vaginosis on Gram stain using Nugent’s score.

Blood specimens will be collected for syphilis, Hepatitis B virus (HBV) and HSV-2 serology as per schedule of evaluation.

Routine laboratory evaluations

Blood specimens will be collected for:
- Hematology assays (full blood count);
- Biochemistry assays (urea and electrolytes, creatinine clearance, liver function tests, calcium, magnesium, phosphate, blood glucose and HbA1C, cholesterol, LDL, triglycerides);
- On-site rapid testing for HIV antibodies (in Phase II, if applicable);
- Hemoglobin at Enrollment into Phase II; and
• Urine specimen will be collected for on-site β-HCG pregnancy testing as well as Uricheck Dipstick, if indicated.

On-site laboratory point-of-care assays, based on availability and appropriate validation of assays, may be utilized for safety monitoring and other tests required as per the schedule of evaluation. These tests include, but are not limited to the following: HIV-1 viral load, CD4+ T cell count, creatinine clearance, HBA1C, cholesterol assays, alanine aminotransferase (ALT) and STI testing. These assays will ensure that results are available on the day of study visits for efficient clinical management of study participants thereby minimising the need for return clinic visits. In addition, implementation of point-of-care assays aim to reduce cost and simplify laboratory testing.

Throughout the course of HIV-1 infection, a number of complications are commonly seen which can be attributed to the immunosuppressive effects of HIV infection itself or to medications. There are several laboratory tests that aid in monitoring for the development of these complications.

The complete blood count is important in documenting anemia and thrombocytopenia, which are found in 30-40% of patients infected with HIV-1. Likewise, monitoring of renal function with urine dipstick and serum urea and electrolytes (U&E) and modified liver function tests (mLFT) is commonly advocated in the initial evaluation of HIV infection and would be of benefit in follow up of participants during the early stages of infection. Liver function tests, in particular, would help detect the presence of subclinical hepatitis, which is commonly found in patients at risk for HIV. Early studies of HIV-1 infected patients revealed abnormal liver function tests in up to 75%, and severe abnormalities in 20% of patients. Likewise, a number of renal abnormalities have been described in patients with HIV infection, either due to the direct cytotoxic effect of the virus resulting in HIV-related nephropathy, or due to concomitant opportunistic infections, hepatitis C, or medication effect. Renal dysfunction in patients with HIV disease is usually diagnosed incidentally when patients present with opportunistic infections or on a routine laboratory evaluation. Some renal abnormalities are reported in over 25% asymptomatic patients and according to an early study, there was no difference in abnormalities of electrolytes and liver function tests when stratified by CD4 count (Freedberg et al., 1994).

**Cellular immunology evaluations**

Blood specimens will be collected according to schedule in Appendix 4. PBMCs will be isolated as described in Appendix 8.

CD4 + T cell counts will be measured using the FACSCalibur flow cytometer.

Immunology assays will be carried out according to the algorithm for measuring cellular immune responses (Appendix 9a). Fresh PBMC will be screened to identify the number of epitopes and magnitude of response. The remainder of the assays will be performed from cryopreserved PBMC (Appendix 9a).

Blood CD8+ and CD4+ T cell antigen-specific IFN-gamma production will be assessed using IFN-gamma ELISPOT assays on fresh PBMCs. These assays will measure CD8+ and CD4+ T cell cellular responses to pools of Gag, Nef, gp160, Pol, Rev, Tat, Vpu, Vpr, and Vif peptides. Peptides are all subtype C-based.
Further analysis will be performed from cryopreserved PBMC when pools of peptides have been identified that correspond to HIV-1 protein regions (Appendix 9).

CD8+ and CD4+ T cell antigen-specific intracellular cytokine production will be confirmed using FACS analysis on cryopreserved PBMC to more accurately delineate CD4+ and CD8+ T cell responses. CD8+ and CD4+ T cell function will also be determined using proliferation assays.

CD8+ and CD4+ T cell phenotypes will be assessed using FACS analysis on cryopreserved PBMC. Cytokine profiles in plasma will be examined by ELISA and multiplex assays.

MHC tetramer staining will be performed on cryopreserved PBMC specimens from selected participants for whom reagents are available so that the frequency of epitope-specific cells can be assessed and a closer analysis of T cell phenotypes.

**Host genetic evaluations**

Class I and II molecular HLA typing will be performed on all participants at high resolution.

Host genes associated with resistance to HIV infection or impacting on HIV disease progression or are AIDS restricting genes, such as polymorphisms in the CCR5 gene, will be defined using molecular techniques including PCR, restriction fragment length polymorphism, sequencing and microarray. Genetic profiles may be performed to confirm sample identity. For some of these factors, such as KIR, LEDGF, APOBEC3G and TRIM5αhu, we will investigate whether expression levels, as measured by flow cytometry or mRNA quantification, are inversely correlated with viral load during acute HIV-1C infection and/or chronic infection. We further plan to characterize the genetic variants of these molecules in order to determine the allelic frequencies of various variants and to determine if there may be a modifying role of genetic variants of these genes on HIV-1 disease progression, and that the effect on viral set point is an early indicator of this modifying role. For HLA class II, KIR genes and PD-1, our studies will focus on characterization of genetic variants within South Africa and the role that genetic variation may play in differential outcome at different phases of HIV-1 infection.

**Antibody laboratory evaluations**

Serum binding antibodies in blood reacting with purified HIV proteins or peptides from env and gag will be assessed in an ELISA assay. The end-point titers will be determined relative to a known standard.

Serum neutralizing activity of anti-envelope antibodies will be assessed in a single-cycle infection assay in cell culture. Primary virus neutralization will be performed using a pseudovirion-based neutralization assay in JC53-bl cells with a luciferase read-out. The reciprocal titer at which sera reduce luciferase expression by 80% will be calculated.

Autologous neutralizing antibody assays will be performed on a subset of patients where an early isolate is obtained (preferably the enrolment sample). All 19 follow-up serum samples will be tested against the initial isolate to determine the kinetics of the autologous neutralizing antibody response. In patients where later isolates are available these may be used to determine how
quickly neutralization-escape occurs and whether the escape variants are later targeted for neutralization and subsequent rounds of escape.

Neutralization assays will be performed initially on all patients at 6, 12, 24, 36 and 48 months against a small panel of subtype C pseudoviruses. The earliest time point to show potent activity in either this assay, in assays with the early autologous isolate or both assays will be tested against an expanded panel of pseudoviruses of subtypes B and C to assess the strength and breadth of neutralizing antibody activity as well as the durability of this response. The panel of pseudoviruses will include pseudotyped Du151 (subtype C vaccine strain which is relatively neutralization-resistant), Du174 (a subtype C strain that is more sensitive to neutralization), QH0692.42 and TRO.11 (subtype B viruses from acutely infected individuals). Other viruses may be added as more data is obtained on additional isolates (e.g., early isolates from the cohort).

Targets of these neutralizing antibodies will be identified through generation of monoclonal monoclonal antibodies. This will be done by EBV-transformation of peripheral blood mononuclear cells or in some instances these will be used to generate an antibody phage-display library. Culture supernatants will be screened for IgG secretion and for anti-HIV-1 antibodies by ELISA. Those that are positive will be screened for neutralization using pseudoviruses with autologous envelopes and/or epitope-specific pseudoviruses. Positive wells will be sub-cultured and continually screened for specificity before a stable monoclonal cell line is produced. Large quantities of mAb of interest will be generated for further analyses. The targets of these antibodies will be identified by analysing neutralizing escape using cloned envelopes from later time-points or by generating resistant variants in vitro. We also propose to generate monoclonal reagents once more cross-reactive antibodies develop (after 2 years of infection). Collectively these data will help us identify the targets of type-specific antibodies which force envelope escape as well as the targets of antibodies that are more broadly cross-reactive. Such targets will be useful in the design of an HIV vaccine immunogen aimed at inducing neutralizing antibodies.

Virology laboratory evaluations

Detection of HIV-1 RNA
The ROCHE COBAS Ampliprep-COBAS TaqMan version 2.0 assay will be used to measure viral loads. The test quantifies HIV-1 RNA over a range of 20 – 10 000 000 cp/ml. The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test uses reverse transcription and PCR amplification primers that define a sequence within the highly conserved region of the HIV-1 gag gene. The gag region encodes the group-specific antigens or core structural proteins of the virion. The nucleotide sequence of the primers has been optimized to yield comparable amplification of group M subtypes of HIV-1.

Monitoring Viral Diversity

Direct sequencing of PCR products gives the predominant sequence in the population but reveals little information concerning the number of sequence variants. A second approach is to clone the PCR products and sequence them individually. While this gives information about the precise sequence of different members of the population, it is a very time-consuming and limited approach to understanding the population structure, i.e. how many genotypic species are there and what is their relative proportion. This study will use two gel based screening methods, heteroduplex mobility assay (HMA) and heteroduplex tracking assay (HTA), as a genotypic sampling strategy. HMA will be used to identify intraperson diversity as complex mixtures of
quasispecies are visualized as a defuse smear on a gel. HTA differs from HMA in that one strand of the heteroduplex is a labeled DNA probe, and if only a single strand of the probe is labeled then each band in the gel represents a different genotype. HTA allows the detection of minor species that represent as little as 3% of the total population, it is very accurate in its sampling of the population of mixed viral genotypes, and it is labor-efficient allowing many samples to be analyzed.

Genetic diversity studies will be performed according to the algorithm outlined in Appendix 9b and 9c. To monitor viral evolution over time, initially samples collected at 6 monthly intervals will be screened. Should these samples be of sufficient interest such as evidence of immunological escape or dual infections, shorter sampling times will be analyzed. Individuals with low diversity identified using HMA will have direct PCR sequencing, whereas samples with high diversity will be cloned, and clones representing different HMA migration patterns will be sequenced. This study will focus on three regions of the genome (gag, env and nef). HMA can resolve viral populations with diversity >1% in C2-C3 and will be used to screen for intra-subtype dual infections. Dual infections will be confirmed by cloning and sequencing of multiple clones. HTA will be used to determine the number of viral variants within an individual. A modified version of HTA may be investigated which utilizes a single stranded RNA probe, enabling direct amplification and sequencing of products separated by HTA.

To monitor for changes in genetic diversity associated with escape from cellular or humoral immune responses, viral populations will be analyzed as outlined in Appendix 9b. Regions will be sequenced only where a change in response is seen (either responses to non-response or vice versa). Direct PCR sequencing will be performed except where there is evidence of high diversity as determined by HMA or HTA. To correlate genetic changes associated with viral escape from neutralizing responses, direct PCR sequencing of gp160 will be performed on early and late isolates identified as possible escape variants. In addition, direct PCR sequencing of gp160 plasma virus will be done from patients with broadly cross-reactive antibodies. Overriding selection pressures on viral gene sequences will be assessed through analysis of the site-adjusted frequency of synonymous (dS) and non-synonymous (dN) site changes within individual codons.

Amplification of near full-length genomes of a subset of well-characterized specimens performed in order to assess viral evolution and compensatory changes across the genome (Appendix 9b). Amplification will be optimized for cultured isolates, as well as DNA and RNA extracted from PBMCs and plasma. It may be necessary to amplify the genome in three or more large, overlapping fragments.

Determination of the potential impact of tenofovir levels on viral dynamics at acute infection. For women who were referred from the CAPRISA 004 Microbicide gel study, additional genital specimens including cytobrush and biopsy will be taken. These specimens will be collected at enrolment into phase II.

**Viral Isolation**
HIV will be isolated from PBMCs by co-culture with activated donor PBMC and monitored for p24 antigen production for 4 weeks. Cultures that are p24 antigen positive will be expanded to generate viral stocks. The replication rate of each isolate will be determined by establishing PBMC cultures with a standard innoculum and monitored for p24 antigen every 3-4 days for 2 weeks.
Monitoring Viral Resistance
Plasma and genital tract secretions from cytobrush and CVL specimens will be assessed for tenofovir resistance mutations at the earliest post-seroconversion time-point and at 3 month post-infection using HIV-1 genotyping (population sequencing of pol gene) for viral isolation, and Real-time allele-specific PCR for K65R (minority populations of K65R) and Ultra-deep pyrosequencing for resistance detection.

Persistence of resistant viruses will be assessed from subsequent time-points if either of these two early time-points demonstrate resistance mutations utilizing similar specimens and assays as above.

Sizing of the latent cellular reservoir
Replication competent viruses from the latent cellular reservoir are quantified using a viral outgrowth assay (QVOA) wherein resting CD4+ T cells, purified from a 200ml blood draw from an individual suppressed on ART, are stimulated with PHA and IL2 and plated at 300 000 cells per well of a 12-well plate. Cells are then incubated with uninfected, irradiated donor PBMC and cultures are maintained for up to 19 days. Viral expansion is measured by p24 ELISA and reservoir size is given as infectious units per million cells.

Intracellular viral DNA copy number (gag and pol copies per million PBMC) is quantified using digital droplet PCR (ddPCR) following extraction of genomic DNA from stored PBMC from individuals suppressed on ART.

Coreceptor Phenotypes
Coreceptor usage will be determined on virus isolates by measuring virus replication in coreceptor-transfected cells lines. U87.CD4.CCR5 and U87.CD4.CXCR4 cell lines will be used initially to determine whether viruses use CCR5 (R5 viruses), CXCR4 (X4 viruses) or both CCR5 and CXCR4 (R5X4 or dual tropic isolates). Virus replication will be measured by assessing cytopathic effects (syncitium formation) and p24 antigen production on days 4, 8 and 10 after infection. A virus will be characterized as R5 when p24 antigen in the CCR5-transfected cells exceed those in the CXCR4-transfected cells by 90% and there is extensive CPE (>50% of the cells involved in syncitium formation) in the CCR5-transfected cells with little to no evidence of CPE in the CXCR4 cells. Dual-tropic viruses are those that show equivalent levels of replication in both cell lines. These assessments are usually made on day 8 data although some rapidly replicating viruses are assessed on day 4 while some slower growing viruses can take up to 10 days before assigning a coreceptor phenotype.

In select cases (such as those patients where a number of longitudinal isolates are available or where unusual replication patterns are seen in U87 cells), viruses will also be assessed for alternate coreceptor usage using GHOST(3) cell lines. The assays are performed essentially as above except that virus replication can also be monitored by fluorescence microscopy or flow cytometry as a result of activation of green fluorescent protein. However, reported data will make use of p24 antigen and CPE as described above. The receptors that can be assessed in this assay are extended to include CCR1, CCR2b, CCR3, CCR8, Bob, Bonzo and GPR1. In the case of CXCR4-using viruses assessments of alternate coreceptor usage requires the addition of AMD3100 to block endogenous CXCR4 on GHOST(3).
5.6 Unscheduled Visits
Subjects may be seen on an unscheduled basis for clinical or administrative reasons not related to the study. If data collection is warranted, it will be captured on participant medical records and Case Report Forms.

Participants presenting with clinical complaints will be evaluated by the study staff and, if required, referred for further care in the appropriate public health clinics.

5.7 Unscheduled Visits and Clinical Suspicion of Acute Retroviral Syndrome
If an unscheduled visit occurs during the Phase I (HIV-1 negative sex worker cohort), the participant will be screened for clinical symptoms of acute retroviral syndrome (ARS) as outlined in Appendix 1. If there is a high clinical suspicion of ARS, HIV-1 RNA and two rapid antibody tests will be performed according to the procedures outlined in Appendix 7.

5.8 Visits Conducted Over Multiple Days (“Split Visits”)
All procedures required by the study protocol to be performed at a particular follow-up visit should be completed at a single visit on a single day. In the event that all required procedures cannot be completed on a single day, the remaining procedures will be completed within the allowable visit window period.

5.9 Missed Visits
For participants who do not complete scheduled visits within the allowable window, the missed visit will be documented on case report forms.
Allowable Visit Windows by Phase:
Phase I: HIV negative +/- 2 weeks (Completed)
Phase II: Acute Infection +/- 1 week
Phase III: Early Infection +/- 2 weeks
Phase IV: Established Infection +/- 1 month
Phase V: Antiretroviral therapy +/- 1 month

5.10 Final Visit
For participants enrolled into the Phase I cohort who do not seroconvert during the study period, the penultimate visit would include HIV testing and testing for other STIs (final visit was 24 months post-Enrollment into Phase I). A final “exit” contact is required to provide the participant with final study test results, post-test counseling, and referral for treatment if needed. Participants were given the opportunity to review their consent to have their samples stored. A withdrawal of consent is documented on the original sample storage consent form under the section, “Withdrawal of Consent.”

6.0 DATA COLLECTION

6.1 Type of Data Collection
The types of data that will be collected are:
Locator data and demographic characteristics;
Clinical data;
Laboratory data; and
Compliance with screening and enrollment criteria.
All data will be entered onto CRFs (case report forms).
6.2 Data Collection Plan
CRFs will be provided for each participant. The participants will not be identified by name on any of the CRFs. Subjects will be identified by the participant identification number (PID) provided by the CAPRISA data management centre upon enrollment.

The CRFs will be completed by the study staff at the research site, checked for QC purposes and either faxed through or directly entered into the data management system located at the CAPRISA data management centre. All data entry procedures will follow the CAPRISA data management Standard Operating Procedures (SOP). Instruction regarding the recording of study data on CRFs will be provided by the protocol team in conjunction with the Data Management Centre.

Prior to study initiation, the SOPs for study data management and QA will be established. These SOPs will specify procedures and staff responsibilities for recording, verifying and otherwise ensuring the completeness and accuracy of study data. Study staff will be trained in source documentation requirements and proper forms completion techniques as well as in the specification of the site SOPs.

It is the responsibility of the CAPRISA Data Management Centre to assure the quality of computerized data for each CAPRISA study.

6.3 Data Storage
There will be only one hardcopy of the CRF as the data is faxed through and captured electronically at the data management centre. The original CRF will stay on site at the research clinic and an electronic version will be stored at the Data Management Centre. All CRFs will be stored according in a double-locked location with secure and restricted access. Alternatively, data will be entered directly into the database.

Data will be stored on the server and backed up according to the SOPs to ensure against loss of data. User security is provided by password access control (both network and software) as well as restricting access within the software to allow only authorized users to perform certain functions.

6.4 Quality Assurance/Quality Control of Data
Quality assurance and quality control of data will be undertaken according to the SOPs.

7.0 STUDY MANAGEMENT

7.1 Scheduled Termination
For participants in Phase I that do not seroconvert during the study period, the scheduled termination date is 24 months after Enrollment. Participants enrolled into Phase II-IV, will be followed-up until they are initiated on ART and thereafter will be transitioned into phase V where follow-up will be for a minimum of 5 years.

7.2 Criteria for Discontinuation
Participants may voluntarily withdraw from the study for any reason at any time. The participant’s right to withdraw from the study will be respected at all times. For participants who terminate their involvement prior to the planned termination period, an exit interview will be conducted to elicit reasons for early withdrawal.
Participants may also be prematurely withdrawn from the study by the investigators in order to protect their safety, e.g. due to exacerbation of a concomitant medical condition or if they are unable to comply with required study procedures. In this case participants will be encouraged to continue to attend scheduled clinics and complete the remaining scheduled study visits in order to access care and referral mechanisms for the duration of the study.

Participants who withdraw from the study will be provided the opportunity to review and withdraw their consent for specimen storage. Withdrawal of consent for specimen storage will be documented on the source documents, and the applicable samples will be discarded in this event.

7.3 Retention Plans

Once a participant enrolls in this study, the study site will make every effort to retain him/her in follow-up to minimize possible bias associated with loss-to-follow-up. An average annual retention rate of 90% is targeted. Study site staff will develop and implement standard operating procedures to achieve this goal. Components of such procedures will include:

Thorough explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit.

Collection of detailed locator information at the study screening visit, and active review and updating of this information at each subsequent visit.

Where homesteads or places of residence are not easily identified by conventional addresses, maps reflecting relevant landmarks (roads, buildings, physical features, etc.) and the location of homesteads will be drawn.

Use of appropriate and timely visit reminder mechanisms. These will include visit cards listing the follow-up dates and the use of short text messages sent by cellular phone to those participants who are willing to receive these messages.

Immediate follow-up on missed visits.
Mobilization of trained outreach workers or “tracers” to complete in-person contact with participants at their homes and/or other community locations. In Vulindlela, the existing network of Community Health Workers will be utilized. For the female sex worker cohort, the Community Liaison Persons and the Community Liaison Officer will be utilized.

Regular communication with the study community at large to increase awareness of HIV/AIDS and explain the purpose of HIV prevention research and the importance of completing research study visits.

A participant tracking database to facilitate visit scheduling and timely identification and follow-up on missed visits will be established. The CAPRISA Data Manager will generate weekly reports on the number and percentage of participants completing the quarterly follow-up visits at which the primary study endpoints will be assessed. The data manager will track retention rates closely and work with cohort managers as needed to address below-target accrual or retention rates.
A participant will be deemed lost to follow-up when the above-mentioned methods have been used and the study staff have been unable to trace the participant, or if the participant indicates to study staff or community workers/tracers that they are no longer willing to take part. In the latter case, every effort will be made to conduct an exit interview to determine the reasons for discontinuation.

**Sex Worker Cohort (Recruitment and follow up of the female sex worker cohort has been completed)**

In past work with this cohort, a group of Community Liaison Persons (CLPs) was elected by the sex workers with one woman representing each of the FSW stops. Contact has been retained with this network of CLPs, and this will be utilized to ensure retention and follow-up of participants. For instance, where recruitment is conducted at new recruitment sites, existing CLPs will be utilized to set up and extend the CLP network to new sites. The CLP acts as an advocate for the women at the site. The elected community liaison persons also assist women at the sites by providing them with counseling, support, condoms and health education materials. They will assist with recruitment by informing new arrivals at the site about the study. They will also ensure that the sex workers recruited at their sites attend regular follow-up visits by assisting them with transport arrangements and reminding them of follow-up visit dates.

All CLPs will receive training and education in human subjects research, as well as any other relevant training for assisting in cohort maintenance.

**Microbicide cohort, Vulindlela community cohort, and Other research cohorts**

Once recruited into the Acute Infection study, these cohorts will be followed-up using the retention strategy plan outlined in 7.3 above.

### 7.4 Access to HIV Related Care

**HIV testing**

HIV diagnosis testing will be done in this study using 2 different rapid HIV tests (for example, Determine and Capillus). Those who test positive on the screening will be referred to their respective public health care facility for confirmation and further post-test counseling. If negative, pooled sera for HIV RNA PCR will be done and confirmed individually as illustrated in Appendix 7. Participants with positive HIV RNA PCR will have a later confirmatory EIA test done.

The screening phase of the study has been completed. Participants enrolling in Phase II of the study will already have a positive HIV RNA PCR from the study they participated in previously. A confirmatory EIA test will be done at enrolment into the study.

**HIV counseling**

HIV pretest, risk reduction, and posttest counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study. Counseling will be provided by Open Door, or a similar organization, in accordance with a standard study counselling manual. In accordance with the core inclusion criteria of the study, participants must be willing to receive their HIV test result in order to take part in the study.
There will be ongoing counseling and risk reduction education provided to participants throughout the study period.

The screening phase of the study has been completed. Counselling is done by a trained counsellor.

**Referral mechanism**
All research participants who need to be referred to public health facilities will have a referral note written to the respective health facility. This note gains them access to a patient card of that facility at a nominal fee. Once a hospital card is obtained, this will give the patient free access to all hospital facilities including medication.

A direct referral mechanism exists between CAPRISA and the public health clinics in and around Durban and Vulindlela. The clinics, in turn, have direct referral procedures to all clinics and services offered by the public hospitals. If participants require any care or procedures not offered by the clinics, they will be referred to the relevant public referral hospital.

**Condoms and treatment of STIs**
Condoms will be freely available to all participants throughout the duration of their participation. Participants will be examined and symptomatic participants will be treated on site or referred to the relevant local public health care facility to be treated syndromically. All participants will be routinely screened for STIs at annual intervals for the duration of the study and those requiring treatment will be recalled and referred to the appropriate public health care facility or treated on site. Syndromic management of STIs is standard practice in all South African Primary Health Care Clinics. Treatment guidelines and drugs are available in these facilities.

**Ongoing monitoring**
Ongoing monitoring for disease progression including viral load measurements and CD4+ T cell counts will be done according to the Schedule of Evaluation (Appendix 4).

**Treatment and Prophylaxis of Opportunistic Infections**
Patients in need of treatment or prophylaxis for opportunistic infections will receive the treatment on site or be referred to a healthcare facility of their choice. At these sites participants will be provided, or referred for, treatment and/or prophylaxis according to the standard of care available in the public sector in South Africa.

**Pregnancy**
While every effort will be made to encourage women enrolled in the Acute Infection study to use some form of contraception, including barrier methods, it is possible that some women will fall pregnant during the course of the study.

Once enrolled onto the study, pregnancy testing will be conducted on clinical suspicion, or if requested by the participant.

Women who fall pregnant during the study period will be managed according to current South African standards of care, but remain eligible for participation in the Acute Infection Study. These women will be referred to their local antenatal clinic for routine pregnancy management (see Appendix 10).
Antiretroviral Therapy (ART)
Highly active antiretroviral therapy (HAART) is currently available in South Africa. The Department of Health has recently announced a plan to provide ART to every HIV positive individual with a CD4 count of less than 500 from January 2015. It is anticipated that treatment will be offered to everyone at diagnosis later in 2015. Table 3 (Section 5.2.3) provides the current guidelines for ART initiation. Participants (from both eThekwini and Vulindlela Clinical Trial Sites) who require ARV therapy will be initiated on ART at the respective site and referred to their closest ART treatment site 6 months post ART initiation or when their viral load is undetectable.

8.0 ADVERSE EVENTS REPORTING
As this study does not involve an investigational drug, sites are not required to submit Serious Adverse Event (SAE) forms. However, clinical signs and symptoms with severity of > Grade 3 (see Appendix 5) and which are not associated with a study-specific diagnosis, must be reported to team via the study CRFs as clinical events. These participants should be referred to the appropriate clinics at appropriate public health care facility for management. Unexpected adverse events will be reported to the local IRB/EC.

Participants will be monitored after blood draws prior to leaving the clinical site to make sure they do not have an adverse clinical event.

9.0 STATISTICAL CONSIDERATIONS
A 10% attrition rate in year 1 and 5% in year 2 are expected, which would yield 135 evaluable participants. Given a fixed sample size of 135 evaluable participants, we focus instead on the attainable power for detecting an effect size for the two primary endpoints, i) viral load at 12 months post infection and ii) time to progression of HIV disease to CD4+ T cell count below 350 cells/mm$^3$, AIDS-defining illness or initiation of antiretroviral therapy.

9.1 Power Projection for the Primary Objective #1
To determine whether the magnitude and breadth of HIV-1 specific CD8+ T cell responses at three months post infection correlates with viral load at 12 months post infection.

The two predictor variables are the number of IFN-g spots in the ELISPOT assay and the number of epitopes recognized by HIV-specific CD8+ T cells. The outcome variable is viral load at 12 months post infection. As mentioned above, we wish to determine the attainable power for declaring whether the correlation between the number of IFN-g spots and viral load at 12 months post infection (or the number of epitopes recognized and viral load at 12 months post infection) is significantly larger than, for example 0.6 or 0.8. Specifically, with 135 evaluable participants, a two-sided Fisher Z-transform correlation test will have 80% power (alpha=0.025$^3$) to declare a correlation coefficient $r = .60$ as significantly (different) larger than 0.4 (null hypothesis, $H_0 : \rho_0 = .4$, weak correlation). It will have 83% power (alpha=0.025) to declare a correlation coefficient $r = .75$ as significantly larger than 0.6, ($H_0 : \rho_0 = .6$, moderate correlation). Similarly, the attainable power is 82% to declare an $r = .88$ as significantly larger than 0.8 ($H_0 : \rho_0 = .8$, high correlation).

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$^3$ A bonferroni adjusted 2-sided alpha of 0.05/2 to allow for the two primary comparisons
9.2 Power Projection for the Primary Objective #2

To determine whether viral load at 12 months post infection is related to the subsequent progression of HIV disease as defined by: 1) CD4+ T cell count below 350 cells/mm³, 2) AIDS-defining illnesses or 3) initiation of ART.

Time to progression data (Mlisana et al., 2003) of 31 seropositive participants were used to estimate the 3-year cumulative progression rates for subjects grouped above and below their median viral loads (median ≈ 9500 copies/mL). For subjects with viral load below and above 9500 copies/mL the 3-year cumulative rates were 10% and 30% respectively. Assuming 2 years of accrual, a study duration of 5.5 years, and a 5% common exponential dropout rate, then with 135 subjects we shall have 80% power to detect an absolute effect size of 20% (2-sided alpha=0.025).

9.3 Analysis Plans:
All analyses prior to treatment initiation pertain to the sample of 135 evaluable participants. All statistical tests will be 2-sided (alpha=0.05).

Primary Objective #1
To determine whether the magnitude and breadth of HIV-1 specific CD8+ T cell responses in early infection correlates with viral load at 12 months post infection.

Ordinary multiple linear regression modeling will be used. The two predictor variables are the number of IFN-g spots in the ELISPORT assay and the number of epitopes recognized by HIV-specific CD8+ T cells. The outcome variable is viral load at 12 months post infection. In the regression model, we shall use log viral load, which is approximately normally distributed, thus satisfying the statistical assumption of linear regression models. Interaction effects between predictors will also be evaluated.

Primary Objective #2
To determine whether viral load at 12 months post infection is related to the subsequent progression of HIV disease as defined by: 1) CD4+T cell count below 350 cells/mm³, 2) AIDS-defining illnesses or 3) initiation of ART.

The predictor variable will be log viral load at 12 months post infection and the outcome will be the time-to-event endpoint (CD4+ T cell count < 350 cells/mm³; or AIDS defining illness; or initiation of ART). The time-to-event distribution will be modeled using a proportional hazards (PH) model (Cox, 1972) with log viral load at 12 months post infection as covariate. If the proportional hazards assumption (PH) is violated, several approaches may be considered. One approach would be to use a stratified proportional hazards model in which the proportional hazards assumption holds within each stratum (Kalbfleisch and Prentice, 1980). Hazard ratios (log viral load) and 95% confidence intervals will be calculated.

Secondary Objective #1
To describe virological, immunological and clinical course of disease in subtype C infection.

Viral loads, CD4+ T cell counts, hematological and biochemical measures will be collected according to the schedule of events, Appendix 4. Clinical assessment over time, i.e. the clinical
course of infection will be monitored utilizing a clinical assessment tool including criteria for classification of functional status together with the Karnofsky score.

Means and changes from baseline (with 95% confidence limits) for viral loads/CD4+ T cell counts and other continuous variables will be plotted over time. Repeated measures ANOVA will be conducted to test for trend in (mean) viral load, CD4+ T cell count (and other continuous variables) over time. The analysis will use mixed effects models (Diggle, Liang and Zeger, 1994). Adding linear contrasts to the model will enable one to compare changes in viral load from baseline to each of the subsequent follow-up times. Similar analyses will be conducted for CD4+ T cell counts and other continuous variables.

Discrete outcome variables will be analyzed for trend over time using a repeated measures categorical data analysis. Repeated responses induce a correlation structure that can be utilized in the analysis. These analyses will use the generalized estimating equation (GEE) approach (Diggle et al. op. cit., 1994) for correlated binary outcomes or ordinal GEE (Lipsitz et al., 1994) for correlated ordinal outcomes.

**Secondary Objective #2**

To determine if viral load at 6 and/or 18 months is a better predictor than viral load at 12 months of progression of HIV disease, defined as CD4+ T cell count below 350 cells/mm$^3$, AIDS-defining illness or initiation of ART.

The time to progression of HIV disease to CD4+ T cell count below 350 cells/mm$^3$, AIDS-defining illness or initiation of antiretroviral therapy (composite endpoint) will be modeled using a proportional hazards (PH) model (Cox, 1972). Three separate models will be fitted using as covariate the log viral loads at 6, 12 and 18 months respectively. The model with the smallest Akaike information criterion (AIC; Akaike, 1974) will determine viral load at the corresponding timepoint as the best predictor of progression. Alternatively, all three predictors may be used in conjunction with a backwards and stepwise selection option in the regression model to eliminate non-significant predictors. These selection regression procedures may result in a model with more than one significant predictor. However, if interest remains in the best viral load predictor then the first approach using the Akaike information criterion for the 3 separate models will be the arbiter.

**Secondary Objective #3**

To determine whether CD4+ and CD8+ T cell responses are associated with viral load trajectory.

CD4+ and CD8+ T cell responses will be measured using five different methods. The methods and their resulting outcomes are displayed in the following table:

<table>
<thead>
<tr>
<th>Method</th>
<th>Outcome measurement</th>
<th>Scale</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 γ-interferon ELISPOT assay</td>
<td># spots /10$^6$ cells</td>
<td>Numerical</td>
<td>Number of CD4 and CD8 epitopes</td>
</tr>
<tr>
<td>2 Intracellular cytokine/antigen staining</td>
<td>Proportion cells positive for cytokine or antigen</td>
<td>Percentage</td>
<td>Anti-HIV CD4 and CD8 numbers</td>
</tr>
</tbody>
</table>
Each method with its associated outcome will be a covariate in the regression model. For example, Method 1 will be represented by a numerical variable measured as the number of spots per \(10^6\) cells. There will be at least 3 and at most 5 covariates available for each subject. Specifically, methods (Outcomes) 1, 4 and 5 will be done on each sample. Method 2 will only be done if Method 1 gives a response and Method 3 will only be done if Method 4 gives a response.

In the analysis a repeated measures analysis of covariance will be used, which takes into account the fact that viral load will be measured repeatedly. The analysis will use mixed effects models (see Section 9.3.3). The fixed effects will be the covariates mentioned above and the subjects will be the random effects. A step-down likelihood ratio test will be used sequentially to eliminate covariates not significantly associated with the viral load (outcome).

Test of trend in viral load over time will be performed using orthogonal polynomial contrasts in the regression models.

**Secondary Objective #4**

To determine the association between neutralizing antibody responses and viral load trajectory.

An analysis similar to that described in Section 9.3.3 will be conducted. The outcome variable will be the repeatedly measured viral load, the predictor variables (fixed effects) will be the number of isolates neutralized (breadth) and HIV-1 neutralizing antibody titers (strength). The subjects will be the random effects.

**Secondary Objective #5**

To describe the dynamics of viral evolution in subtype C infection and to determine if viral genetic changes associated with neutralizing antibody and cellular immune responses are associated with increased viral load.

Here, we wish to assess the effect of neutralizing antibody and cellular immune (CD4+ and CD8+ T cell) responses on amino acid changes in targeted regions of the genome. Endpoints are amino acid changes in targeted regions of the genome. Changes in amino acid sequences will be measured by the estimated ratio of non-synonymous substitution rate to synonymous substitution rate. Let \(\lambda_{NS}\) = non-synonymous substitution rate, and \(\lambda_S\) = synonymous substitution rate. Both these rates are unknown but their ratio, \(\mu\), can be estimated by the ratio of two quantities, \(dN\) and \(dS\):

\[
\frac{\lambda_{NS}}{\lambda_N} = \mu = \frac{dN}{dS}
\]

where \(dN\) = number of non-synonymous substitutions/non-synonymous site and \(dS\) = number of synonymous substitutions/synonymous site. Both these quantities are random variables (r.v.) and the ratio of two random variables is also a random variable. Preliminary analysis of the codon data, supplied by the P.I., indicates that the ratios \(\mu\) are approximately lognormally distributed (Shapiro Wilk test for normality (P>0.05)).
As noted previously (Section 9.3.5), cellular immune (CD4+ and CD8+ T cell) responses can be measured using five different methods and that each subject will have at least 3 and at most 5 such covariates. We shall use multiple linear regression to determine the association between immune response, of neutralizing antibody (covariates) and amino acid changes (response variable).

For the relationship between viral load that may or may not be increasing, a repeated measures analysis of variance will be used. The analysis will use mixed effects models (see Section 9.3.3). The fixed effects will be the cellular immune response. Tests of trend in viral load over time will be performed using orthogonal polynomial contrasts in the regression model.

**Cutpoint determination**

Also of interest is determining cutpoints for this ratio, \( \mu \), that would be indicative of evolution phenomena.

Values of

- \( \mu >> 1 \) suggest adaptive evolution,
- \( \mu < 1 \) suggest purifying evolution and
- \( \mu \approx 1 \) suggest neutral evolution.

Instead of determining cutpoints by inspection or heuristically, binary recursive partitioning will be used (Ciampi *et al.*, 1987; Clark and Pregibon, 1992). This approach enables one to determine meaningful categories for \( \mu \) as a means of distinguishing between adaptive, purifying and neutral evolution respectively.

**Secondary Objective #6**

To determine if dual infection with two genotypically distinct HIV strains is associated with changes in viral load at 12 months and disease progression.

The outcome variable will be the log viral load at 12 months and the predictor variable will be the presence or absence of HIV-1 dual infection in a participant. The association between outcome and predictor will be determined using ordinary linear regression.

In addition, rapid progression to AIDS will be modeled using a proportional hazards (PH) model (Cox, 1972) with the number of HIV-1 dual infections as covariate. If the proportional hazards assumption (PH) is violated, several approaches may be considered. One approach would be to use a stratified proportional hazards model in which the proportional hazards assumption holds within each stratum (Kalbfleisch and Prentice, 1980). Hazard ratios and 95% confidence intervals will be calculated.

**Secondary Objective #7**

To categorize clinical signs and symptoms during acute HIV-1 infection and to correlate the presence or severity of these symptoms/signs (as assessed by a quantitative scoring system) with subsequent virologic, immunologic and clinical progression.
Outcome variables include viral load, CD4+ T cell count and progression to HIV related signs and symptoms. The first two outcomes are repeated measures (longitudinal) endpoints and the 3rd is a time-to-event endpoint. Predictor variables (clinical signs and symptoms) will include diarrhea, fever, skin rash, oral candidiasis, lethargy, pharyngitis/sore throat, all defined on a severity scale from 0-16. These variables will be collected once only (upon enrollment in the acute infection phase of the study).

Outcome variables viral load and CD4+ T cell count will be analyzed using mixed effects models (as proposed in Section 9.3.3). Progression to HIV related signs and symptoms will be analyzed using a proportional hazards (PH) model (as proposed in Section 9.3.2).

Secondary Objective #8

To assess nutritional and metabolic status in participants with acute and early HIV-1 infection at baseline and during the course of the study and to correlate this with HIV-1 related disease progression.

The outcome variable is HIV-1 related disease progression (time-to-event). The predictor variables (covariates) are a) Morphologic changes that will include Body Mass Index (BMI), waist-to-hip ratio, arm circumference, triceps skin fold measurement and b) Metabolic levels that include total cholesterol, LDL, triglycerides, random glucose levels. Since these predictors are measured repeatedly and the outcome is time-to-event, we shall again use a proportional hazards model in which the covariates will be time dependent. In this instance, a modification (Cox, 1975) of the standard PH-model is used.

Secondary Objective #9

To describe the psychosocial and behavioral changes following acute infection.

The Functional Assessment of HIV Infection (FAHI) quality of life (QOL) instrument, other risk behavior questionnaires and the records of incident treatable STIs will be used. QOL data will be collected at baseline, month 3 and bi-annually thereafter. In order to explore these changes a repeated measure ANOVA model will be used. However, it is important to stress, that attempting to collect longitudinal data at so many time points may result in the occurrence of missing data. Missing data arising in longitudinal studies can seriously affect the analysis.

There are several approaches to the analysis of longitudinal QOL data (Fairclough, 1998a, b), which depend primarily on the research hypothesis of interest. The most common views the study as a repeated measures design. We propose to carry out the analyses using mixed effects models (Diggle, Liang and Zeger, 1994). Mixed effects modeling will be implemented using the procedure PROC MIXED in SAS. A linear contrast statement in PROC MIXED can be used to compare changes in QOL from baseline to each of the subsequent follow-up times.

The types of analyses will be affected by the type and amount of missing data. Every effort will be made to ensure that the amount of missing data is kept at a minimum since their presence complicate the statistical analyses.

In general, multivariate analyses require complete data vectors or that the proportion of subjects with missing assessments should be small (less than 5%) and missing completely at random.
(MCAR). That is, “missingness” is completely unrelated to the participant’s QOL measurement and covariates. The MCAR assumption is strong and, if violated, the estimates of treatment effect could be biased. In MAR, a weaker assumption than MCAR, “missingness” depends on covariates and the non-missing outcomes (QOL) but is independent of the value of the missing outcomes (QOL). In both MAR and MCAR the missing data are ignored and one can simply perform the standard repeated measures analysis as outlined above. Fortunately, mixed effects models are sufficiently robust in the presence of up to 20% missing outcomes, even if the outcomes are missing at random (MAR) (Fairclough, 1998b). We shall investigate the reasons for any missing data as the study proceeds, which should enable us to check whether the MAR assumption is tenable.

In the worst case scenario, the missing outcomes are not missing at random (NMAR). In NMAR, “missingness” depends on the value of the missing observation. For example, the missing observations arise when subjects do not return for follow-up, due to death or severity of illness. The remaining subjects may appear healthier (also known as informative censoring) because the sample is shrinking down to an even smaller complement of healthier subjects. We therefore propose to use imputation schemes if missing data are NMAR. The motivation for imputation is that balanced statistical data methods such as MANOVA can be used to analyze the imputed data sets. Multiple imputation (Rubin, 1987 & 1996, Rubin and Schenker, 1991) can deal with both non-random (NMAR) and random missingness (MAR) by imputing non-randomly missing values using a statistical model for the outcome given the missing value indicator.

Secondary Objective #10

To characterize the degree and dynamics of restoration of cellular immune function during ART. T cell phenotype and function will be measured using different methods. The methods and their resulting outcomes are displayed in the following table:

<table>
<thead>
<tr>
<th>Method</th>
<th>Outcome measurement</th>
<th>Scale</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Memory cell phenotypes</td>
<td>Proportion cells positive for specific memory phenotype</td>
<td>Percentage</td>
<td>CD4 and CD8 numbers</td>
</tr>
<tr>
<td>2 Activation phenotypes</td>
<td>Proportion activated cells</td>
<td>Percentage</td>
<td>CD4 and CD8 numbers</td>
</tr>
<tr>
<td>3 Intracellular cytokine/antigen staining</td>
<td>Proportion cells positive for cytokine or for proliferation</td>
<td>Percentage</td>
<td>Anti-HIV CD4 and CD8 numbers</td>
</tr>
</tbody>
</table>

A generalized estimating equation (GEE) regression model will be used to model T cell memory phenotypes and T cell function, assessing how this changes over time and how this is affected by HIV infection and ART initiation, while adjusting for repeated measurements for the same participants. The effect of viral load and CD4 count on immune function will be evaluated using a GEE regression model. In addition to modelling T cell function, T cell activation and T cell memory phenotypes separately, these outcomes will also be adjusted for each other by fitting the rest as covariates into the model.

Secondary Objective #11
To determine whether use of 1% tenofovir gel induces low levels of tenofovir resistant virus that are not detected by conventional genotyping:

Data will be presented in a standard curve used for quantification of the K65R allele-specific PCR. HIV-1 RT amplicons from different samples containing the K65R resistance mutation will be presented in a table depicting allele-specific PCR and clonal analysis results. Correlation between these 2 methods will be presented.

**Secondary Objective #12**

To quantify tenofovir concentrations in vaginal secretions, vaginal tissue and blood plasma and tenofovir diphosphate concentrations in cervical cells, vaginal tissue and PBMC’s

Descriptive statistics will be used to summarize tenofovir and tenofovir diphosphate concentrations in the biological matrices under investigation. A pharmacokinetic model for tenofovir and tenofovir diphosphate concentrations in blood plasma, genital secretions, and vaginal and cervical tissues will be modelled.

**Secondary Objective #13**

To co-localise the HIV (anti- p24) within target cells in the tenofovir exposed versus tenofovir unexposed across the genital tract mucosa:

The ultrastructural study is purely a qualitative examination of tissue. No statistical analysis will be done for this objective.

### 10.0 SUBJECT PROTECTION

This protocol, the informed consent documents (Appendix 5), and any subsequent modifications will be reviewed and approved by the Institutional Review Board or Ethics Committee responsible for oversight of the study. Written informed consent will be obtained from the subject (or parent or legal guardian of subjects who cannot consent for themselves, or if the IRB/Ethics committee does not determine that parental consent can be waived). The informed consent will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject (or parent or legal guardian), and this fact will be documented in the participant’s record.

### 10.1 Informed Consent

The informed consent process will cover all elements of informed consent required by research regulations, as required by the local IRB and the sponsor(s). Written informed consent will be obtained from each participant prior to screening or enrollment. During the process of consenting a participant, the following topics of importance to this study will be addressed:

- The purpose of the study;
- The study procedures;
- The risks of participation;
- The need to practice safer sexual behaviors;
- The importance of adherence to the study schedule;
The potential social harms associated with study participation (and what to do if such harms are experienced);
The benefits of study participation;
The distinction between research and clinical care; and
The right to withdraw from the study at any time without penalty.

The consent form includes a description of the study, the information obtained from participant interviews, the participant tracking procedures, and the potential risks and benefits associated with participation. The procedures ensuring confidentiality, a list of those with access to the data, the assurance that non-participation or withdrawal from the study will not jeopardize patient care, and the names and telephone numbers of contacts in case of questions are also included in the consent form. Participants will be given a copy of the consent form.

The informed consent forms will be translated into isiZulu and the accuracy of the translation will be verified through independent translation.

In addition to the informed consent forms, the Study Team members will work with study staff and community representatives to develop appropriate information materials about the study and a standardized approach to the informed consent process to be implemented.

The informed consent process will include an assessment of each potential participant’s understanding, prior to enrollment, of concepts identified by the Study Team as essential to the informed consent decision. Based on input from study staff and community representatives, this assessment may take the form of a “quiz”. Participants who are not able to demonstrate adequate understanding of key concepts after exhaustive educational efforts will not be enrolled in the study.

A standardized approach to obtaining and documenting the informed consent process will be implemented.

CAPRISA researchers will periodically assess the participants’ understanding of the study, as well as assess the process and understanding of consent.

Due to the diverse nature of the procedures and assessments at each phase of this study, participants will have to give informed consent at various stages. Consent for specimen storage will be required if specimens are to be retained for future testing. Participants do not have to consent for specimen storage to participate in the study and may withdraw consent for specimen storage at any time.

Participants, who are recruited into Phase II of the study, will not have to be reconsented for Phase III, as this is the natural progression of the follow-up schedule. The informed consent given at recruitment into Phase II will include consent for Phase II, III, and IV. Participants, who are recruited into Phase V of the study, will be reconsented for Phase V.

10.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

Ethics approval for this protocol and the template informed consent forms attached as Appendix 5 will be obtained from the Ethics Committee of the Nelson R. Mandela School of Medicine (FWA #00000678). The IRB requires annual progress reports, a final project report, and has full rights
to inspect the study site and procedures. Annual reports will include the total number of participants enrolled in the study, the number of participants who complete the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others.

Additional ethics approval needs to be obtained from the Ethics Committees of the University of the Witwatersrand and the University of Cape Town (UCT) for the analysis of human specimens:

University of Cape Town: FWA00001938; and University of Witwatersrand (for National Institute of Communicable Diseases): FWA00000715.

All the relevant ethics committees meet monthly and require annual reports on the study.

10.3 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring, or at the request of the IRB and/or the sponsor(s).

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality to the extent possible. A standard operating procedure for confidentiality protection that reflects the study implementation plan will be established with input from study staff and community representatives to identify potential confidentiality issues and strategies to address them.

In addition to these protections, all individuals who are not bound by professional ethics to participant confidentiality, but who may have to obtain confidential information (for example, the sex worker Community Liaison Persons or community members who assist in tracing defaulters), will sign confidentiality agreements. Participant study information will not be released without their written permission, except as necessary for monitoring.

10.4 Risks to Participants

Study participants may feel discomfort during blood and biological specimen collection as well as during clinical examination. Some people get a bruise where the needle is placed to draw blood and/or feel faint after blood has been drawn. Participants may become embarrassed, worried or anxious when completing their Behavioral Risk Assessment and/or receiving HIV counseling. They also may become worried or anxious while waiting for their HIV and/or pregnancy test results. Trained counselors will be available to help participants deal with these feelings.

Research participants may be exposed to some psychosocial risks related to:

1) Negative outcomes of disclosure: It is possible that participants who share HIV test results with their sexual partners will experience negative reactions. This risk is one that many face, regardless of Enrollment in this study. Counselling will be provided in this regard. Counsellors will screen all women for risk of violence following disclosure. While counselors will strongly encourage and support participants making voluntary disclosure to their sexual partners, in
instances where it is envisaged that the women’s life is, or might likely be put, at risk from the disclosure, counselors will advise women to delay disclosure until the participant (and relevant counseling agencies, if applicable) can develop a safety plan to protect the participant from the envisaged harm.

2) Precipitation of emotional crisis: It is possible that the qualitative and survey interviews will precipitate an emotional crisis among women who have not had the opportunities to talk about these emotionally charged topics in the past. To address this risk, the interviewers will be trained to respond empathetically to the women. However, since interviewers are not trained counselors, they will also refer participants to Counsellors for ongoing support and counseling.

10.5 Benefits to Participants

There may be no direct benefits to participation in this study. However, participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like microbicides and vaccine trials, and the effectiveness of HIV risk reduction and prevention messages. Study participants will receive HIV and STI counseling and testing, a physical exam, and pelvic exams. Participants will be referred for other medical conditions identified as part of the follow-up procedures. Participants who reach a clinical endpoint will be approached for enrolment into Phase V or referred to a local clinic of their choice, where they will be offered care and ART.

10.6 Incentives

Participants will not receive any incentives but will be compensated for their time and effort in this study, and/or be reimbursed for costs associated with travel to study visits, time away from work, and child care. Reimbursement amounts will be specified in the study informed consent process and approved by all applicable Ethics Committees.

10.7 Specimen Storage

Separate informed consent will be obtained for the storage of samples for possible future research testing. However, consenting to specimen storage is not a requirement for participation. Participants may withdraw one, or both, of the informed consent agreements at any time.

Any residual specimens from participants who do not consent to long-term storage and additional testing will be destroyed at the end of the study, after all study-specific and quality assurance testing has been completed. Use of stored material obtained during the study will be governed by guidelines drafted by the CAPRISA Scientific Review Committee (Appendix 10). All new ancillary studies that wish to use stored material require approval from the Scientific Review Committee and a Research Ethics Committee prior to their commencement.

11. SITE MONITORING

Internal monitors, under the direction of the CAPRISA Quality Assurance manager will visit the research sites to review the individual records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians’ progress notes, nurses’ notes, individuals’ hospital charts). This is to ensure the protection of study subjects, compliance with
the protocol, and accuracy and completeness of the records. The monitors will also inspect the sites’ regulatory files to ensure that regulatory requirements are being followed.

The Protocol Chair, PI, project co-ordinator, project manager, study epidemiologist, and project Data Manager, and the biostatistician will make regular visits to monitor the quality of data collection and data entry. A minimum of two total site visits per year will be conducted.

11.1 Access to Source Documents

The principal investigator will make study documents (e.g., consent forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRBs, the site monitors, internal monitors, or the sponsors’ designee for confirmation of the study data.

12. PUBLICATIONS OF RESEARCH FINDINGS

Presentation and publication of the results of this study will be governed by the CAPRISA Scientific Review sub-committee. The CAPRISA publication policy is attached as Appendix 11.

13. BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institute of Health.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72. Please also refer to individual carrier guidelines, e.g., FedEx, Airborne, for specific instructions.

Blood and other biological specimens will be collected as described in Appendix 8 and specimen storage and CAPRISA specimen repository, University of KwaZulu-Natal. Fresh blood will be transported to NICD for PBMC isolation and storage. Specimens will also be transported to the University of Cape Town. Transportation of all specimens will abide by IATA regulations. All sites will implement SOPs for processing, tracking, and retrieving specimens. Specimens will also be stored for future use. Use of these specimens will be subject to ethical approval and approval of CAPRISA Scientific Review Committee.

Any residual specimens of participants who do not consent to long-term storage and additional testing will be destroyed at the end of the study, after all protocol-required quality assurance testing has been completed.
14. REFERENCES


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Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS),
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Vanhems et al. Incubation time of Acute HIV Infection and Duration of Acute HIV Infection are independent prognostic factors of progression to AIDS. JID 2000; 182:334-7.


15. LIST OF APPENDICES

Appendix 1  
Clinical Evaluations

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Study definition
15.1 Appendix 1: Clinical Evaluations

Phase I HIV Negative FSW: Screening Evaluation (The recruitment and follow-up of this cohort has been completed)

Targeted Physical Exam at entry to the Study
A. Blood pressure, temperature, HEENT (head, eyes, ears, nose and throat), look for ulcers, lymphadenopathy and skin rashes
B. Screen for signs and symptoms of acute seroconversion syndrome. Specify if occurring within the last 2 weeks of visit.

Phase I HIV Negative FSW: Clinical Evaluation of a febrile illness possibly consistent with acute infection: Unscheduled visits during phase I

These participants will be evaluated for the presence of acute HIV infection by testing for HIV antibodies and HIV RNA according to the algorithm outlined in Appendices 6 and 7 and performing a targeted clinical evaluation for:

- Fever (temperature ≥ 101ºF/38ºC)
- Reported fever within 5 days of clinic visit
- And at least one of the following:
  - Rash
  - Lymphadenopathy
  - Oral, genital, or rectal ulcers
  - Exudative pharyngitis
  - Aseptic meningitis (headache, photophobia, stiff neck)
  - Myalgia/arthralgia
  - Fatigue
  - Weight loss
  - Night sweats
  - Anorexia

Participants with clinically evident alternative diagnosis at the time of visit will not be included in the study and their care will be referred to the Infectious Disease Unit for further evaluation.

Phase II Acute Infection

Baseline Clinical Examination at recruitment into Acute Infection Phase
A. Medical History: History of Diabetes Mellitus (DM), Hypertension (HTN), Coronary Artery Disease (CAD), Cardiovascular accident (CVA), Malignancy and Tuberculosis (TB).
B. Family Medical History (circle one)
   a. DM Yes/No
   b. CAD Yes/No
   c. CVA Yes/No
   d. Not available
C. Physical Exam will include
   a. Vital signs including BP, Temp, wt, ht (calculate Body Mass Index)
   b. HEENT exam including evaluation for oral ulcers, lymphadenopathy, heart, lung, abdominal exam to document organomegaly and skin rashes
   c. Morphologic Assessment: Waist circumference
D. Screen for signs and symptoms of acute seroconversion syndrome. Specify, if occurring within the last 2 weeks of visit. (See Evaluation Tool below)

Targeted On-Study Clinical Assessment

A. Record Vital signs including Temp, weight
B. Screen for HIV-related symptoms and signs:
   a. Thrush
   b. Leukoplakia
   c. Zoster
   d. Weight loss (quantify)
C. Morphologic Assessment during annual visits.
   a. Waist circumference,

D. Record signs and symptoms of AIDS-defining illnesses (see Appendix 2). Confirmed AIDS-defining illness recorded as clinical endpoint.
E. Symptoms driven evaluation will include referral to clinic of choice. (nic)

Phase III/IV Early/Established Infection

Targeted On-Study Clinical Assessment (as in Phase II above)

Phase V: ART initiation
Targeted On-Study Clinical Assessment (as in Phase II above)

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**Guidelines for obtaining morphologic and body composition measurements during Acute HIV Infection** (Durnin et al., 1974; Gerrior et al., 2001; Wanke et al., 2003)

1. Waist to hip ratio: Waist circumference will be measured at the thinnest area below the rib cage and above the umbilicus. The hip will be measured at the greatest circumference around the hip or buttocks with the subject standing, relaxed, with feet together and arms by the side. The waist hip ratio will be calculated from these measures; norms for men are <0.9 and <0.85 for women.
2. Triceps and biceps skinfold measurements will be performed using a calliper. This measurement will always be done on the same side of the body with the subject standing.
3. Mid arm circumference will be measured at the midpoint of the arm to the nearest 0.1 cm. Measurements of the same arm will be obtained during follow up.
4. Definitions of body wasting: loss of 10% of body weight in 1 year, loss of 5% of body weight in 6 months, and a decrease in BMI to <20 kg/m²
5. Body mass index will be calculated as follows:
   a. BMI = \( \frac{\text{weight in kilograms}}{\text{height in meters}^2} \)
   OR
   b. BMI = 704 x \( \frac{\text{weight in pounds}}{\text{height in inches}^2} \)
Clinical Evaluation of a febrile illness possibly consistent with acute infection: Unscheduled visits during phase I

These participants will be evaluated for the presence of acute HIV infection by testing for HIV antibodies and HIV RNA according to the algorithm outlined in Appendix 7.

- Fever (temperature $\geq 101^\circ/38^\circ\text{C}$)
- Reported fever within 5 days of clinic visit
- And at least one of the following:
  - Rash
  - Lymphadenopathy
  - Oral, genital, or rectal ulcers
  - Exudative pharyngitis
  - Aseptic meningitis (headache, photophobia, stiff neck)
  - Myalgia/arthralgia
  - Fatigue
  - Weight loss
  - Night sweats
  - Anorexia

Participants with clinically evident alternative diagnosis at the time of visit will not be included in the study and their care will be referred to the Infectious Disease Unit for further evaluation.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Y</th>
<th>N</th>
<th>Number of Days</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Depression</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Confusion</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Photophobia</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Stiff neck</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Retro-orbital pain</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Night sweats</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
<tr>
<td>Parasthesias</td>
<td>Y</td>
<td>N</td>
<td>Number of Days</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Signs at the time of the clinical evaluation

Fever ($\geq 38^\circ\text{C}$) Y N
Rash Y N
  - maculopapular/morbilliform/ erythema nodosum (circle one)
  - face/trunk/extremities (circle)
Lymphadenopathy Y N
  - Tenderness Y N
  - Size ____________
  - Location ____________
Pharyngitis Y N
  - erythematous/exudative (circle one)
Arthritis Y □ N □
   o joints affected (location) ________-
   o number of joints affected: mono/oligo/poly (circle one)
Aseptic meningitis / Aseptic meningoencephalitis Y □ N □
Peripheral neuropathy Y □ N □
   o acute peripheral neuropathy, myelopathy, mononeuritis multiplex (circle one)
Thrush (vaginal/oral) Y □ N □
Esophageal candidiasis Y □ N □
Mucocutaneous ulceration Y □ N □
   o oral ulcers, genital ulcers, anal ulcers (circle)
Conjunctivitis Y □ N □
Hepatomegaly Y □ N □
Splenomegaly Y □ N □

Laboratory phenomena
Anemia (Hb <10.5) Y □ N □
Thrombocytopenia (Platelet Count <150,000/L) Y □ N □
Leukopenia (Leukocytes <400,000/L) Y □ N □
Elevated hepatic enzyme levels (AST, alkaline phosphatase >120 IU/L) Y □ N □
Quantitative Scoring System for Signs and Symptoms of Acute HIV Infection (see below for severity grading system)

**Diarrhea**
- Mild (1)
- Moderate (2)
- Severe (3)

**Fever**
- Temperature $\geq 38^\circ C$ (1)

**Skin Rash**
- Mild (1)
- Moderate (2)
- Severe (3)

**Oral candidiasis**
- Mild (1)
- Moderate (2)
- Severe (3)

**Lethargy**
- Mild (1)
- Moderate (2)
- Severe (3)

**Pharyngitis or sore throat**
- Mild (1)
- Moderate (2)
- Severe (3)

*Total Score __________*
**Estimating Severity Grade of Signs and Symptoms of Acute HIV**
Adapted from the Division of AIDS (National Institutes of Health, USA) Table for Grading Severity of Adult Adverse Experiences. August 1992

Grade 1 or Mild symptoms:
Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required

Grade 2 or Moderate symptoms:
Mild to moderate limitation in activity as a result of the signs/symptoms. No or minimal medical intervention needed.

Grade 3 Severe:
Marked limitation in activity, some assistance usually required; medical intervention, therapy required.

Examples how to grade the severity of signs and symptoms and how to assign score:

**Diarrhea:**
- **Mild Diarrhea:** mild or transient; 3-4 loose stools per day OR mild diarrhea lasting <1 week
- **Moderate or persistent diarrhea:** 5-7 loose stools per day OR diarrhea lasting ≥ week
- **Severe Diarrhea:** Bloody diarrhea OR orthostatis hypotension or >7 loose stools/day OR IV therapy required

**Pharyngitis:**
- **Mild Pharyngitis/Sore throat:** mild discomfort, no difficulty in swallowing
- **Moderate Pharyngitis/Sore throat:** difficulty swallowing but able to drink and eat food
- **Severe Pharyngitis/Sore throat:** Unable to swallow food due to discomfort

**Rash:**
- **Mild Rash:** Erythema, pruritus
- **Moderate Rash:** Diffuse maculopapular rash
- **Severe Rash:** Maculopapular and diffuse, involving more than one location (face, trunk, extremities)
### Karnofsky Score

<table>
<thead>
<tr>
<th>Condition</th>
<th>%</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Able to carry on normal activity and to work. No special care is needed.</td>
<td>100</td>
<td>Normal, no complaints, no evidence of disease.</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>Able to carry on normal activity, minor signs or symptoms of disease.</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>Normal activity with effort, some signs or symptoms of disease.</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>Cares for self. Unable to carry on normal activity or to do active work.</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>Requires occasional assistance, but is able to care for most of his needs.</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Requires considerable assistance and frequent medical care.</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Disabled, requires special care and assistance.</td>
</tr>
<tr>
<td>Unable to work. Able to live at home, care for most personal needs. A varying degree of assistance is needed.</td>
<td>30</td>
<td>Severely disabled, hospitalization is indicated although death not imminent.</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Hospitalization necessary, very sick, active supportive treatment necessary.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Moribund, fatal processes progressing rapidly.</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Dead.</td>
</tr>
</tbody>
</table>
## 15.2 Appendix 2: AIDS-Defining Illnesses: (Modified WHO Stage IV criteria) to be Evaluated as Clinical Endpoints

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Presumptive</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extrapulmonary cryptococcosis</strong></td>
<td>Clinical history and physical examination AND Positive serum cryptococcal antigen</td>
<td>Presumptive Diagnosis PLUS Lumbar puncture with either positive India Ink smear OR CSF cryptococcal antigen</td>
</tr>
<tr>
<td><strong>Tuberculosis (extrapulmonary)</strong></td>
<td>Clinical history and exam PLUS Positive AFB smear from other affected sites other than the lungs</td>
<td>Presumptive Diagnosis PLUS Positive MTB culture from affected site</td>
</tr>
<tr>
<td><strong>Kaposi’s sarcoma</strong></td>
<td>Characteristic lesions of skin or mucous membranes: A characteristic gross appearance of an erythematous or violaceous plaque-like lesion on skin or mucous membrane. (NB: A presumptive diagnosis of Kaposi’s sarcoma will not be made by clinicians who have only seen few cases).</td>
<td>Presumptive Diagnosis PLUS Histopathology</td>
</tr>
<tr>
<td><strong>Cerebral toxoplasmosis</strong></td>
<td>Suggestive clinical history and improvement on anti-toxoplasmosis therapy; recent onset of a focal neurological abnormality consistent with intracranial disease or a reduced level of consciousness; and positive serum antibody to Toxoplasma or successful response to therapy for toxoplasmosis.</td>
<td>Presumptive PLUS Positive Toxo IgG AND Consistent CT findings (if available)</td>
</tr>
<tr>
<td><strong>Candidiasis of the esophagus/trachea or lungs</strong></td>
<td>Esophageal Candidiasis: Odynophagia/dysphagia AND Improvement with fluconazole treatment Pulmonary Candidiasis: Clinical examination</td>
<td>Presumptive diagnosis and biopsy proven fungal infection</td>
</tr>
<tr>
<td><strong>HIV-Encephalopathy</strong></td>
<td>Clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection which could explain the findings.</td>
<td>Same</td>
</tr>
<tr>
<td><strong>HIV-associated wasting</strong></td>
<td>Weight loss of &gt;10% of body weight, plus either unexplained chronic diarrhea (&gt;1 month) or chronic weakness and unexplained prolonged fever (&gt;1 month).</td>
<td>Same</td>
</tr>
<tr>
<td><strong>Cytomegalovirus (CMV) disease of an organ other than liver, spleen or lymph nodes</strong></td>
<td>CMV retinitis: Ophthalmologic exam consistent with CMV retinitis CMV disease (non-retinitis): Clinical history AND Endoscopic appearance suggestive of CMV disease</td>
<td>Presumptive Diagnosis PLUS CMV antigen positivity Presumptive Diagnosis PLUS Histopathology OR CMV antigen &gt;2/100,000 cells</td>
</tr>
<tr>
<td><strong>Any disseminated endemic mycosis (i.e., Histoplasmosis, coccidiomycosis)</strong></td>
<td>Coccidiodomycosis (disseminated and extrapulmonary): History and positive serology Disseminated Histoplasmosis: No presumptive diagnosis?</td>
<td>Direct examination and culture (sputum or other clinical specimens) Isolation from body fluids or tissues: Microscopy (histology or cytology), culture or detection of antigen in a specimen obtained directly from the affected tissues or a fluid from those tissues.</td>
</tr>
<tr>
<td><strong>Atypical mycobacteriosis: Mycobacterium avium intracellulare (MAI) complex,</strong></td>
<td>Clinical examination, Chest X-Ray and Microscopy of a specimen from stool or normally sterile body fluids, or tissue from a site other than lungs, skin or cervical or hilar lymph nodes that shows acid-fast bacilli of a species not identified by culture.</td>
<td>Presumptive diagnosis plus CT scan plus Positive culture from pulmonary and or non pulmonary sites</td>
</tr>
<tr>
<td><strong>Progressive multifocal leukoencephalopathy</strong></td>
<td>Definitive diagnosis only Microscopy (histology or cytology).</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>History/Clinical findings</td>
<td>Diagnostic Method(s)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------</td>
</tr>
<tr>
<td>Chronic herpes simplex (&gt;1 month duration)</td>
<td>History and Clinical examination Hydroxyurea (HSV) IgG positive serology</td>
<td>Presumptive AND Positive culture</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td>Definitive diagnosis only Microscopy (histology or cytology).</td>
</tr>
<tr>
<td>Salmonella septicaemia</td>
<td>Clinical examination Stool culture positive</td>
<td>Positive blood culture</td>
</tr>
<tr>
<td>PCP</td>
<td>Clinical history AND Consistent chest x-ray AND Improvement on PCP therapy</td>
<td>Presumed diagnosis plus positive sputum examination (silver stain)</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>Diarrhea &gt; 1 month And/or performance scale 4: bed-ridden, &gt; 50% of the day during the last month.</td>
<td>Positive fecal smear (Modified Acid Fast Stain)</td>
</tr>
</tbody>
</table>
Appendix 3: FAHI and Risk Behavioral Assessment Questionnaire

Viral Set Point and Clinical Progression in HIV-1 Subtype C Infection

Quality of Life FAHI

Below is a list of statements that other people with your illness have said are important. By checking one (1) box per line, please indicate how true each statement has been for you during the past week (7 days).

<table>
<thead>
<tr>
<th>PHYSICAL WELL-BEING</th>
<th>Not at all</th>
<th>A little bit</th>
<th>Somewhat</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP1 I have a lack of energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP2 I have nausea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP3 Because of my physical condition, I have trouble meeting the needs of my family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP4 I have pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP5 I am bothered by side effects of treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP6 I feel ill</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP7 I am forced to spend time in bed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1 I have been short of breath</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5 I am bothered by a change in weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMT6 I get tired easily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI7 I feel fatigued</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI12 I feel weak all over</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2 I have been coughing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Date: 11-AUG-04 v1.3 FINAL

Completed by: [Signature]

Phase Visit Interim #
### Quality of Life FAHI

*By checking one (1) box per line, please indicate how true each statement has been for you during the past week (7 days).*

<table>
<thead>
<tr>
<th>Emotional Well-Being/Living with HIV</th>
<th>Not at all</th>
<th>A little bit</th>
<th>Somewhat</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE1 I feel sad</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE4 I feel nervous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE5 I worry about dying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE6 I worry that my condition will get worse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI1 I am unhappy with my appearance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI2 It is hard to tell other people about my infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI4 I worry about spreading my infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI5 I am concerned about what the future holds for me</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B7 I worry about the effect of stress on my illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI10 I am embarrassed by my illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Date:** 11-AUG-04

**Completed by:**

**Signature:**
## Quality of Life FAHI

By checking one (1) box per line, please indicate how true each statement has been for you during the past week (7 days).

<table>
<thead>
<tr>
<th>Functional and Global Well-Being</th>
<th>Not at all</th>
<th>A little bit</th>
<th>Somewhat</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF1 I am able to work (include work at home)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF2 My work (include work at home) is fulfilling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF3 I am able to enjoy life</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF4 I have accepted my illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF5 I am sleeping well</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF6 I am enjoying the things I usually do for fun</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF7 I am content with the quality of my life right now</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE2 I am satisfied with how I am coping with my illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE3 I am losing hope in the fight against my illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6 I feel sexually attractive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6 I feel motivated to do things</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H11 I am hopeful about the future</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11-AUG-04 v1.3 FINAL  Date: [dd MMM yy]  Completed by: [ ]

Signature: [ ]
Quality of Life FAHI

By checking one (1) box per line, please indicate how true each statement has been for you during the past week (7 days).

<table>
<thead>
<tr>
<th>SOCIAL WELL-BEING</th>
<th>Not at all</th>
<th>A little bit</th>
<th>Somewhat</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS1 I feel close to my friends</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>GS2 I get emotional support from my family</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>GS3 I get support from my friends</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>GS4 My family has accepted my illness</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>GS5 I am satisfied with family communication about my illness</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>GS6 I feel close to my partner (or the person who is my main support)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>HI3 I have people to help me if I need it</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

Q1 □ Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box and go to the next section.

GS7 I am satisfied with my sex life | □ | □ | □ | □ | □ |

<table>
<thead>
<tr>
<th>COGNITIVE FUNCTIONING</th>
<th>Not at all</th>
<th>A little bit</th>
<th>Somewhat</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 My thinking is clear</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>HI8 I have trouble concentrating</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>HI9 I have trouble remembering things</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

11-AUG-04 v1.3 FINAL  
Date: dd MMM yy  
Completed by: □ □ □  
Signature: __________________________
Risk Behaviour Assessment

A. SOCIO-DEMOGRAPHIC INFORMATION

We need to collect information about who you are.

1. What is your date of birth (preferable, if available)?
   
   or
   
   How old are you (completed years)?

2. Gender
   
   □ Male  □ Female

3. What is your marital status?
   
   □ Single, no partner  □ Divorced
   
   □ Married  □ Widowed
   
   □ Stable partner  □ Many partners
   
   □ Separated  □ Refused

4. Observed race?
   
   □ White  □ Black
   
   □ Coloured  □ Indian
   
   □ Other, specify: __________________________

Race is recorded to assess the representation of the population in the sample.

5. Highest level of school passed?
   
   □ Grade (see notes for Grade table)

6. Does anyone depend on you financially?
   
   □ Yes  □ No  □ Refused

   If yes, please indicate number

   6a. □ Adults  6b. □ Children

11-AUG-04 v1.3 FINAL

Date: dd MMM yy

Completed by: __________________________

Signature: __________________________
B. SEXUAL HISTORY AND BEHAVIOURAL RISK SECTION

We would like to ask you about your life and your sexual behaviour in the recent past.

7. Age at first sexual intercourse?  [ ] Years old

8. Last sexual contact?  [ ] Days ago

9. Number of sexual partners in the last 3 months. A steady partner is one that you see most of the time, often over a period of time. A casual partner is one that you may see only occasionally or even only once [sex worker cohort - include only those who do not pay you for sex].

   a. Steady  [ ]  [ ]  [ ] Too many to remember  [ ] Refused
   b. Casual  [ ]  [ ]  [ ] Too many to remember  [ ] Refused

10. Total lifetime sexual partners [sex worker cohort - include only those who do not pay you for sex].

   a. Steady  [ ]  [ ]  [ ] Too many to remember  [ ] Refused
   b. Casual  [ ]  [ ]  [ ] Too many to remember  [ ] Refused

For all partners:

11. Have you ever had peno-vaginal sex?  [ ] Yes  [ ] No  [ ] Refused  go to 12.

   If yes, on average how many times per month?

   [ ]  [ ]  [ ] per month, or  [ ] Less than once a month  [ ] Less than once a year
   [ ] Only once  [ ] Refused

12. Have you ever had anal sex?  [ ] Yes  [ ] No  [ ] Refused  go to 13.

   If yes, on average how many times per month?

   [ ]  [ ]  [ ] per month, or  [ ] Less than once a month  [ ] Less than once a year
   [ ] Only once  [ ] Refused

11-AUG-04 v1.3 FINAL

Date:  [ ]  [ ]  [ ]  [ ]  [ ]  [ ]  [ ] Completed by:  [ ]

Signature: __________________________
Risk Behaviour Assessment

13. How safe do you think anal sex is compared to peno-vaginal sex when it comes to HIV infections?
   □ The same risk □ More risk
   □ Less risk □ Don’t know

14. Have you ever had oral sex? □ Yes □ No □ Refused
   If yes, on average how many times per month?
   □ per month, or □ Less than once a month □ Less than once a year
   □ Only once □ Refused

15. How safe do you think oral sex is compared to peno-vaginal sex when it comes to HIV infections?
   □ The same risk □ More risk
   □ Less risk □ Don’t know

16. Do you do anything to prevent yourself from falling pregnant?
    □ Yes □ No □ Refused
    a. If yes, how do you prevent yourself from becoming pregnant?
       □ Yes □ No □ Don’t know □ Refused
       1. Condom ........................................
       2. Pill ...........................................
       3. IUD ..........................................
       4. Injections ...................................
       5. Diaphragm / foam / jelly ....................
       6. Female sterilisation .........................
       7. Rhythm / Calendar method ..................
       8. Withdrawal ..................................
       9. Herb / Traditional remedies ............... 
      10. Quinine ....................................
      11. Thigh sex ..................................
      12. Anal sex ..................................
      13. Other, specify _______________________

11-AUG-04 v1.3 FINAL
Date:  dd MMM yy
Completed by: _______________________
Signature: _______________________

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Risk Behaviour Assessment

17. Some women report that they use substances to clean their vaginas after and between sexual encounters (douching). Do you use any substance to do this? □ Yes □ No □ Refused
   If yes, please describe what you use and how often:
   1. ________________________________ □ More than once a day □ Almost every day □ About once a week □ Once a month
   2. ________________________________
   3. ________________________________

18. Have you ever had sex while drunk from alcohol or taking any other drugs/substances? □ Yes □ No □ Don't know □ Refused
   18a. If yes, how often?
   □ □ per month, or □ Less than once a month □ Refused
   18b. How often was a condom used in this situation?
   □ Every time □ More than half the time
   □ Less than half the time □ Never
   □ Don't know □ Refused

19. How often can you insist on using a condom if you want to? (sex workers - not clients)
   a. With a steady partner? Never □ Occasionally □ Less than half the time □ More than half the time □ Every sexual act □ No partners
   b. With casual partners?

20. Was your last sexual encounter with a steady or casual partner/client? □ Steady □ Casual
   a. Was a condom used? □ Yes □ No
   b. Why? ________________________________

Date: dd □ MMM □ yy
Completed by: ________________________________
Signature: ________________________________
C. HIV/AIDS SECTION

21. Have you ever heard about HIV/AIDS? □ Yes □ No → End of Form

22. What are all the ways that you think HIV/AIDS is spread? (tick appropriate, without prompting)

☐ Vaginal sex (no condom) ☐ Oral sex (no condom) ☐ Anal sex (no condom)
☐ Breastfeeding ☐ Touching infected person ☐ Contact with infected blood
☐ Using public toilets ☐ Mosquito bites ☐ From mother to child during pregnancy
☐ Sharing the same cup or eating utensils with someone who is infected
☐ Other, specify: ____________________________

23. What are all the ways that you think we can prevent the spread of HIV/AIDS? (tick appropriate, without prompting)

☐ Having a good diet ☐ Using condoms ☐ Staying faithful to one partner
☐ Abstaining from sex ☐ Making sure all injections are done with clean needles
☐ Avoid sharing razors ☐ Other, specify: ____________________________

24. If you have any other sexually transmitted infection, do you think you are more likely to get HIV/AIDS than someone who does not have an infection?

☐ Yes ☐ No ☐ Only sometimes ☐ Unsure

Why? __________________________________________

25. Do you think that HIV/AIDS can be cured? That is, is there something you can do to get rid of the HIV infection once you have it?

☐ Yes ☐ No ☐ Unsure

If yes, how? __________________________________________

26. Do you think that HIV/AIDS can be treated? That is, is there something you can do to keep well even though you have been infected with HIV?

☐ Yes ☐ No ☐ Unsure

If yes, how? __________________________________________
Phase I cohort only

This section refers only to sex for compensation

1. How long have you been in sex work? □□ Years □□ Months □ Refused

2. At what age did you start sex work? □□ Years old

3. How many days a week do you perform sex work? □□ Days

4. At how many sites do you work over a year? □□ Sites/year

5. On an average working day, how many clients do you see? □□ Clients

6. In the last week, how many clients did you have? □□ Clients

7. Per week, how many of your sessions with clients are
   a. Short sessions/jobs □□
   b. Overnight stays □□

8. In an overnight stay, on average how many times do you have sexual contact with the client? □□ Times

9. How many condoms were used during last week with your clients? □□ condoms

10. How often was a condom used with your clients during the last month?
    □ Never □ Sometimes, less than half □ Often, more than half
    □ Always (100%) □ No clients last month

11. If always, was a new condom used for every act if you had more than one sex act with the same client? □ Yes □ No

11-AUG-04 v1.3 FINAL Date: □□ □□ MMM yy Completed by: □□ □□ □□
Signature: ____________________________
Viral Set Point and Clinical Progression in HIV-1 Subtype C Infection

Risk Behaviour Assessment Followup

To be administered during follow-up visits in phases II, III, and IV to end of study (Risk indicators).

This set of questions is designed to ask you about your sexual behaviour in the recent past, particularly since your last visit at the clinic. It is important for us to know what kinds of sexual behaviour you have been engaging in.

1. Number of sexual partners in the last 3 months. A steady partner is one that you see most of the time, usually over a period of time. A casual partner is someone that you may see only occasionally, or even only once [sex worker cohort - but does not pay you for sex].
   a. Steady
   b. Casual

2. Sex worker cohort only (this question only)
   How many clients did you see last week?

3. Have you been treated (or treated yourself) for a sexually transmitted infection since your last visit?
   Yes  No  Refused
   go to 4.
   If yes, how many times?

4. In the last month, have you had peno-vaginal sex?
   Yes  No  Refused
   go to 5.
   If yes, on average how many times in a week?
   per week, or
   Less than once a week
   Only once
   Refused
   Was a condom used?
   Every time
   Often, more than half the time
   Sometimes, less than half the time
   Never
   Refused

5. In the last month, have you had anal sex?
   Yes  No  Refused
   End of Form.
   If yes, on average how many times in a week?
   per week, or
   Less than once a week
   Only once
   Refused
   Was a condom used?
   Every time
   Often, more than half the time
   Sometimes, less than half the time
   Never
   Refused

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Date:  
Completed by:  
Signature:  

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6. How safe do you think anal sex is compared to peno-vaginal sex when it comes to HIV infections?  
   □ The same risk □ More risk  
   □ Less risk □ Don't know

7. In the last month, have you had oral sex?  □ Yes  □ No  □ Refused  
   If yes, on average how many times in a week?  □ □ □ per week, or  
   □ Less than once a week  □ Only once  □ Refused
   Was a condom used?  □ Every time  □ Often, more than half the time  
   □ Sometimes, less than half the time □ Never  □ Refused

8. How safe do you think oral sex is compared to peno-vaginal sex when it comes to HIV infections?

9. In the last month, have you had sex while drunk from alcohol or taking any other drugs/substances?  □ Yes  □ No  □ Don't know □ Refused  
   If yes, how often?  □ □ □ per week, or  
   □ Less than once a week  □ Only once  □ Can't recall □ Refused
   How often was a condom used in this situation?  □ Every time  □ Often, more than half the time  
   □ Sometimes, less than half the time □ Never  □ Don't know  □ Refused

Date: □ □ □ MMM yy
Completed by: □ □ □  
Signature: __________________________
Appendix 4: Schedule of Evaluations
15.5 Appendix 5: Informed Consents

1. Sample Informed consent form (Screening: HIV Negative Phase Screening) – This Phase of the study has been completed.

2. Sample Informed Consent form (Enrolment: Phase I - HIV Negative) – This Phase of the study has been completed

3. Sample Informed Consent form (Enrolment: Phase II-IV - Acute Infection)

4. Sample Informed Consent: Specimen Storage Phase II-IV

5. Sample Adolescent Informed Consent Form for Enrolment (Phase II-IV)

6. Sample Adolescent Consent for Specimen Storage for Possible Future Research (Phase II-IV)

7. Sample Parent/guardian Informed Consent Form for Adolescent Enrolment (Phase II-IV)

8. Sample Parent/guardian Consent for Specimen Storage for Possible Future Research (Phase II-IV)

9. Sample Informed Consent form (Enrolment: Phase V – Antiretroviral therapy initiation)

10. Sample Informed Consent: Specimen Storage Phase V

11. Sample Adolescent Informed Consent Form for Enrolment (Phase V)

12. Sample Adolescent Consent for Specimen Storage for Possible Future Research (Phase V)

13. Sample Parent/guardian Informed Consent Form for Adolescent Enrolment (Phase V)

14. Sample Parent/guardian Consent for Specimen Storage for Possible Future Research (Phase V)
1. Informed Consent form (Screening: Monitoring Phase Screening) - V3.02

Title of the Research: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS - OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 2604453 (Dr Nigel Garrett)

INTRODUCTION
You are being asked to take part in a research study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal's Nelson Mandela School of Medicine are doing this study. Over the period of two years, we hope to enroll approximately 298 subjects, from sites in and around Durban and Vulindlela. You will be asked to take part for as long as you are willing and able, up to a maximum period of five and a half years. The follow-up visits for this study will take place at the Medical Research Institute in Durban.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic. HIV is the virus that causes AIDS.

PURPOSE OF THE STUDY
You are being screened for potential enrollment into study. This study will look at the very early stages of HIV infection by following up HIV negative people who may become infected as well as people who have been found to have been recently infected. We will be screening participants for 24 months. All participants who remain HIV negative at the end of this 24-month period will finish the study at that time. If individuals become infected, they will be followed up for at least 42 months post-infection. The longest time that anyone can take part in the study is five and a half years. Researchers are interested in what happens in a person's body when they become infected with the virus and what determines how well their body is able to cope with HIV infection. Researchers are also interested in determining what factors influence the progression of HIV disease in South Africa. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. This study is an observational study. That is, participants will not be provided with treatment on this study, but will be referred for the treatment that is currently used at the government health facilities.

INFORMED CONSENT
You are being asked to volunteer for screening tests to find out if you are eligible for research in the study named above. The research is for people who are at risk for HIV infection. The screening tests will include a set of questions and you will also be screened for HIV.
YOUR PARTICIPATION IS VOLUNTARY
The information on this form will be discussed with you. Once you understand the screening process and the tests that will be used, and if you agree to take part, you will be asked to sign your name or make your mark on this form. You will be given a copy to keep.

Before you learn more about the screening tests, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to have the screening tests, or to withdraw from the screening tests at any time, without losing the benefits of your routine medical care.
- If you decide not to have the screening tests, you can still join another research study later, if one is available and you qualify.
- You are only being asked to have the screening tests at this time. Even if you agree to have the screening tests, you do not have to join the research study.

PURPOSE OF THE SCREENING TESTS
The research that you will be screened for involves the study of HIV virus. The first phase of the study will be monitoring people who are not infected. The screening tests are therefore looking for people who are HIV negative. For this reason, if you have already been infected with HIV you will not be able to join this study and you will be referred to the nearest appropriate public health clinic. Women who are pregnant at screening will also not be able to take part in the study. If you are HIV negative, a sample of your urine will be tested for signs of pregnancy. If you are pregnant, you will be referred to your local public health care clinic for management of your pregnancy.

Some individuals will not be able to take part in the study based on the information that is found during the screening tests. The screening tests are done on site, and you will be able to get the results the same day.

PROCEDURES
At this visit, you will be able to read, discuss and sign this screening consent form. The study staff will ask you where you live and other questions about you, your health and your sexual practices. If your answers to these questions indicate that you may qualify (be eligible) for this study, you will then undergo a rapid test for HIV.

The study staff will talk to you about the HIV test, and what it may mean for you to know the results of this test. You will be asked to talk about how you would feel about knowing your HIV status, and whether you would be prepared to be tested for HIV. You will also receive information about how to prevent yourself from being infected with the virus and other sexually transmitted infections.

Finding out the result of an HIV test can be very difficult. You will talk to the study staff about the meaning of your result, and how you feel about it. You will need to receive your HIV test result in order to be enrolled and stay in the study.

If you are prepared to have the test, one tube of blood will be drawn. If you test positive or the test results are inconclusive, you will be referred to the nearest public health clinic or Voluntary Counseling and Testing (VCT) centre for more tests, counseling and related health care. You will also receive counseling on how to stay healthy, and assistance with obtaining any services for which you may be eligible. You will be told of any other research projects that you might be able to take part in, if there are any.

If your results show that you have not been infected with HIV, you will be tested for pregnancy using a urine sample.

If you are HIV negative and not pregnant, you could be able to take part in the study. The study staff will explain the purpose of the study to you, and you will be asked to sign another consent form to take part in the study.

All of these screening procedures should be completed within one to two hours.

RISKS AND/OR DISCOMFORTS
You may feel some pain or discomfort during the blood collection. A few people feel faint when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the blood draw.
You may be embarrassed, worried, or anxious when you are discussing your sexual practices, ways to protect yourself and others from infections passed on during sex. You may also feel worried or anxious while waiting for and finding out your test results. If you find that you have HIV or any other infection, this could also cause you anxiety and problems at home or with your friends. A trained counselor will help you deal with any feelings that you may have. You will also be referred for medical and support services available that will be able to help you live in a more healthy and positive way.

**BENEFITS**

You will get no direct benefit from taking part in the screening tests. However, you will get counseling and testing for HIV. If you are infected with HIV, you will be referred for medical care, counseling and other services that are available to help you. If you are pregnant, you will be referred to the nearest public health clinic that can provide care and management of your pregnancy.

If you are enrolled onto the Acute Infection Study, you will receive regular monitoring of your health. This would allow you to be referred for treatment for many conditions that you may develop; perhaps earlier than if you were not receiving regular checkups.

**CONFIDENTIALITY**

Research records of your participation in the study will be kept confidential and will not be released without your permission, unless we are required by law to do so.

We will make every effort to protect your privacy and confidentiality during the screening procedures. Your visit will take place in private. Only your doctor and study staff will know the outcome of your screening procedures. However, it is possible that others may learn that you have been here and assume that you are at high risk for HIV infection. Because of this, others may treat you differently or discriminate against you.

Medical records that identify you by name may be inspected by representatives of the agency that is funding this study and by the research team who is responsible for keeping information for this study.

**REASONS WHY YOU MAY BE WITHDRAWN FROM THE SCREENING TESTS WITHOUT YOUR CONSENT**

You may be removed from the screening tests without your consent for the following reasons:

- The research study is stopped or cancelled.
- The study staff feels that having the screening tests may be harmful to you.
- You are not willing to receive your HIV test result.

**COSTS OF THE STUDY**

There is no cost to you for the tests that you will be done during the course of this study.

**COMPENSATION**

You will receive compensation for your time and effort at the scheduled screening visit. You will also receive a refund for the costs incurred by you in order to attend the scheduled clinic (for example, transport costs). You need to find out from the study nurse what the amount of this compensation is before agreeing to take part in the screening tests.

**RESEARCH RELATED INJURIES**

It is very unlikely that you could be injured as a result of the screening tests. In the case of a research related injury, you will be referred to King Edward VIII Hospital for treatment. The cost of this treatment will be borne by the researchers. There is, however, no compensation provided for research related injuries. In the event of a research related injury, contact:

- Nelsile Majola (Ethekwini Site Study Coordinator) 031 260 1924
- Nelsiwe Ngcobo (Vulindlela Site Study Coordinator) 033 260 6880
- Dr. Nigel Garrett (Project Manager) 031 260 4453

**PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS**

If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following:
You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may call:
The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
email: buccas@ukzn.ac.za

SIGNATURES

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.
- I acknowledge that I have been informed by the clinic staff concerning the possible advantages and possible adverse effects that may result from my involvement in the abovementioned study.
- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of: Dr Garrett and Professor SS Abdooll Karim.
- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.
- I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed: ________________________________ Date:__________
Subject/Parent/Guardian

Signed: ________________________________ Date:__________
Witness

Signed: ________________________________ Date:__________
Researcher

For illiterate subjects:

Mark with an ‘X’: ________________________________ Date:__________

Independent Witness: ________________________________ Date:__________

Title and Name: ________________________________

Telephone Number: ________________________________
Title of the Research: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 - 2604453 (Dr Nigel Garrett)

INTRODUCTION
You are being asked to take part in a research study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal’s Nelson Mandela School of Medicine are doing this study. Over the period of two years, we hope to enroll approximately 298 subjects, from sites in and around Durban and Vulindlela. You will be asked to take part for as long as you are willing and able, up to a maximum period of five and a half years, as this is how long the whole study will continue. The follow-up visits for this study will take place at the Medical Research Institute in Durban.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic. HIV is the virus that causes AIDS. If you do become infected with HIV during the study, we will continue to do tests that will tell us how your body is responding to the infection. This is an observational study and no treatment will be given to you by the study staff. Should you become ill or reach a point where you require treatment, you will be referred to the Family Clinic at King Edward VIIIth Hospital for the standard treatment that is currently offered to patients in South Africa. You may then also be offered an opportunity to take part on other available studies offering drugs that treat HIV infection, should you be eligible.

INFORMED CONSENT
You are being asked to volunteer for the research study named above. This is a study for people who have recently been screened for HIV infection and have tested negative. Before you decide whether or not you would like to take part in the study, we would like to explain the study to you. You will learn what the study is about, what procedures will form a part of the study, what the risks and benefits of the study are to you, and what is expected of you as a research participant.

YOUR PARTICIPATION IS VOLUNTARY
Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.

PURPOSE OF THE STUDY
Overall, this study will look at the very early and later stages of HIV infection by following up people who have been found to have been infected very recently. These individuals will be followed up for at least 42 months after they have been informed of the diagnosis of HIV infection and have been enrolled onto the study, and up to a maximum of 66 months (five and a half years).
Researchers are interested in what happens in a person’s body to determine how well the body of people who have become infected is able to cope with HIV infection. Researchers are also interested in determining what factors influence the progression of HIV disease in South Africa. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people’s thoughts, feelings and behaviors.

You have been recruited into the HIV negative phase (called Phase I) of this study as you recently tested HIV negative. You will be asked to come to the clinic once a month to be tested for HIV infection and to receive HIV counseling. In this phase of the study, you will receive condoms and counseling and education on how to protect yourself from sexually transmitted infections, especially HIV. You will also have a physical exam at these visits. If we find that you have any illness and infection at these visits, you will be referred to the Family Clinic at King Edward Hospital for the appropriate treatment and care. This clinic may refer you to other clinics or services at the hospital should you require this. Participation in this study should not increase your risk of infection. All those participants who remain HIV negative will be followed up for 2 years only.

If at one of these visits it is learned that you have been infected with HIV, the virus that causes AIDS, you will be offered an opportunity to be enrolled onto Phase II of this study. This phase looks very closely at what happens in the body during the early stages of infection with HIV. Participants will be asked to come to the clinic on a regular basis for a check-up. For the first 3 weeks you will be asked to come to the clinic once a week and then every other week until 3 months after your diagnosis (5 visits). The next 9 months is called Phase III and will require you to come to the clinic once a month. On Phase IV of the study you will be asked to come to the clinic once every three months until the end of the study, which would be a maximum of five and a half years after your diagnosis.

We will also be providing participants with regular medical check-ups and referral for treatment of any infections or illnesses that they may have. If you become eligible for the second and third phases of this study, you will be provided with more information about those phases of the project. You will be asked to sign an informed consent form to take part in those phases. By agreeing to take part in this phase of the study, you are not compelled to take part in later phases if you become eligible for those. You will always have the right to withdraw from the study at any time.

If, at any time, during this study, the study doctor or you think that you might have fallen pregnant, a sample of your urine will be tested. If you are pregnant, you will be encouraged to stay in the study. The study staff will be able to refer you to available sources of medical care and other services that you and your baby may need.

By taking part in this study, you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic.

PROCEDURES
At this visit, you will be able to read, discuss and sign this consent form. If you decide to consent, the study staff will ask you where you live and other questions about you, your health and your sexual practices. You will also undergo medical tests.

You will also have a physical examination done, which will include looking for clinical evidence of sexually transmitted infections. Specimens to test for sexually transmitted infections, including HIV, will be collected from you. This will include a wash and a swab from your vagina. The study staff will talk to you about the infections that are passed on during sex. If you are having problems with one of these sexually transmitted infections, the study staff will refer you to King Edward Hospital where you will be given medicine to treat the infection.

You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give just less than 50 mls of blood at each visit. This blood will be drawn in around 9 tubes. The blood will be used to test you for signs of early HIV infection, and some samples will be used to check on your general health. Every six months you will be asked to give samples for testing for other sexually transmitted infections. Urine samples will be taken every 6 months to check on your health. If you or the study doctor think you may be pregnant, a sample of your urine will be tested for signs of pregnancy. Some of the specimens that are collected from you may be stored. You will be asked to sign a consent form to indicate whether you agree to allow the team to store some of your samples.
Your visit should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

**BENEFITS**
There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like microbicides and vaccine trials, and the effectiveness of HIV risk reduction and prevention messages.

However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be referred to KEH VIII hospital for treatment. You will receive free condoms, which you can use to reduce your risk of HIV infection. You will receive information on how to use condoms properly to reduce risk.

**PARTICIPANT RESPONSIBILITY**
By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinics. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study, please tell this to the study nurse or doctor. We will encourage you to come to the clinic for a final exit interview and to receive any test results that you may not have received yet.

**COMPENSATION**
You will be compensated for your transport costs when you come in to the scheduled clinics. If your clinic visit takes up most of your day, you will be provided with food and refreshments for the time that you are at the clinic.

**RISKS/DISCOMFORT**
Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of genital secretions (wash and swab from your vagina) are pain and discomfort.

You may learn that you have become HIV positive while on this study. Learning that you are HIV positive can be a very stressful experience. In addition to this, you may be treated badly by friends and family if your HIV status becomes known to others. You may find it difficult to find work should others learn of your HIV status, and you may find that members of your family or community discriminate against you.

Some of the tests being used for this study are very sensitive and can pick up an infection much earlier than the tests used at public health facilities. This means that we will sometimes be able to detect infection before we can actually tell you for sure that you have been infected. We will be able to tell you that you have been exposed, but will have to wait for the standard test to come back positive before we can confirm whether you have been infected. This period is likely to be very stressful while you wait for the test result.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

**NEW FINDINGS**
You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to gain access to them.

**REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT**
You may be removed from the study without your consent for the following reasons:
- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

**ALTERNATIVES TO PARTICIPATION**
There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about. There may also be other places where you can go for HIV counseling and testing. We will also tell you about those places if you wish.

**COSTS OF THE STUDY**
There is no cost to you for the physical examination or laboratory tests that you will receive during the course of this study. You will receive treatment for all sexually transmitted infections that are detected while you take part in this study. You will be referred to King Edward VIIIth Hospital for any other non-study related illnesses that may be detected at the clinic.

**CONFIDENTIALITY**
Research records of your participation in the study will be kept confidential and will not be released without your permission, unless we are required by law to do so.

We will make every effort to protect your privacy and confidentiality during the screening procedures. Your visit will take place in private. Only your doctor and study staff will know the outcome of your screening procedures. However, it is possible that others may learn that you have been here and assume that you are at high risk for HIV infection. Because of this, others may treat you differently or discriminate against you.

Medical records that identify you by name may be inspected by the agency who are funding this study and by the research team who are responsible for keeping information for this study.

In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission. No publications based on this research will contain your name. Only the project and clinic co-ordinators will know both your name and your identification number. This is necessary so that the co-ordinators can ensure that you will receive the correct tests and that you are called in to the correct clinics. The co-ordinators will not release your number and name to anyone else on the research team.

**RESEARCH RELATED INJURIES**
It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred to the King Edward hospital for treatment. The cost of this treatment will be borne by the research team. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form. In the event of a research related injury, contact:

- Nelisile Majola (Ethekwini Site Study Coordinator) 031 260 1924
- Neliswe Majola (Vulindlela Site Study Coordinator) 033 260 6880
- Dr Nigel Garrett (Project Manager) 031 260 4453

**PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS**
If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following:

- Project Manager and Co-chair: Dr Nigel Garrett 031 260 4453
- Principal Investigator: Professor SS Abdool Karim 031 260 4550 (Norma Hatcher)
You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research subject, you may call:
The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
email: brec@ukzn.ac.za

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.
- I acknowledge that I have been informed by the clinic staff and doctor (Dr Garrett) concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.
- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of: Dr Garrett and Professor SS Abdool Karim.
- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.
- I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed:__________________________________________  Date:___________
Subject/Parent/Guardian

Signed:__________________________________________  Date:___________
Witness

Signed:__________________________________________  Date:___________
Researcher

For illiterate subjects:
Mark with an ‘X’:__________________________________________  Date:___________
Independent Witness:__________________________________________  Date:___________

Title and Name:__________________________________________
3. Informed Consent form (Enrolment: Phase II-IV - Acute Infection) – V7.00

Title of the Research: CAPRISA 002: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

Principal Investigator: Professor Salim Abdool Karim
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Telephone No: 031 260 1611 (Fathima Sayed)
Office hours (8am-5pm): 033 260 6863 (Duduzile Nkosi)
031 260 4453 (Nigel Garrett)

INTRODUCTION
You are being asked to take part in a study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal's Nelson Mandela School of Medicine are doing this study. We hope to enroll 300 subjects, from sites in and around Durban and Vulindlela. You will be asked to take part in this study until you decide to initiate antiretroviral therapy. The follow-up visits for this study will take place at the eThekwini and Vulindlela Clinical Research Sites. Each study visit lasts approximately 3 hours.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

INFORMED CONSENT
You are being asked to volunteer for the study named above. This is a study for people who have recently been infected with HIV. Before you decide whether or not you would like to take part in the study, we would like to explain the study to you. You will learn what the study is about, what procedures will form a part of the study, what the risks and benefits of the study are to you, and what is expected of you as a research participant.

OTHER RESEARCH STUDIES
If you have been referred from other studies, it will be necessary for us to look at your HIV test results from previous HIV testing. By signing this form, you are agreeing to your HIV test records being reviewed and information collected about your HIV test results documented in the previous study you were enrolled in.

YOUR PARTICIPATION IS VOLUNTARY
Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.
- If you decide not to take part in this study, you can still join another study later, if one is available and you qualify.

PURPOSE OF THE STUDY
This study will look at several important elements of the spread of HIV in South Africa. HIV is the virus that causes AIDS. After initial infection, the level of virus measured in the blood is usually high, and then it drops down over
time often reaching a point where it does not change much over time. The amount of virus that can be measured in the blood is referred to as the viral load. This study aims to better understand the relationship between the viral load over time and disease progression in HIV infection in South Africa. That is, we are trying to find out whether the amount of virus that can be measured at certain times (at 6, 12 and 18 months) is related to how quickly someone with HIV infection develops AIDS illnesses. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people’s thoughts, feelings and behaviors.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype ‘C’ is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections in the developed world, there is limited information available to determine what influences the viral load (amount of virus that can be measured in your blood) in subtype C infections.

Since the point at which the viral load stabilizes is currently the best indication of progression to illness and AIDS, the results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV and AIDS. This study will also provide important information for future treatment interventions, i.e. interventions aimed at preventing AIDS after HIV has occurred.

You have been referred to this study because you are known to have been recently infected with HIV. By taking part in this study you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your own situation directly. That is, it may not provide a cure or alter the course of your illness. However, you will be monitored closely and referred for care and treatment relating to any HIV-related illness that you may develop.

As people become ill after HIV infection, their blood cells known as CD4+ T cells (protective cells) become reduced. This is an indication of the immune system’s failure to control infection. Most infections occur when the level of these cells drop below 200 copies/ml. At this time people can become sick very easily.

PROCEDURES
Entering the Study
At this visit, you will be able to read, discuss and sign this consent form. If you decide to sign, the study staff will ask you where you live and other questions about you, your health and your sexual practices.

You will also have a physical examination done, which will include looking for evidence of sexually transmitted infections (STIs) and the presence of any AIDS related illnesses. You will be asked to give specimens for the testing of STIs. If you are a woman this could include a urine sample and swabs from your vagina. The study staff will talk to you about the infections that are passed on during sex. If you have clinical signs and symptoms of STIs, you will be treated as soon as possible after diagnosis or you may be referred to a public health clinic where you will be given medicine to treat those infections.

You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give around 90 mls of blood (around 15 tubes) at your first visit. The blood will be used for tests that look at the way in which your body is responding to HIV infection. It will also be used to look at the virus that you have been infected with. Some of this blood together with a urine specimen, will be used to check on your general health. If you are a woman and you or the study doctor suspect that you are pregnant, this sample will be checked for signs of pregnancy. If you are pregnant, you will be encouraged to stay in the study. You will also be referred to your nearest public health clinic for care relating to your pregnancy.

You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle. You will also be evaluated for the presence of any AIDS related illnesses.

During the Study
The study nurse will notify you of your scheduled clinic visits. The number of times that you will be called in to the clinic will vary over time. You may leave the study at any time.

At first you will be asked to come in approximately every month for six months. After this you will come in every alternate month until one year after starting, and then once every three months until the end of the study. Each
study visit will last approximately 3 hours. At these visits, you will also be given a medical examination by one of the study nurses or doctors. You will be asked to give tubes of blood at each visit. The amount of blood varies between 8 and 15 tubes. The blood will be used to see how your body is responding to HIV infection, and to check on the type of virus that you have been infected with. The virus that infects you, changes over time, and we want to study the way in which the virus changes.

Once a year you will be tested for any STIs. This will be done by testing your blood, and by using a vaginal swab and urine specimen. You will be treated or referred for treatment for any STIs that you have. You will be treated or referred for treatment for any STIs that you have. You will be asked questions about your sexual behaviour, general health as well as other questions related to your lifestyle.

People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity. These are known as ‘opportunistic infections’. Any opportunistic infections that you may develop will be detected at the scheduled clinic visits and you will be treated or referred to the relevant public health clinic where you will receive treatment for any treatable opportunistic infections. Your body has cells in it that help you fight off infection and these helper cells are called CD4+ T cells. The level of CD4+ cells in your body can be an indication of severity of disease. You are encouraged to start treatment for HIV as soon as you are ready, but latest when your immune system weakens indicated by a CD4 count of less than 500 cells/mm$^3$. Treatment will be initiated at the eThekwini or Vulindlela sites and you may be referred to your closest ART treatment site 6 months after initiation of ART or when your viral load is undetectable. You will be given the opportunity to participate (if eligible) in any antiretroviral drug therapy trial or treatment programmes available. However, your participation in our study, is no guarantee that you will participate in such other programmes or trials. You will be told about the care and treatment that you will receive at the clinic by the clinic staff. You will also be able to get counselling on how to stay as healthy as possible and how to get any services that you may be eligible to receive.

You will be asked to tell the study staff about any medical problems you may have during the study, especially those related to your genitals or to STIs. You must contact the study staff between your regularly scheduled visits if you need to report these problems. The study staff will examine you and they will treat or refer you to a clinic for medical care, if needed. If you are a woman and you become pregnant during this study you will be encouraged to stay in the study. The study staff will be able to refer you to available sources of medical care and other services you and your baby may need.

At each visit the study staff will update information on where you live and how to keep in contact with you. They will use this information to remind you of your scheduled visits. If you miss a visit, the study staff will try to contact you to find out why you missed a visit. They may also visit your home to try to find you. They will try to reach you through the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you. If you have a cellular phone, study staff may ask if they can use this number to contact you.

Your visit should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

Some of the specimens collected may be stored. You will be asked to sign a separate consent form to indicate whether you agree to allow the team to store you specimens.

**BENEFITS**

There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like microbicides and vaccine trials, and the effectiveness of HIV risk reduction and prevention messages.

However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from STIs and HIV infection. If you contract a treatable STI during the course of this study, you will be treated on site or referred to a public health clinic for treatment. You will receive free condoms and be given information on how to use condoms properly to reduce your risk of STIs.
PARTICIPANT RESPONSIBILITY
By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinics. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study please tell the study nurse or doctor. We will encourage you to come to the clinic for a final exit interview and to receive any test results that you may not have received yet.

REIMBURSEMENT
You will be compensated for your time, travel and inconvenience. If your visit is 3 hours or less you will be given R200. Clinic visits may take up most of your day. If you have to be at the clinic for the day you will be provided with food and refreshments for the time that you are at the clinic.

RISKS/DISCOMFORT
Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals. Although you may choose to be involved in a study of HIV/AIDS medicines, these studies are also researching the effectiveness of medicines and taking those medicines would have their own risks.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of vaginal specimens are pain and discomfort. Being HIV positive can be a very stressful experience. In addition to this, you may be treated badly by friends and family if your HIV status becomes known to others. You may find it difficult to find work should others learn of your HIV status, and you may find that members of your family or community discriminate against you.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

NEW FINDINGS
You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT
You may be removed from the study without your consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION
There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about. There may also be other places where you can go for HIV counseling and testing. We will also tell you about those places.

COSTS OF THE STUDY
There will be no financial cost to you for participation in this study. You will be treated on site or referred for treatment for all treatable STIs that are detected while you take part in this study.

CONFIDENTIALITY
Research records of your participation in the study will be kept confidential and we will not give this information to anyone, unless require by law to do so.

We will make every effort to protect your privacy and confidentiality during the screening procedures. Your visit will take place in private. Only your doctor and study staff will know the outcome of your screening procedures. However, it is possible that others may learn that you have been here and assume that you are at high risk for HIV infection. Because of this, others may treat you differently or discriminate against you.
Medical records that identify you by name may be inspected by the agency who are funding this study and by the research team who are responsible for keeping information for this study.

In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission. No publications based on this research will contain your name. However, in some instances we may request additional consent from you if there is a possibility that you may be identified in a publication by description of your characteristics. Only the project and clinic coordinators will know both your name and your identification number. This is necessary so that the coordinators can ensure that you will receive the correct tests and that you are called in to the correct clinics. The coordinators will not release your number and name to anyone else on the research team.

**RESEARCH RELATED INJURIES**

It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred for treatment. The cost of this treatment will be free. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form. In the event of a research related injury, contact during office hours (8am-5pm):

- Fathima Sayed (eThekwini Site) 031 2601611,
- Hlengiwe Shozzi (eThekwini Site) 031 2601943,
- Duduzile Nkosi (Vulindlela Site) 033 2606863, or
- Dr Nigel Garrett (Project Manager) 031 2604453

**PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS**

If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following during office hours (8am-5pm):

- Fathima Sayed (eThekwini Site) 031 2601611,
- Hlengiwe Shozzi (eThekwini Site) 031 2601943,
- Duduzile Nkosi (Vulindlela Site) 033 2606863, or
- Dr Nigel Garrett (Project Manager) 031 2604453

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.
- I acknowledge that I have been informed by the clinic staff and doctor concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.
- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of: Dr Garrett and Professor SS Abdool Karim.

- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.

- I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Subject

Signed: __________________________ Date:________
Subject

Name: __________________________________________
Researcher

Signed: __________________________ Date:________
Researcher

**For illiterate subjects:**

Mark with an 'X': __________________________ Date:________

Independent Witness: __________________________ Date:________

Title and Name: __________________________
4. Informed Consent: Specimen Storage (Phase II-IV – Acute Infection) – V7.00

Title of the Research: CAPRISA 002: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 2601611 (Fathima Sayed)
Office hours (8am-5pm) 033 260 6863 (Duduzile Nkosi)
031 260 4453 (Nigel Garrett)

INTRODUCTION
You have been enrolled into an HIV acute infection study. While you are taking part in this study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This consent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to sign this consent form. You will be given a copy to keep. The results of tests on your stored samples will not usually be made known to you, and the researchers do not intend sharing this information with anyone else. If the researchers believe that information from tests on your stored material is important, they will make this available to you through your regular doctor. Please make sure that you update your contact information with the study staff so that they can contact you if the need arises.

BLOOD AND BIOLOGICAL SAMPLES
At your clinic visits, blood and other biological samples will be taken from you. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a number and not your name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES
The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your samples may be analyzed in laboratories outside of South Africa. Your samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify the study doctor who will try to contact you or your regular doctor. If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this kind of test result, you need to provide the study team with the contact details of your regular doctor.

STORAGE OF SAMPLES
Your samples will be stored at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is no time limit on how long your samples may be stored.
BENEFITS
There is no direct benefit to you through having your samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

RISKS
There is very little risk to you when you have your samples stored. There is a small risk that others may find out information about your HIV status from stored samples. This risk is reduced as your sample is stored under a study number, and not your name. You are entitled to the same protections of confidentiality and privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from these tests may cause distress to you.

Some genetic testing may be done on your stored samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

CONFIDENTIALITY
The results of future tests of your samples will not go onto your medical record. Although every effort is made to make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your personal information may be disclosed.

Medical records that identify you by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.

PARTICIPANT RIGHTS
The decision to allow your samples to be stored is completely voluntary. You may decide not to allow your samples to be stored after the tests that are needed for this study have been done or you decide to stop participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your samples to be stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, “Withdrawal of Consent” so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your samples.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS
If you have any questions about the storage of samples for this study, or would like to know more about the storage of blood, please call either of the following during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,
Hlengiwe Shozi (eThekwini Site) 031 2601943,
Duduzile Nkosi (Vulindlela Site) 033 2606863, or
Dr Nigel Garrett (Project Manager) 031 2604453

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za
SIGNATURES
Please read the statement below and think about your choice. No matter what you decide, it will not affect your care.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_______ Yes
_______ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Subject
Signed: __________________________________________ Date:__________
Subject

Name: __________________________________________
Researcher
Signed: __________________________________________ Date:__________
Researcher

For illiterate subjects:
Mark with an ‘X’:____________________________________ Date:__________
Independent Witness: ________________________________ Date:__________
Title and Name: ______________________________________

Withdrawal of Consent
I hereby withdraw my consent for the storage of my biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed: __________________________________________ Date:__________
Subject

Signed: __________________________________________ Date:__________
Researcher
For illiterate subjects:

Mark with an ‘X’: __________________________________________ Date:__________

Independent Witness: ______________________________________ Date:__________

Title and Name: ____________________________________________
If the participant is younger than 18 years of age, this administrative section must be completed prior to completing the consent form for enrolment.

1. Has the participant’s age been verified? Yes ☐ No ☐
   1.1 If yes, indicate below how the participant’s age has been verified:
   ☐ Birth Certificate
   ☐ Identification Document (ID)
   ☐ Other: Specify:

2. Who has provided consent for this participant to participate in this study:
   ☐ Parent
   ☐ Legal Guardian

If consent has not been given to participate in this study, please record reason why consent was not given:

3. Has the participant completed the literacy assessment? Yes ☐ No ☐
4. Does the participant require an impartial witness? Yes ☐ No ☐

Completed by: __________________________

Staff Note: If the participant cannot read, the consent form must be read to the participant exactly as written, in the participant’s language of choice, and a witness must sign this form to confirm that the correct information was given to the participant and that the participant freely consents to be in this study.

If you have indicated NO to Question 1 or 3 above please do not proceed any further.
INTRODUCTION:
You are being asked to take part in a study named above. This is a study for people who have recently been infected with HIV. This document gives you information about the study that will be discussed with you by the study doctor or nurses. You are asked to read (or to have read to you) this consent form in a language of your choice. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. This consent form may contain words or terms that you don’t understand. Please ask us to explain anything you may not understand. To be sure you understand the contents of this form we will ask you a few questions after you have read this consent form or have had the consent form read to you. If you decide to join this study, we will ask you to sign this form. We will give you a copy of this form to keep or if you prefer we can keep this form for you.

Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Scientists associated with the University of KwaZulu-Natal’s Nelson Mandela School of Medicine are doing this study. We hope to enroll 300 subjects, from sites in and around Durban and Vulindlela. You will be asked to take part for as long as you are willing and able to. The follow-up visits for this study will take place at the eThekwini and Vulindlela Clinical Research Sites. Each study visit will last approximately 3 hours.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

OTHER RESEARCH STUDIES
If you have been referred from other research studies, it will be necessary for us to look at your HIV test results from previous HIV testing. By signing this form, you are agreeing to your HIV test records being reviewed and information collected about your previous HIV test results.

YOUR PARTICIPATION IS VOLUNTARY
Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.
- If you decide not to take part in this study, you can still join another study later, if one is available and you qualify.
- Please note that since you are younger than 18 years of age, we also require your parent or guardian to agree to your participation in this study. Your parent or guardian has been given a similar consent form to read and sign.
• If your parent/ guardian agrees to your participation in CAPRISA 002 we will still require you to agree to participate in this study.
• You can agree to take part in this study at a later date, but prior to study completion.

The rest of this consent form will describe to you the purpose of CAPRISA 002, when each study procedure will take place, procedures for contacting you if necessary, the risks and benefits of participating in this study, and your rights as a study participant.

This study has been reviewed and approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (BREC).

PURPOSE OF THE STUDY
This study will look at several important elements of the spread of HIV in South Africa. HIV is the virus that causes AIDS. After initial infection, the level of virus measured in the blood is usually high, and then it drops down over time often reaching a point where it does not change much over time. The amount of virus that can be measured in the blood is referred to as the viral load. This study aims to better understand the relationship between the viral load over time and disease progression in HIV infection in South Africa. That is, we are trying to find out whether the amount of virus that can be measured at certain times (at 6, 12 and 18 months) is related to how quickly someone with HIV infection develops AIDS illnesses. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people’s thoughts, feelings and behaviors.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype ‘C’ is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections in the developed world, there is limited information available to determine what influences the viral load in subtype C infections.

Since the point at which the viral load stabilizes is currently the best indication of progression to illness and AIDS, the results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV and AIDS. This study will also provide important information for future treatment interventions, i.e. interventions aimed at preventing AIDS after HIV has occurred.

You have been referred to this study because you are known to have been recently infected with HIV. By taking part in this study you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your own situation directly. That is, it may not provide a cure or alter the course of your illness. However, you will be monitored closely and referred for care and treatment relating to any HIV-related illness that you may develop.

As people become ill after HIV infection, their blood cells known as CD4+ T cells (protective cells) become reduced. This is an indication of the immune system’s failure to control infection. Most infections occur when the level of these cells drop below 200 copies/ml. At this time people can become sick very easily.

PROCEDURES
Entering the Study
At this visit, you will be able to read, discuss and sign this consent form. If you decide to sign, the study staff will ask you where you live and other questions about you, your health and your sexual practices.

You will also have a physical examination done, which will include looking for evidence of sexually transmitted infections (STIs) and the presence of any AIDS related illnesses. You will be asked to give specimens for the testing of STIs. If you are a woman this could include a urine sample and swabs from your vagina. The study staff will talk to you about the infections that are passed on during sex. If you have clinical signs and symptoms of STIs, you will be treated as soon as possible after diagnosis or you may be referred to a public health clinic where you will be given medicine to treat those infections.
You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give around 90 mls of blood (around 15 tubes) at your first visit. The blood will be used for tests that look at the way in which your body is responding to HIV infection. It will also be used to look at the virus that you have been infected with. Some of this blood together with a urine specimen, will be used to check on your general health. If you are a woman and you or the study doctor suspect that you are pregnant, this sample will be checked for signs of pregnancy. If you are pregnant, you will be encouraged to stay in the study. You will also be referred to your nearest public health clinic for care relating to your pregnancy.

You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle. You will also be evaluated for the presence of any AIDS related illnesses.

**During the Study**

The study nurse will notify you of your scheduled clinic visits. The number of times that you will be called in to the clinic will vary over time. You may leave the study at any time.

At first you will be asked to come in approximately every month for six months. After this, you will come in every alternate month until one year after starting, and then once every three months until you decide to start ART. At these visits, you will also be given a medical examination by one of the study nurses or doctors. You will be asked to give tubes of blood at each visit. The amount of blood varies between 8 and 15 tubes. The blood will be used to see how your body is responding to HIV infection, and to check on the type of virus that you have been infected with. The virus that infects you, changes over time, and we want to study the way in which the virus changes.

Once a year you will be tested for any STIs. This will be done by testing your blood and by taking a vaginal swab and urine specimen. You will be treated or referred for treatment for any STIs that you have. You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle.

People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity. These are known as ‘opportunistic infections’. Any opportunistic infections that you may develop will be detected at the scheduled clinic visits and you will be treated or referred to the relevant public health clinic where you will receive treatment for any treatable opportunistic infections. Your body has cells in it that help you fight off infection and these helper cells are called CD4+ T cells. The level of CD4+ cells in your body can be an indication of severity of disease. You are encouraged to start treatment for HIV as soon as you are ready, but latest when your immune system weakens indicated by a CD4 count of less than 500 cells/mm³. Treatment will be initiated at the eThekwini or Vulindlela sites and you may be referred to your closest ART treatment site 6 months after initiation of ART or when your viral load is undetectable. You will be given the opportunity to participate (if eligible) in any antiretroviral drug therapy trial or treatment programmes available. However, your participation in our study is no guarantee that you will participate in such other programmes or trials. You will be told about the care and treatment that you will receive at the clinic by the clinic staff. You will also be able to get counseling on how to stay as healthy as possible and how to get any services that you may be eligible to receive.

You will be asked to tell the study staff about any medical problems you may have during the study, especially those related to your genitals or to STIs. You must contact the study staff between your regularly scheduled visits if you need to report these problems. The study staff will examine you and they will treat or refer you to a clinic for medical care, if needed.

If you are a woman and you become pregnant during this study you will be encouraged to stay in the study. The study staff will be able to refer you to available sources of medical care and other services you and your baby may need.

At each visit the study staff will update information on where you live and how to keep in contact with you. They will use this information to remind you of your scheduled visits. If you miss a visit, the study staff will try to contact you to find out why you missed a visit. They may also visit your home to try to find you. They will try to reach you through the contact people that you provided. If they talk to these people, they will not tell them why they are trying to reach you. If you have a cellular phone, study staff may ask if they can use this number to contact you.
Your visit should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

Some of the specimens collected may be stored. You will be asked to sign a separate consent form to indicate whether you agree to allow the team to store you specimens.

**BENEFITS**

There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like microbicides and vaccine trials, and the effectiveness of HIV risk reduction and prevention messages.

However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be treated on site or referred to relevant public health clinic for treatment. You will receive free condoms and information on how to use condoms properly.

**PARTICIPANT RESPONSIBILITY**

By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinics. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study, please tell the study nurse or doctor. We will encourage you to come to the clinic for a final exit interview and to receive any test results that you may not have received yet.

**REIMBURSEMENT**

You will be compensated for your time, travel and inconvenience. If your visit is 3 hours or less you will be given R200. Clinic visits may take up most of your day. If you have to be at the clinic for the day you will be provided with food and refreshments for the time that you are at the clinic.

**RISKS/DISCOMFORT**

Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals. Although you may choose to be involved in a study of HIV/AIDS medicines, these studies are also researching the effectiveness of medicines and taking those medicines would have their own risks.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of genital specimens are pain and discomfort.

Being HIV positive can be a very stressful experience. In addition to this, you may be treated badly by friends and family if your HIV status becomes known to others. You may find it difficult to find work should others learn of your HIV status, and you may find that members of your family or community discriminate against you.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

**NEW FINDINGS**

You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.
REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from the study without your consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION
There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about.

COSTS OF THE STUDY
There will be no financial cost to you for participation in this study.

CONFIDENTIALITY
Research records of your participation in the study will be kept confidential and we will not give this information to anyone, unless required by law to do so. We will make every effort to protect your privacy and confidentiality. Your visits will take place in private. However, it is possible that others may learn that you have been here and assume that you are HIV-infected. Because of this, others may treat you differently or discriminate against you.

Medical records that identify you by name may be inspected by the research team who are responsible for keeping information for this study. In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission.

No publications based on this research will contain your name. However, in some instances we may request additional consent from you, if there is a possibility that you may be identified in a publication by description of your characteristics. Only the project investigators and clinic coordinators will know both your name and your identification number. This is necessary so that the coordinators can ensure that you are called in to the correct clinics. These investigators will not release your number and name to anyone else on the research team.

RESEARCHRELATED INJURIES
It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred for treatment. The cost of this treatment will be free. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form.

In the event of a research related injury, contact during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,
Hlengiwe Shozi (eThekwini Site) 031 2601943,
Duduzile Nkosi (Vulindlela Site) 033 2606863, or
Dr Nigel Garrett (Project Manager) 031 2604453

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS
If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,
Hlengiwe Shozi (eThekwini Site) 031 2601943,
Duduzile Nkosi (Vulindlela Site) 033 2606863, or
Dr Nigel Garrett (Project Manager) 031 2604453

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):
If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.

- I acknowledge that I have been informed by the clinic staff and doctor concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.

- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.

- I agree that the study will be conducted under the supervision of: Dr Garrett and Professor SS Abdool Karim.

- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.

- I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Participant
Signed: __________________________ Date: ____________
Participant

Name: __________________________________________
Researcher
Signed: __________________________ Date: ____________
Researcher

Name: __________________________ Date: ____________
Witness
Signed: __________________________ Date: ____________
Witness
For illiterate participants:

Mark with an ‘X’: ___________________________ Date: ________

Independent Witness: ______________________ Date: ________

Title and Name: ____________________________

Was a copy of the signed copy given to the study participant:       Yes ☐      No ☐

If no, why not:

_________________________________________________________________________________________
_________________________________________________________________________________________
_________________________________________________________________________________________

ADOLESCENT CONSENT FOR SPECIMEN STORAGE FOR POSSIBLE FUTURE RESEARCH
(PHASE II-IV)
VERSION 1.0, 13 OCTOBER 2017

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 260 1611 (Fatima Sayed)
033 260 6863 (Duduzile Nkosi)
031 260 4453 (Nigel Garrett)

INTRODUCTION
You have been enrolled into a study named above. While you are taking part in this study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This assent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to note this on the consent form. You will be given a copy of this form to keep. The results of tests on your stored samples will not usually be made known to you, and the researchers do not intend sharing this information with anyone else. If the researchers believe that information from tests on your stored material is important, they will make this available to you through your regular doctor. Please make sure that you update your contact information with the study staff so that they can contact you if the need arises.

Please note that since you are younger than 18 years of age, we would like your parent or guardian to agree to the storage of your specimens. Your parent or guardian has been given a similar consent form to read and sign. Even if your parent/legal guardian agrees to the storage of your specimens we will still require you to agree to the storage of your specimens.

BLOOD AND BIOLOGICAL SAMPLES
At your clinic visits, blood and other biological samples (vaginal swabs and urine) will be taken from you by study staff. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a confidential Participant Identification (PID) number and not your name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES
The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your samples may be analyzed in laboratories outside of South Africa. Your samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify the study doctor who will try to contact you or your regular doctor.
If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this kind of test result, you need to provide the study team with the contact details of your regular doctor.

**STORAGE OF SAMPLES**
If you agree to have your samples stored they will be stored with your confidential PID number at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is a possibility that your stored samples may be shipped and analysed overseas at specialized laboratories if a test is unavailable locally. There is no time limit on how long your samples may be stored.

**BENEFITS**
There is no direct benefit to you through having your samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

**RISKS**
There is very little risk to you when you have your samples stored. There is a small risk that others may find out information about your HIV status from stored samples. This risk is reduced as your sample is stored under a confidential PID number, and not your name. You are entitled to the same protections of confidentiality and privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from these tests may cause distress to you.

Some genetic testing may be done on your stored samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

**CONFIDENTIALITY**
The results of future tests of your samples will not go onto your medical record. Although every effort is made to make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your personal information may be disclosed. Medical records that identify you by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.

**PARTICIPANT RIGHTS**
The decision to allow your samples to be stored is completely voluntary. You may decide not to allow your samples to be stored after the tests that are needed for this study have been done or you decide to stop participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your samples to be stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, “Withdrawal of Consent” so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your samples.

**PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS**
If you have any questions about the storage of samples for this study, or would like to know more about the storage of blood, please call either of the following during office hours (8am-5pm):
You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone:  +27 (0)-31-260-4769 / 260-4553
Fax:  +27 (0)-31-260-4609
Email:  brec@ukzn.ac.za

SIGNATURES

Please read the statement below and think about your choice. No matter what you decide, it will not affect your care or participation in the CAPRISA 002 Study.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_______ Yes
_______ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Volunteer

Signed: ____________________________ Date: ___________
Volunteer

Name: __________________________________________
Researcher

Signed: ____________________________ Date: ___________
Researcher

Name: __________________________________________ Date: ___________
Witness

Signed: ____________________________ Date: ___________
Witness
For illiterate participants:

Mark with an ‘X’: _______________________________ Date:_________

Independent Witness: _______________________________ Date:_________

Title and Name: _______________________________

Was a copy of the signed copy given to the study participant: Yes ☐ No ☐
If no, why not:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Withdrawal of Consent

I hereby withdraw my consent for the storage of my biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed: _______________________________ Date:_________
Volunteer

Signed: _______________________________ Date:_________
Researcher

For illiterate subjects:

Mark with an ‘X’: _______________________________ Date:_________

Independent Witness: _______________________________ Date:_________

Title and Name: _______________________________
If the participant is younger than 18 years of age, this administrative section must be completed with the participant’s parent/guardian prior to the participant enrolling into the study.

1. Who has provided consent for this learner to participate in this study:
   - Parent
   - Legal Guardian

   If consent has not been given to participate in this study, please record reason why consent was not given:

2. Has the parent/guardian completed the literacy assessment?  □ Yes  □ No
3. Does the parent/guardian require an impartial witness?  □ Yes  □ No

Completed by: __________________________

Staff Note: If the parent/guardian cannot read, the consent form must be read to the parent/guardian exactly as written, in the parent/guardian’s language of choice, and a witness must sign this form to confirm that the correct information was given to the parent/guardian and that the parent/guardian freely consents to be in this study.
CAPRISA 002: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

PARENT/GUARDIAN INFORMED CONSENT FORM FOR ADOLESCENT ENROLMENT
(PHASE II-IV)
VERSION 1.0, 13 OCTOBER 2017

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 260 1611 (Fathima Sayed)
(Office hours 8am-5pm) 033 260 6863 (Duduzile Nkosi)
031 260 4453 (Nigel Garrett)

INTRODUCTION:
Your child/ward has been approached to join the CAPRISA 002 study. This is a study for people who have recently been infected with HIV. This document gives you information about the study that will be discussed with your child/ward by the study doctor or nurses. You are asked to read (or to have read to you) this consent form in a language of your choice. Once you understand the study and if you agree for your child/ward to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. This consent form may contain words or terms that you don’t understand. Please ask us to explain anything you may not understand. To be sure you understand the contents of this form we will ask you a few questions after you have read this consent form or have had the consent form read to you. If you decide to allow your child/ward to join this study, we will ask you to sign this form. We will give you a copy of this form to keep or if you prefer we can keep this form for you.

Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree for your child/ward to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Scientists associated with the University of KwaZulu-Natal’s Nelson Mandela School of Medicine are doing this study. We hope to enroll 300 subjects, from sites in and around Durban and Vulindlela. Your child/ward will be asked to take part for as long as he/she is willing and able to. The follow-up visits for this study will take place at the eThekwini and Vulindlela Clinical Research Sites. Each study visit will last approximately 3 hours.

By taking part in this study, your child/ward is contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

OTHER RESEARCH STUDIES
If your child/ward has been referred from other research studies, it will be necessary for us to look at their HIV test results from previous HIV testing. By signing this form, you are agreeing to your child/ward’s HIV test records being reviewed and information collected about their previous HIV test results.

YOUR CHILD/WARD’S PARTICIPATION IS VOLUNTARY
Before you learn more about this study it is important that you know the following:

• Please note that since your child/ward is younger than 18 years of age, we require your permission to allow your child/ward to participate in this study. Your child/ward will be given a similar consent form to read and sign.
• Your child/ward’s participation is entirely voluntary.
• You may decide not to allow your child/ward to take part in the study, or to withdraw from the study at any time, without losing the benefits of their routine medical care.
• If you decide not to allow your child/ward to take part in this study, he/she can still join another study later, if one is available and they qualify.
• Your child/ward can agree to take part in this study at a later date, but prior to study completion.
The rest of this consent form will describe to you the purpose of CAPRISA 002 study, when each study procedure will take place, procedures for contacting your child/ward if necessary, the risks and benefits of participating in this study, and your child/ward’s rights as a study participant.

This study has been reviewed and approved by the University of KwaZulu-Natal’s Biomedical Research Ethics Committee (BREC).

PURPOSE OF THE STUDY
This study will look at several important elements of the spread of HIV in South Africa. HIV is the virus that causes AIDS. After initial infection, the level of virus measured in the blood is usually high, and then it drops down over time often reaching a point where it does not change much over time. The amount of virus that can be measured in the blood is referred to as the viral load. This study aims to better understand the relationship between the viral load over time and disease progression in HIV infection in South Africa. That is, we are trying to find out whether the amount of virus that can be measured at certain times (at 6, 12 and 18 months) is related to how quickly someone with HIV infection develops AIDS illnesses. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people’s thoughts, feelings and behaviors.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype ‘C’ is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections in the developed world, there is limited information available to determine what influences the viral load in subtype C infections.

Since the point at which the viral load stabilizes is currently the best indication of progression to illness and AIDS, the results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV and AIDS. This study will also provide important information for future treatment interventions, i.e. interventions aimed at preventing AIDS after HIV has occurred.

Your child/ward has been referred to this study because he/she is known to have been recently infected with HIV. By taking part in this study your child/ward is contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your child/ward’s own situation directly. That is, it may not provide a cure or alter the course of his/her illness. However, your child/ward will be monitored closely and referred for care and treatment relating to any HIV-related illness that he/she may develop.

As people become ill after HIV infection, their blood cells known as CD4+ T cells (protective cells) become reduced. This is an indication of the immune system’s failure to control infection. Most infections occur when the level of these cells drop below 200 copies/ml. At this time people can become sick very easily.

PROCEDURES
Entering the Study
At this visit, you and your child/ward will be able to read, discuss and sign consent forms. If you and your child/ward decide to sign, the study staff will ask your child/ward where he/she lives and other questions about him/her, his/her health and sexual practices.

Your child/ward will also have a physical examination done, which will include looking for evidence of sexually transmitted infections (STIs) and the presence of any AIDS related illnesses. Your child/ward will be asked to give specimens for the testing of STIs. If she is female this could include a urine sample and swabs from her vagina. The study staff will talk to your child/ward about the infections that are passed on during sex. If your child/ward has clinical signs and symptoms of STIs, he/she will be treated as soon as possible after diagnosis or may be referred to a public health clinic where he/she will be given medicine to treat those infections.

Your child/ward will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). Your child/ward will be asked to give around 90 ml of blood (around 15 tubes) at his/her first visit. The blood will be used for tests that look at the way in which your child/ward’s body is responding to HIV infection. It will also be used to look at the virus that he/she have been infected with. Some of this blood together with a urine specimen, will be used to check on their general health. If your child/ward is female and she or the study doctor suspects that she is pregnant, this sample will be checked for signs of pregnancy. If your child/ward is
pregnant, she will be encouraged to stay in the study. She will also be referred to her nearest public health clinic for care relating to her pregnancy.

Your child/ward will be asked questions about their sexual behavior, general health as well as other questions related to their lifestyle. He/she will also be evaluated for the presence of any AIDS related illnesses.

**During the Study**
The study nurse will notify your child/ward of their scheduled clinic visits. The number of times that he/she will be called in to the clinic will vary over time. Your child/ward may leave the study at any time.

At first your child/ward will be asked to come in approximately every month for six months. After this, you will come in every alternate month until one year after starting, and then once every three months until he/she decide to start ART. At these visits, your child/ward will also be given a medical examination by one of the study nurses or doctors. Your child/ward will be asked to give tubes of blood at each visit. The amount of blood varies between 8 and 15 tubes. The blood will be used to see how your child/ward's body is responding to HIV infection, and to check on the type of virus that he/she have been infected with. The virus that infects your child/ward, changes over time, and we want to study the way in which the virus changes.

Once a year your child/ward will be tested for any STIs. This will be done by testing their blood and by taking a vaginal swab and urine specimen. Your child/ward will be treated or referred for treatment for any STIs that he/she may have. Your child/ward will be asked questions about his/her sexual behavior, general health as well as other questions related to their lifestyle.

People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity. These are known as 'opportunistic infections'. Any opportunistic infections that your child/ward may develop will be detected at the scheduled clinic visits and he/she will be treated or referred to the relevant public health clinic where he/she will receive treatment for any treatable opportunistic infections. Your child's/ward's body has cells in it that help you fight off infection and these helper cells are called CD4+ T cells. The level of CD4+ cells in your child's/ward's body can be an indication of severity of disease. Your child/ward is encouraged to start treatment for HIV as soon as he/she is ready, but latest when his/her immune system weakens indicated by a CD4 count of less than 500 cells/mm³. Treatment will be initiated at the eThekwini or Vulindlela sites and your child/ward may be referred to his/her closest ART treatment site 6 months after initiation of ART or when his/her viral load is undetectable. Your child/ward will be given the opportunity to participate (if eligible) in any antiretroviral drug therapy trial or treatment programmes available. However, your child/ward's participation in our study, is no guarantee that he/she will participate in such other programmes or trials. Your child/ward will be told about the care and treatment that he/she will receive at the clinic by the clinic staff. Your child/ward will also be able to get counseling on how to stay as healthy as possible and how to get any services that he/she may be eligible to receive.

Your child/ward will be asked to tell the study staff about any medical problems he/she may have during the study, especially those related to their genitals or to STIs. Your child/ward must contact the study staff between their regularly scheduled visits if you need to report these problems. The study staff will examine you and they will treat or refer you to a clinic for medical care, if needed.

If your child/ward is female and becomes pregnant during this study she will be encouraged to stay in the study. The study staff will be able to refer her to available sources of medical care and other services she and her baby may need.

At each visit the study staff will update information on where your child/ward lives and how to keep in contact with him/her. They will use this information to remind your child/ward of their scheduled visits. If your child/ward misses a visit, the study staff will try to contact him/her to find out why he/she missed a visit. They may also visit your home to try to find your child/ward. They will try to reach your child/ward through the contact people that he/she provided. If they talk to these people, they will not tell them why they are trying to reach your child/ward. If your child/ward has a cellular phone, study staff may ask if they can use this number to contact him/her.

Your child/ward’s visit should not last more than three hours. Your child/ward will usually receive his/her results at their next scheduled visit. Should the study team feel that your child/ward needs to receive results sooner he/she will be contacted to come to the clinic. For this reason, it is important to make sure that your child/ward’s contact details are up-to-date and that he/she has indicated how he/she would prefer to be contacted.
Some of the specimens collected may be stored. Your child/ward will be asked to sign a separate consent form to indicate whether he/she agrees to allow the team to store his/her specimens.

**BENEFITS**
There may be no direct benefits to your child/ward by participating in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like vaccines.

However, your child/ward may benefit from the advice of the research team and/or the test results he/she will receive as a result of their participation in the study. For example, he/she will receive HIV/STI prevention education. This may help him/her protect themselves and others from sexually transmitted illnesses. If your child/ward contracts a treatable STI during the course of this study, he/she will be treated or referred to a relevant public health clinic for treatment. Your child/ward will receive free condoms and information on how to use condoms properly.

**PARTICIPANT RESPONSIBILITY**
By signing this informed consent form, you are allowing your child/ward to make themselves available to attend the scheduled clinic visits. You are not, however, giving up their right to freely withdraw from this study at any time. If your child/ward does decide to withdraw from the study, he/she needs to please tell the study nurse or doctor.

**REIMBURSEMENT**
Your child/ward will be compensated for their time, travel and inconvenience. If their visit is 3 hours or less he/she will be given R200. Clinic visits may take up most of his/her day. If your child/ward has to be at the clinic for the day he/she will be provided with food and refreshments for the time that they are at the clinic.

**RISKS/DISCOMFORT**
Your child/ward’s participation in this study is for research purposes. His/her involvement does not mean that they will receive special medicines or treatments that other people cannot get from clinics or hospitals.

Your child/ward may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. He/she may have bruising at the site of the blood draw. There is a small chance that your child/ward may get an infection in the site of the specimen draws. The risks of collection of genital specimens (swab from their vagina) are pain and discomfort.

Some genetic testing may be done on your child/ward’s samples. The greatest risk is to their privacy. It is possible that if others found out information about your child/ward that is learned from these tests (such as information about their genes), it could cause problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify your child/ward by name and they do not become part of your medical records.

**NEW FINDINGS**
Your child/ward will be told of any new information learned during this study that might cause him/her to change their mind about staying in the study. Your child/ward will be told when the results of the study may be available, and how to learn about them.
REASONS WHY YOUR CHILD/WARD MAY BE WITHDRAWN FROM THE STUDY WITHOUT THEIR CONSENT

Your child/ward may be removed from the study without their consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to your child/ward.
- Your child/ward is not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION

There may be other HIV research studies going on at the clinic or in your community that your child/ward may be eligible for. We will tell him/her about the other studies that we know about.

COSTS OF THE STUDY

There will be no financial cost to you or your child/ward for participation in this study.

CONFIDENTIALITY

Research records of your child/ward’s participation in the study will be kept confidential and we will not give this information to anyone, unless required to do so by law.

We will make every effort to protect their privacy and confidentiality. Your child/ward’s visits will take place in private. However, it is possible that others may learn that your child/ward has been here and assume that he/she is HIV-infected. Because of this, others may treat them differently or discriminate against them.

Medical records that identify your child/ward by name may be inspected by the research team who are responsible for keeping information for this study.

In order to protect your child/ward’s right to confidentiality, he/she will be assigned a code number. This will be used to identify them, rather than their name. Your child/ward’s personal information will not be released without their permission. No publications based on this research will contain your child/ward’s name. However, in some instances we may request additional consent from you both, if there is a possibility that your child/ward may be identified in a publication by description of their characteristics. Only the project investigators and clinic coordinators will know both their name and their identification number. This is necessary so that the coordinators can ensure that your child/ward is called in to the correct clinics. These investigators will not release your child/ward’s number and name to anyone else on the research team.

RESEARCH RELATED INJURIES

It is very unlikely that your child/ward will become injured as a result of involvement in this study. However, in the case of a research related injury, he/she will be referred for treatment. The cost of this treatment will be free. There is, however, no compensation provided for research related injuries. Your child/ward does not give up any legal rights by signing the consent form. In the event of a research related injury, contact during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,  
Hlengiwe Shozi (eThekwini Site) 031 2601943,  
Duduzile Nkosi (Vulindlela Site) 033 2606863, or  
Dr Nigel Garrett (Project Manager) 031 2604453

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you have any questions about your child/ward’s involvement in this study, or would like to know more about the study, please call either of the following during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,  
Hlengiwe Shozi (eThekwini Site) 031 2601943,  
Duduzile Nkosi (Vulindlela Site) 033 2606863, or  
Dr Nigel Garrett (Project Manager) 031 2604453
You are not giving up your child’s/ward’s legal rights by signing the informed consent document. If you have any questions about your rights as a parent of research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

SIGNATURE:
If you have read the informed consent (or if you have had it read to you) and understand the information, and you voluntarily agree for your child/ward to join this study please sign your name on the informed consent form signature page. This will assist us to verify that the participant has your permission to participate in this study.

PARENT/GUARDIAN SIGNATURE PAGE:
If you have read the informed consent (or if you have had it read to you) and understand the information, and you voluntarily agree for your child/ward to join this study please sign your name below.

_________________________________________  ___________________________  ___________________________
Parent/Guardian name  Parent/Guardian signature  Date
(print name as it appears in ID book/Birth certificate)

_________________________________________  ___________________________  ___________________________
Name of staff member who administered consent (print)  Staff member’s signature  Date

_________________________________________  ___________________________  ___________________________
Witness’ name (print)  Witness’ signature  Date

For illiterate subjects:

Mark with an ‘X’: ____________________________________________________________________________ Date:________

Independent Witness: ________________________________________________________________________ Date:________

Title and Name: _____________________________________________________________________________

Was a copy of the signed copy given to the parent/guardian:  ☐ Yes  ☐ No

If no, why not:

___________________________________________________________________________________________
___________________________________________________________________________________________
___________________________________________________________________________________________

PARENT/GUARDIAN CONSENT FOR SPECIMEN STORAGE FOR POSSIBLE FUTURE RESEARCH
(PHASE II-IV)
VERSION 1.0, OCTOBER 2017

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 260 1611 (Fathima Sayed)
(Office hours 8am-5pm) 033 260 6863 (Duduzile Nkosi)
031 260 4453 (Nigel Garrett)

INTRODUCTION
You have agreed for your child/ward to take part in a study named above. There may be some remaining blood and biological specimens taken from your child/ward during this study that might be useful for future research. You are being asked to agree to the storage of your child/ward’s blood, vaginal specimens and urine for possible future research that will include additional testing. This is research that will be conducted in the future that may or may not be related to the study.

This consent form gives you information about the collection, storage, and use of your child/ward’s blood and urine for possible future research. The study staff will talk to you about this information. Please ask if you have any questions. If you agree to the storage of your child/ward’s blood and urine for possible future research, you will be asked to note this on this consent form. You will get a copy of this form to keep. It is important that you know the following:

- Your child/ward does not have to agree to have their specimens stored if you don’t want them to OR if they do not want to.
- Please note that since your child/ward is younger than 18 years of age, we would like your permission to allow for your child/ward’s specimens to be stored. Your child/ward has been given a similar assent form to read and sign. If you agree for your child/ward's specimens to be stored we will still require their agreement for storage.

HOW WILL YOU GET THE BLOOD AND URINE FROM MY CHILD/WARD?
The CAPRISA 002 study staff will collect your child/ward’s blood and other biological samples (vaginal swabs and urine) as part of the CAPRISA 002 study that you have consented your child/ward to participate in. This blood, urine and vaginal specimens is needed to carry out the regular tests for the CAPRISA 002 research study. If you agree to have your child/ward’s specimens stored for possible future research, we will store the remainder of this blood, vaginal and urine specimens after the tests for the CAPRISA 002 have been completed.

As with your child/ward’s other samples, only a confidential Participant Identification (PID) number and not your child/ward’s name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

HOW WILL YOU USE MY CHILD/WARD’S STORED BLOOD AND URINE?
The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your child/ward’s cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your child/ward’s samples may be analyzed in laboratories outside of South Africa. Your child/ward’s samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.
The researchers do not plan to contact you or your child/ward's regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your child's/ward's health, the researchers will notify the study doctor who will try to contact your child/ward or your child's/ward's regular doctor. If you or your child/ward wish to be notified of this type of test result, you or your child/ward need to make sure that you contact the study nurse or doctor with any changes to your child’s/ward’s phone number or address. If you want your child's/ward's regular doctor to be told about this kind of test result, you or your child/ward need to provide the study team with the contact details of your regular doctor.

WHERE WILL MY CHILD/WARD’S BLOOD AND URINE BE STORED?
If you agree to have your child/ward’s samples stored they will be stored with your child/ward’s confidential PID number at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is a possibility that your child’s/wards stored samples may be shipped and analysed overseas at specialized laboratories if a test is unavailable locally. There is no time limit on how long your samples may be stored.

HOW LONG WILL YOU KEEP MY CHILD/WARD’S BLOOD AND URINE?
There is no time limit on how long your child/ward’s blood and urine will be stored.

DOES STORAGE OF MY CHILD/WARD’S BLOOD, VAGINAL AND URINE SPECIMENS BENEFIT MY CHILD/WARD?
There is no direct benefit to you or your child/ward through having his/her samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

WHAT ARE THE RISKS FOR MY CHILD/WARD?
There is very little risk to your child/ward when he/she has his/her samples stored. There is a small risk that others may find out information about his/her HIV status from stored samples. This risk is reduced as his/her sample is stored under a confidential PID number, and not his/her name. He/she is entitled to the same protections of confidentiality and privacy for stored samples as he/she is for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your child’s/ward’s health we cannot currently test for. Results from these tests may cause distress to your child/ward.

Some genetic testing may be done on your child/ward’s stored samples. The greatest risk is to your child/ward’s privacy. It is possible that if others found out information about your child/ward’s that is learned from these tests (such as information about your genes), it could cause him/her problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify your child/ward by name and they do not become part of your child's/ward’s medical records.

WHAT ABOUT CONFIDENTIALITY?
The results of future tests of your child/ward’s samples will not go onto your child's/ward's medical record. Although every effort is made to make sure that your child/ward’s samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your child’s/ward’s personal information may be disclosed. Medical records that identify your child/ward by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.
WHAT ARE MY CHILD/WARD’S RIGHTS?
The decision to allow your child/ward’s samples to be stored is completely voluntary. You may decide not to allow your child/ward’s samples to be stored after the tests that are needed for this study have been done or if your child/ward decides to stop participating in the study. If you do decide to allow your child/ward’s samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your child/ward’s samples to be stored any more. If you decide to do this, your child/ward’s samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, “Withdrawal of Consent” so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your child’s/ward’s samples.

WHAT DO I DO IF I HAVE QUESTIONS?
If you have any questions about the storage of your child’s/ward’s samples for this study, or would like to know more about the storage of blood, please call either of the following during office hours (8am-5pm):

eThekwini Site: Fathima Sayed 031 260 1611
   Hlengi Shozi 031 260 1943
Vulindlela Site: Duduzile Nkosi 033 260 6863
Project Director: Nigel Garrett 031 260 4453

You are not giving up your child’s/ward’s legal rights by signing the informed consent document. If you have any questions about your rights as a parent of research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

PARENT/GUARDIAN SIGNATURE PAGE:
Please read the statement below and think about your choice. No matter what you decide, it will not affect your child’s/ward’s care or participation in the CAPRISA 002 Study.

I agree to allow some of my child/ward’s biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_______ Yes
_______ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Parent/Guardian
Signed: ____________________________ Date: __________

Name: __________________________________________
Researcher
Signed: ____________________________ Date: __________
Name: _______________________________ Date: ________________
Witness

Signed: _______________________________ Date: ________________
Witness

For illiterate subjects:

Mark with an ‘X’: _______________________________ Date: ____________

Independent Witness: _______________________________ Date: ____________

Title and Name: ________________________________

Was a copy of the signed copy given to the parent/guardian: Yes ☐ No ☐
If no, why not:
________________________________________________________
________________________________________________________

Withdrawal of Consent

I hereby withdraw my consent for the storage of my child’s/ward’s biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed: _______________________________ Date: ________________
Volunteer

Signed: _______________________________ Date: ________________
Researcher

For illiterate subjects:

Mark with an ‘X’: _______________________________ Date: ____________

Independent Witness: _______________________________ Date: ____________

Title and Name: ________________________________
9. Informed Consent form (Enrolment: Phase V – Antiretroviral therapy) – V4.00

Title of the Research: CAPRISA 002: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

Principal Investigator: Professor Salim Abdool Karim University of KwaZulu-Natal King George V Avenue Durban 4001

Telephone No: Office hours (8am-5pm): 031 260 1611 (Fathima Sayed) 033 260 6863 (Duduzile Nkosi) 031 260 4453 (Dr. Nigel Garrett) – Project Director

INTRODUCTION
You are being asked to take part in a study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal’s Nelson Mandela School of Medicine are doing this study. Over the period of a few years, we hope to enroll all participants from the CAPRISA Acute Infection study and other CAPRISA studies who require or have now started antiretroviral treatment (ART). You will be asked to take part for as long as you are willing and able after starting treatment, for a minimum of 5 years and thereafter annual follow-up for a further 15 years will be optional. The follow-up visits for this study will take place at the CAPRISA eThekwini and Vulindlela Clinical Research Sites. Each visit will last approximately 3 hours.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

INFORMED CONSENT
You are being asked to volunteer for the study named above. This is a study for people who were taking part in the CAPRISA Acute Infection study, tenofovir gel or tenofovir gel implementation studies or other CAPRISA studies and who require or have now started antiretroviral treatment (ART). You will be asked to take part for as long as you are willing and able after starting treatment, for a minimum of 5 years and thereafter annual follow-up for a further 15 years will be optional. The follow-up visits for this study will take place at the CAPRISA eThekwini and Vulindlela Clinical Research Sites. Each visit will last approximately 3 hours.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

YOUR PARTICIPATION IS VOLUNTARY
Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.
- If you decide not to take part in this study, you can still join another study later, if one is available and you qualify.

PURPOSE OF THE STUDY
This study will look at several important elements of HIV in South Africa. HIV is the virus that causes AIDS. During HIV infection, the CD4+ cells in your blood are destroyed by the virus, and that is why you get sick and need to go onto treatment. HIV also attacks other cells, making them tired from fighting the virus in your blood all the time. Antiretroviral treatment blocks the virus from multiplying in your blood, and so your CD4+ cells can recover again.
In this study we will try to find out if other parts of your immune system can also be ‘rebalanced’ to normal and become functional again when the virus is no longer multiplying in your blood, and how long this takes to happen.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype ‘C’ is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections, and people with subtype B infection that go onto treatment in the developed world, there is limited information available about people infected with subtype C, and their immune system functioning when they go onto treatment.

This study could give us important information about how much an immune system that is damaged by HIV can recover when antiretroviral therapy is taken. For the participants who were enrolled in the tenofovir gel study or the tenofovir gel implementation study, we also want look at whether resistance to Tenofovir occurs and how this impacts ART treatment. The results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV and AIDS.

As ART does not provide a permanent cure for HIV, we also want to study the latent reservoir which is a group of infected CD4 cells that are not actively producing HIV. Latent HIV reservoirs can be found in many places throughout the body, and HIV can hide out for years inside reservoirs. Latent HIV reservoirs can wake up and start making more HIV. If someone with HIV is not taking ART when this happens, the level of HIV in their blood or viral load will start to increase. We will define the size and sequence composition of the reservoir, as well as its dynamic origin and decay and other important characteristics which may tell us how we may eradicate HIV from infected cells that the ART cannot get to.

You have been referred to this study because you participated in either the CAPRISA Acute Infection study or another CAPRISA study and you have now started taking ART. By taking part in this study you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your own situation directly. That is, it will not provide a cure or alter the course of your illness. However, you will be monitored at each study visit and referred for care and treatment relating to any HIV-related illness that you may develop.

**PROcedures**

**Entering the Study**

At this visit, you will be able to read, discuss and sign this consent form. You will have a physical examination done, which will include looking for evidence of sexually transmitted infections (STIs). You will be asked to give urine or vaginal swab specimens for the testing of STIs. The study staff will talk to you about the infections that are passed on during sex. If you have clinical signs and symptoms of STIs, you will be treated as soon as possible or referred to a public health clinic where you will be given medicine to treat those infections.

You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give around 90-100 mls of blood (around 15 tubes) at each visit. The blood will be used for tests that look at the way in which your body is responding to HIV infection. It will also be used to look at the virus that you have been infected with. Some of this blood together with a urine specimen, will be used to check on your general health. If you are a woman and you or the study doctor suspect that you are pregnant, this sample will be checked for signs of pregnancy. If you are pregnant, you will be encouraged to stay in the study. You will also be referred to your nearest public health clinic for care relating to your pregnancy.

After two years and five years on ART, we will do a bigger blood draw of approximately 200mls, which is required for the latent reservoir (cure) studies. We will only collect this large amount of blood if you are clinically well as assessed by a nurse or doctor and your blood levels are within normal limits prior to the blood draw.

You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle. You will be asked questions about how often you take your antiretroviral treatment.

**During the Study**

The study nurse will notify you of your scheduled clinic visits. The number of times that you will be called in to the clinic will vary over time. You may leave the study at any time, but we would like to follow you up for a minimum of 5 years. After 5 years of follow-up you will be given the option to remain in the study for up to another 15 years with only annual follow-up.
You will be asked to come back after 1 month, 3 months and 6 months after starting therapy, and then every 6 months until you have reached 5 years on therapy. If it is more than 6 months since you started therapy, you will only come in every 6 months. At these visits, you will also be given a medical examination by one of the study doctors. You will be asked to give tubes of blood at each visit. The amount of blood will be around 90-100 mls (around 15 tubes). The blood will be used to see how your immune system responds while you are on treatment for HIV.

Once a year you will be tested for any STIs. This will be done by testing your blood, and by using vaginal swab sample and urine if you are a woman, or urine only if you are a man. You will be treated or referred for treatment for any STIs that you have. You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle.

People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity, even sometimes when they are on treatment. For any opportunistic infections that you may develop that are detected at the scheduled clinic visits, or for any problems that you have with your medication, you will be managed at the clinic or referred to your treatment provider at your public health clinic. You will also be able to get counselling on how to stay as healthy as possible and how to get any services that you may be eligible to receive. If you become pregnant during this study you will be encouraged to stay in the study. The study staff will be able to refer you to available sources of medical care and other services you and your baby may need.

At each visit the study staff will update information on where you live and how to keep in contact with you. They will use this information to remind you of your scheduled visits. If you miss a visit, the study staff will try to contact you to find out why you missed a visit. They may also visit your home to try and find you. They will try to reach you through the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you. If you have a cellular phone, study staff may ask if they can use this number to contact you.

Your visit should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

Some of the specimens collected may be stored. You will be asked to sign a separate consent form to indicate whether you agree to allow the team to store your specimens.

**BENEFITS**

There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like vaccines.

However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be treated or referred to a relevant public health clinic for treatment. You will receive free condoms and information on how to use condoms properly to reduce risk your risk of STIs.

**PARTICIPANT RESPONSIBILITY**

By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinic visits. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study please tell the study nurse or doctor.

**REIMBURSEMENT**

You will be compensated for your time, travel and inconvenience. If your visit is 3 hours or less you will be given R200. Clinic visits may take up most of your day. If you have to be at the clinic for the day you will be provided with food and refreshments for the time that you are at the clinic.
RISKS/DISCOMFORT
Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of genital specimens (swabs from your vagina) are pain and discomfort.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

NEW FINDINGS
You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT
You may be removed from the study without your consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION
There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about.

COSTS OF THE STUDY
There will be no financial cost to you for participation in this study.

CONFIDENTIALITY
Research records of your participation in the study will be kept confidential and we will not give this information to anyone, unless require by law to do so.

We will make every effort to protect your privacy and confidentiality. Your visits will take place in private. However, it is possible that others may learn that you have been here and assume that you are HIV-infected. Because of this, others may treat you differently or discriminate against you. Medical records that identify you by name may be inspected by the research team who are responsible for keeping information for this study.

In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission. No publications based on this research will contain your name. However, in some instances we may request additional consent from you, if there is a possibility that you may be identified in a publication by description of your characteristics. Only the project investigators and clinic coordinators will know both your name and your identification number. This is necessary so that the coordinators can ensure that you are called in to the correct clinics. These investigators will not release your number and name to anyone else on the research team.

RESEARCH RELATED INJURIES
It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred for treatment. The cost of this treatment will be free. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form. In the event of a research related injury, contact during office hours (8am-5pm):
PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS
If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following during office hours (8am-5pm):

Fathima Sayed (eThekwini Site)  031 260 1611
Hlengi Shozi (eThekwini Site)  031 260 1943
Duduzile Nkosi (Vulindlela Site)  033 260 6863
Dr. Nigel Garrett (Project Director)  031 260 4453

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone:  +27 (0)-31-260-4769 / 260-4553
Fax:  +27 (0)-31-260-4609
Email:  brec@ukzn.ac.za

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to participate in the study, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.
- I acknowledge that I have been informed by the clinic staff and doctor concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.
- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of Dr. Garrett and Prof Abdool Karim.
- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.
- I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________________________
Subject

Signed: __________________________________ Date: __________
Subject

Name: __________________________________________________________
Researcher

Signed: __________________________________ Date: __________
Researcher
For illiterate subjects:

Mark with an 'X': ___________________________ Date:__________

Independent Witness: ___________________________ Date:__________

Title and Name: ________________________________
10. Informed Consent: Specimen Storage: Phase V - Antiretroviral therapy – V4.00

Title of the Research: CAPRISA 002: VIRAL SET POINT AND CLINICAL PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No:
Office hours (8am-5pm): 031 260 1611 (Fathima Sayed)
033 260 6863 (Duduzile Nkosi)
031 260 4453 (Dr. Nigel Garrett) – Project Director

INTRODUCTION
You have been enrolled into a research study. While you are taking part in this study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This consent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to sign this consent form. You will be given a copy to keep. The results of tests on your stored samples will not usually be made known to you, and the researchers do not intend sharing this information with anyone else. If the researchers believe that information from tests on your stored material is important, they will make this available to you through your regular doctor. Please make sure that you update your contact information with the study staff so that they can contact you if the need arises.

BLOOD AND BIOLOGICAL SAMPLES
At your clinic visits, blood and other biological samples (vaginal swab, urine) will be taken from you. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a number and not your name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES
The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your samples may be analyzed in laboratories outside of South Africa. Your samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify the study doctor who will try to contact you or your regular doctor. If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this kind of test result, you need to provide the study team with the contact details of your regular doctor.

STORAGE OF SAMPLES
Your samples will be stored at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is no time limit on how long your samples may be stored.
**BENEFITS**
There is no direct benefit to you through having your samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

**RISKS**
There is very little risk to you when you have your samples stored. There is a small risk that others may find out information about your HIV status from stored samples. This risk is reduced as your sample is stored under a study number, and not your name. You are entitled to the same protections of confidentiality and privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from these tests may cause distress to you.

Some genetic testing may be done on your stored samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

**CONFIDENTIALITY**
The results of future tests of your samples will not go onto your medical record. Although every effort is made to make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your personal information may be disclosed.

Medical records that identify you by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.

**PARTICIPANT RIGHTS**
The decision to allow your samples to be stored is completely voluntary. You may decide not to allow your samples to be stored after the tests that are needed for this study have been done or you decide to stop participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your samples to be stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, “Withdrawal of Consent” so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your samples.

**PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS**
If you have any questions about the storage of samples for this study, or would like to know more about the storage of blood, please call either of the following during office hours (8am-5pm):

- eThekwini Site: Fathima Sayed 031 260 1611
- Hlengiwe Shoni 031 260 3033
- Vulindlela Site: Duduzile Nkosi 033 260 6863
- Project Director: Nigel Garrett 031 260 4555

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
email: brec@ukzn.ac.za
SIGNATURES
Please read the statement below and think about your choice. No matter what you decide, it will not affect your care.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_______ Yes
_______ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Subject
Signed: ________________________________________ Date: ________________
Subject

Name: __________________________________________
Researcher
Signed: ________________________________________ Date: ________________
Researcher

For illiterate subjects:

Mark with an ‘X’: ______________________________________ Date: ________________

Independent Witness: _____________________________ Date: ________________

Title and Name: ____________________________________
Withdrawal of Consent

I hereby withdraw my consent for the storage of my biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed:__________________________________________ Date:______________
Subject/Parent/Guardian

Signed:__________________________________________ Date:______________
Researcher

For illiterate subjects:

Mark with an ‘X’:________________________________ Date:______________

Independent Witness:________________________________ Date:____________

Title and Name:________________________________________
If the participant is younger than 18 years of age, this administrative section must be completed prior to completing the consent form for enrolment.

1. Has the participant’s age been verified?  Yes ☐ No ☐

1.1 If yes, indicate below how the participant’s age has been verified:
☐ Birth Certificate
☐ Identification Document (ID)
☐ Other: Specify:______________

2. Who has provided consent for this participant to participate in this study:
☐ Parent
☐ Legal Guardian

If consent has not been given to participate in this study, please record reason why consent was not given:_____________________________________

3. Has the participant completed the literacy assessment?  Yes ☐ No ☐

4. Does the participant require an impartial witness?  Yes ☐ No ☐

Completed by: __________________________

Staff Note: If the participant cannot read, the consent form must be read to the participant exactly as written, in the participant’s language of choice, and a witness must sign this form to confirm that the correct information was given to the participant and that the participant freely consents to be in this study.

If you have indicated NO to Question 1 or 3 above please do not proceed any further.
INTRODUCTION:
You are being asked to take part in a study named above. This document gives you information about the study that will be discussed with you by the study doctor or nurses. You are asked to read (or to have read to you) this consent form in a language of your choice. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or to make a mark in front of a witness. This consent form may contain words or terms that you don’t understand. Please ask us to explain anything you may not understand. To be sure you understand the contents of this form I will ask you a few questions after you have read this consent form or have had the consent form read to you. If you decide to join this study, we will ask you to sign this form. We will give you a copy of this form to keep or if you prefer we can keep this form for you.

Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

This is a study for people who were taking part in CAPRISA studies who require or have now started taking antiretroviral treatment (ART). Before you decide whether or not you would like to take part in the study, we will explain the study to you. You will learn what the study is about, what procedures will form a part of the study, what the risks and benefits of the study are to you, and what is expected of you as a research participant. Scientists associated with the University of KwaZulu-Natal's Nelson Mandela School of Medicine are doing this study. Over the period of a few years, we hope to enroll all participants from the CAPRISA Acute Infection (CAPRISA 002) study and other CAPRISA studies who require antiretroviral treatment (ART). You will be asked to take part for as long as you are willing and able after starting treatment, for a minimum of 5 years and thereafter annual follow-up for a further 15 years will be optional. The follow-up visits for this study will take place at the CAPRISA eThekwini and Vulindlela Clinical Research Sites. Each study visit will last approximately 3 hours.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

YOUR PARTICIPATION IS VOLUNTARY
Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.
- If you decide not to take part in this study, you can still join another study later, if one is available and you qualify.
- Please note that since you are younger than 18 years of age, we also require your parent or guardian to agree to your participation in this study. Your parent or guardian has been given a similar consent form to...
• If your parent/ guardian agrees to your participation in CAPRISA 002 we will still require you to agree to participate in this study.
• You can agree to take part in this study at a later date, but prior to study completion.

The rest of this consent form will describe to you the purpose of CAPRISA 002, when each study procedure will take place, procedures for contacting you if necessary, the risks and benefits of participating in this study, and your rights as a study participant.

This study has been reviewed and approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (BREC).

PURPOSE OF THE STUDY
This study will look at several important elements of HIV in South Africa. HIV is the virus that causes AIDS. During HIV infection, the CD4+ cells in your blood are destroyed by the virus, and that is why you get sick and need to go onto treatment. HIV also attacks other cells, making them tired from fighting the virus in your blood all the time. Antiretroviral treatment blocks the virus from multiplying in your blood, and so your CD4+ cells can recover again. In this study we will try to find out if other parts of your immune system can also be ‘rebalanced’ to normal and become functional again when the virus is no longer multiplying in your blood, and how long this takes to happen.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype ‘C’ is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections, and people with subtype B infection that go onto treatment in the developed world, there is limited information available about people infected with subtype C, and their immune system functioning when they go onto treatment.

This study could give us important information about how much an immune system that is damaged by HIV can recover when antiretroviral therapy is taken. The results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV and AIDS.

As ART does not provide a permanent cure for HIV, we also want to study the latent reservoir which is a group of infected CD4 cells that are not actively producing HIV. Latent HIV reservoirs can be found in many places throughout the body, and HIV can hide out for years inside reservoirs. Latent HIV reservoirs can wake up and start making more HIV. If someone with HIV is not taking ART when this happens, the level of HIV in their body or viral load will start to increase. We will define the size and composition of the reservoir, as well as its dynamic origin and decay and other important characteristics which may tell us how we may eradicate HIV from infected cells that the ART cannot get to.

You have been referred to this study because you participated in either the CAPRISA Acute Infection (CAPRISA 002) study, or another CAPRISA study and you have now started taking ART. By taking part in this study you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your own situation directly. That is, it will not provide a cure or alter the course of your illness. However, you will be monitored at each study visit and referred for care and treatment relating to any HIV-related illness that you may develop.

PROCEDURES
Entering the Study
At this visit, you will be able to read, discuss and sign this consent form. You will have a physical examination done, which will include looking for evidence of sexually transmitted infections (STIs). You will be asked to give urine or vaginal swab specimens for the testing of STIs. The study staff will talk to you about the infections that are passed on during sex. If you have clinical signs and symptoms of STIs, you will be treated as soon as possible or referred to a public health clinic where you will be given medicine to treat those infections.

You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give around 90 -100 mls of blood (around 15 tubes) at each visit. The blood will be used for tests that look at the way in which your body is responding to HIV infection.
It will also be used to look at the virus that you have been infected with. Some of this blood together with a urine specimen, will be used to check on your general health. If you are a woman and you or the study doctor suspect that you are pregnant, this sample will be checked for signs of pregnancy. If you are pregnant, you will be encouraged to stay in the study. You will also be referred to your nearest public health clinic for care relating to your pregnancy.

After two years and five years on ART, we will do a bigger blood draw of approximately 200mls, which is required for the latent reservoir (cure) studies. We will only collect this large amount of blood if you are clinically well as assessed by a nurse or doctor and your blood levels are within normal limits prior to the blood draw.

You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle. You will be asked questions about how often you take your antiretroviral treatment.

**During the Study**

The study nurse will notify you of your scheduled clinic visits. The number of times that you will be called in to the clinic will vary over time. You may leave the study at any time, but we would like to follow you up for a minimum of 5 years. After 5 years of follow-up you will be given the option to remain in the study for up to another 15 years with only yearly follow-up.

You will be asked to come back after one month, 3 months and 6 months after starting therapy, and then every 6 months until you have reached a minimum of 5 years on therapy. If it is more than 6 months since you started therapy, you will only come in every 6 months. At these visits, you will also be given a medical examination by one of the study doctors. You will be asked to give tubes of blood at each visit. The amount of blood will be around 90 – 100 mls (around 15 tubes). The blood will be used to see how your immune system responds while you are on treatment for HIV.

Once a year you will be tested for STIs. This will be done by testing your blood, and by using a vaginal swab sample and urine if you are a woman, or urine only if you are a man. You will be treated or referred for treatment for any STIs that you have. You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle.

People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity, even sometimes when they are on treatment. For any opportunistic infections that you may develop that are detected at the scheduled clinic visits or for any problems that you have with your medication, you will be managed at the clinic or referred to your treatment provider, at your public health clinic. You will also be able to get counseling on how to stay as healthy as possible and how to get any services that you may be eligible to receive.

At each visit the study staff will update information on where you live and how to keep in contact with you. They will use this information to remind you of your scheduled visits. If you miss a visit, the study staff will try to contact you to find out why you missed a visit. They may also visit your home to try and find you. They will try to reach you through the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you. If you have a cellular phone, study staff may ask if they can use this number to contact you.

Your visits should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

Some of the specimens collected may be stored. You will be asked to sign a separate consent form to indicate whether you agree to allow the team to store your specimens.

**BENEFITS**

There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like vaccines.
However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be treated or referred to a relevant public health clinic for treatment. You will receive free condoms, with information on how to use condoms properly.

**PARTICIPANT RESPONSIBILITY**

By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinic visits. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study, please tell the study nurse or doctor.

**REIMBURSEMENT**

You will be compensated for your time, travel and inconvenience. If your visit is 3 hours or less you will be given R200. Clinic visits may take up most of your day. If you have to be at the clinic for the day you will be provided with food and refreshments for the time that you are at the clinic.

**RISKS/DISCOMFORT**

Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of genital specimens (swab from your vagina) are pain and discomfort.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

**NEW FINDINGS**

You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.

**REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT**

You may be removed from the study without your consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

**ALTERNATIVES TO PARTICIPATION**

There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about.

**COSTS OF THE STUDY**

There will be no financial cost to you for participation in this study.

**CONFIDENTIALITY**

Research records of your participation in the study will be kept confidential and we will not give this information to anyone, unless required by law to do so. We will make every effort to protect your privacy and confidentiality. Your visits will take place in private. However, it is possible that others may learn that you have been here and assume that you are HIV-infected. Because of this, others may treat you differently or discriminate against you.
Medical records that identify you by name may be inspected by the research team who are responsible for keeping information for this study. In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission.

No publications based on this research will contain your name. However, in some instances we may request additional consent from you, if there is a possibility that you may be identified in a publication by description of your characteristics. Only the project investigators and clinic coordinators will know both your name and your identification number. This is necessary so that the coordinators can ensure that you are called in to the correct clinics. These investigators will not release your number and name to anyone else on the research team.

RESEARCH RELATED INJURIES
It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred for treatment. The cost of this treatment will be free. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form. In the event of a research related injury, contact during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,
Hlengiwe Shozi (eThekwini Site) 031 2601943,
Duduzile Nkosi (Vulindlela Site) 033 2606863, or
Dr Nigel Garrett (Project Manager) 031 2604453

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS
If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,
Hlengiwe Shozi (eThekwini Site) 031 2601943,
Duduzile Nkosi (Vulindlela Site) 033 2606863, or
Dr Nigel Garrett (Project Manager) 031 2604453

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building
University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

• I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.

• I acknowledge that I have been informed by the clinic staff and doctor concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.

• I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.

• I agree that the study will be conducted under the supervision of: Dr Garrett and Professor SS Abdool Karim.

• I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.
• I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: ________________________________
Participant

Signed: ____________________________ Date: __________
Participant

Name: ________________________________
Researcher

Signed: ____________________________ Date: __________
Researcher

Name: ________________________________ Date: __________
Witness

Signed: ____________________________ Date: __________
Witness

For illiterate participants:

Mark with an ‘X’: ________________________________ Date: __________

Independent Witness: ________________________________ Date: __________

Title and Name: ________________________________

Was a copy of the signed copy given to the volunteer: ☐ Yes ☐ No

If no, why not:

___________________________________________________________________________________________
___________________________________________________________________________________________
___________________________________________________________________________________________
INTRODUCTION
You have been enrolled into a study named above. While you are taking part in this study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This assent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to note this on the consent form. You will be given a copy of this form to keep. The results of tests on your stored samples will not usually be made known to you, and the researchers do not intend sharing this information with anyone else. If the researchers believe that information from tests on your stored material is important, they will make this available to you through your regular doctor. Please make sure that you update your contact information with the study staff so that they can contact you if the need arises.

Please note that since you are younger than 18 years of age, we would like your parent or guardian to agree to the storage of your specimens. Your parent or guardian has been given a similar consent form to read and sign. Even if your parent/legal guardian agrees to the storage of your specimens we will still require you to agree to the storage of your specimens.

BLOOD AND BIOLOGICAL SAMPLES
At your clinic visits, blood and other biological samples (vaginal swabs and urine) will be taken from you by study staff. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a confidential Participant Identification (PID) number and not your name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES
The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your samples may be analyzed in laboratories outside of South Africa. Your samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important
information for your health, the researchers will notify the study doctor who will try to contact you or your regular
doctor. If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse
or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this
kind of test result, you need to provide the study team with the contact details of your regular doctor.

**STORAGE OF SAMPLES**
If you agree to have your samples stored they will be stored with your confidential PID number at laboratories that
are specially designed to keep stored samples safely. Only approved researchers working on this project and
related projects will be able to access your samples. The people who work at these laboratories will have access
to your samples when they store them and keep track of them, but they will not know who you are as your
samples will be stored by number. There is a possibility that your stored samples may be shipped and analysed
overseas at specialized laboratories if a test is unavailable locally. There is no time limit on how long your samples
may be stored.

**BENEFITS**
There is no direct benefit to you through having your samples stored. The benefit is to the researchers as they will
be able to make sure that the procedures they are using are accurate and they may learn more about the HIV
virus from your samples.

**RISKS**
There is very little risk to you when you have your samples stored. There is a small risk that others may find out
information about your HIV status from stored samples. This risk is reduced as your sample is stored under a
confidential PID number, and not your name. You are entitled to the same protections of confidentiality and
privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that
tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from
these tests may cause distress to you.

Some genetic testing may be done on your stored samples. The greatest risk is to your privacy. It is possible that
if others found out information about you that is learned from these tests (such as information about your genes), it
could cause you problems with family members (having a family member learn about a disease that may be
passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This
risk is extremely small, because the test results do not identify you by name and they do not become part of your
medical records.

**CONFIDENTIALITY**
The results of future tests of your samples will not go onto your medical record. Although every effort is made to
make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute
confidentiality. If required to do so by law, your personal information may be disclosed. Medical records that
identify you by name may be inspected by representatives from the agency that is funding this study and by the
research team who is responsible for keeping information for this study.

**PARTICIPANT RIGHTS**
The decision to allow your samples to be stored is completely voluntary. You may decide not to allow your
samples to be stored after the tests that are needed for this study have been done or you decide to stop
participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any
time. You must contact the study doctor or nurse and let them know that you do not want your samples to be
stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you wish
all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will
then be asked to sign this form again under the section, “Withdrawal of Consent” so that there is a record of your
decision. At the end of the study you will also be given the opportunity to review your consent for the storage of
your samples.

**PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS**
If you have any questions about the storage of samples for this study, or would like to know more about the
storage of blood, please call either of the following during office hours (8am-5pm):
You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

SIGNATURES
Please read the statement below and think about your choice. No matter what you decide, it will not affect your care or participation in the CAPRISA 002 Study.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_______ Yes
_______ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: __________________________________________
Volunteer
Signed: ___________________________ Date: ____________
Volunteer

Name: __________________________________________
Researcher
Signed: ___________________________ Date: ____________
Researcher

Name: __________________________________________
Witness
Signed: ___________________________ Date: ____________
Witness
For illiterate subjects:

Mark with an ‘X’: ____________________________ Date: _________

Independent Witness: ____________________________ Date: _________

Title and Name: ________________________________

Was a copy of the signed copy given to the study participant:  Yes ☐ No ☐

If no, why not:

___________________________________________________________________________________________

Withdrawal of Consent

I hereby withdraw my consent for the storage of my biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed: ___________________________________ Date: ___________
Volunteer

Signed: ___________________________________ Date: ___________
Researcher

For illiterate subjects:

Mark with an ‘X’: ____________________________ Date: _________

Independent Witness: ____________________________ Date: _________

Title and Name: ________________________________
1. Who has provided consent for this learner to participate in this study:
   - [ ] Parent
   - [ ] Legal Guardian

   If consent has not been given to participate in this study, please record reason why consent was not given:
   ______________________________________________________
   ______________________________________________________

2. Has the parent/guardian completed the literacy assessment?  [ ] Yes  [ ] No

3. Does the parent/guardian require an impartial witness?  [ ] Yes  [ ] No

   Completed by: ______________________________

Staff Note: If the parent/guardian cannot read, the consent form must be read to the parent/guardian exactly as written, in the parent/guardian’s language of choice, and a witness must sign this form to confirm that the correct information was given to the parent/guardian and that the parent/guardian freely consents to be in this study.
INTRODUCTION:
Your child/ward has been approached to join the CAPRISA 002 study. We will talk to you today about what participating in this CAPRISA study will mean for your child/ward. We will be giving you information about this study and if you agree to allow your child/ward to be a part of this study, what we will expect from your child/ward and what your child/ward’s rights are. This information is to help you decide if you want to let your child/ward participate and undergo the procedures that will be required as part of their participation in this study.

This document gives you information about the study that will be discussed with your child/ward by the study doctor or nurses. You are asked to read (or to have read to you) this consent form in a language of your choice. Once you understand the study and if you agree to allow your child/ward to take part, you will be asked to sign the informed consent sheet, or to make a mark in front of a witness. This consent form may contain words or terms that you don’t understand. Please ask us to explain anything you may not understand. To be sure you understand the contents of this form I will ask you a few questions after you have read this consent form or have had the consent form read to you. If you decide to give your child/ward permission to join this study, we will ask you to sign this form. We will give you a copy of this form to keep or if you prefer we can keep this form for you.

Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to allow your child/ward to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

BACKGROUND:

This is a study for people who were taking part in CAPRISA studies who require or have now started taking antiretroviral treatment (ART). Before you decide whether or not you would like your child/ward to take part in the study, we will explain the study to you. You will learn what the study is about, what procedures will form a part of the study, what the risks and benefits of the study are to your child/ward, and what is expected of him/her as a research participant. Scientists associated with the University of KwaZulu-Natal’s Nelson Mandela School of Medicine are doing this study. Over the period of a few years, we hope to enroll all participants from the CAPRISA Acute Infection (CAPRISA 002) study and other CAPRISA studies who require antiretroviral treatment (ART). Your child/ward will be asked to take part for as long as he/she is willing and able after starting treatment, for a minimum of 5 years and thereafter annual follow-up for a further 15 years will be optional to him/her. The follow-up visits for this study will take place at the CAPRISA eThekwini and Vulindlela Clinical Research Sites. Each study visit will last approximately 3 hours.

By taking part in this study, your child/ward is contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.
YOUR CHILD/WARD’S PARTICIPATION IS VOLUNTARY
Before you learn more about this study it is important that you know the following:

- Please note that since your child/ward is younger than 18 years of age, we require your permission to allow your child/ward to participate in this study. Your child/ward will be given a similar consent form to read and sign.
- Your child/ward’s participation is entirely voluntary.
- You may decide not to allow your child/ward to take part in the study, or to withdraw from the study at any time, without losing the benefits of their routine medical care.
- If you decide not to allow your child/ward to take part in this study, he/she can still join another study later, if one is available and they qualify.
- Your child/ward can agree to take part in this study at a later date, but prior to study completion.

The rest of this consent form will describe to you the purpose of CAPRISA 002 study, when each study procedure will take place, procedures for contacting your child/ward if necessary, the risks and benefits of participating in this study, and your child/ward’s rights as a study participant.

This study has been reviewed and approved by the University of KwaZulu-Natal’s Biomedical Research Ethics Committee (BREC).

PURPOSE OF THE STUDY
This study will look at several important elements of HIV in South Africa. HIV is the virus that causes AIDS. During HIV infection, the CD4+ cells in your blood are destroyed by the virus, and that is why you get sick and need to go onto treatment. HIV also attacks other cells, making them tired from fighting the virus in your blood all the time. Antiretroviral treatment blocks the virus from multiplying in your blood, and so your CD4+ cells can recover again. In this study we will try to find out if other parts of your child/ward’s immune system can also be ‘rebalanced’ to normal and become functional again when the virus is no longer multiplying in their blood, and how long this takes to happen.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype ‘C’ is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections, and people with subtype B infection that go onto treatment in the developed world, there is limited information available about people infected with subtype C, and their immune system functioning when they go onto treatment.

This study could give us important information about how much an immune system that is damaged by HIV can recover when antiretroviral therapy is taken. The results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV and AIDS.

As ART does not provide a permanent cure for HIV, we also want to study the latent reservoir which is a group of infected CD4 cells that are not actively producing HIV. Latent HIV reservoirs can be found in many places throughout the body, and HIV can hide out for years inside reservoirs. Latent HIV reservoirs can wake up and start making more HIV. If someone with HIV is not taking ART when this happens, the level of HIV in their body or viral load will start to increase. We will define the size and composition of the reservoir, as well as its dynamic origin and decay and other important characteristics which may tell us how we may eradicate HIV from infected cells that the ART cannot get to.

Your child/ward has been referred to this study because he/she participated in either the CAPRISA Acute Infection (CAPRISA 002) study, or another CAPRISA study and has now started taking ART. By taking part in this study they are contributing to a greater understanding of HIV
infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your child/ward’s own situation directly. That is, it will not provide a cure or alter the course of your child/ward’s illness. However, he/she will be monitored at each study visit and referred for care and treatment relating to any HIV-related illness that they may develop.

PROCEDURES

Entering the Study
At this visit, you and your child/ward will be able to read, discuss and sign the consent forms. Your child/ward will have a physical examination done, which will include looking for evidence of sexually transmitted infections (STIs). He/she will be asked to give urine, and vaginal swab specimens if female, for the testing of STIs. The study staff will talk to your child/ward about the infections that are passed on during sex. If your child/ward has clinical signs and symptoms of STIs, he/she will be treated as soon as possible or referred to a public health clinic where they will be given medicine to treat those infections.

Your child/ward will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). He/she will be asked to give around 90 -100 mls of blood (around 15 tubes) at each visit. The blood will be used for tests that look at the way in which their body is responding to HIV infection. It will also be used to look at the virus that he/she has been infected with. Some of this blood together with a urine specimen, will be used to check on his/her general health. If your child/ward is female and she or the study doctor suspects that she is pregnant, this sample will be checked for signs of pregnancy. If she is pregnant, she will be encouraged to stay in the study. She will also be referred to her nearest public health clinic for care relating to her pregnancy.

After two years and five years on ART, we will do a bigger blood draw of approximately 200mls, which is required for the latent reservoir (cure) studies. We will only collect this large amount of blood if your child/ward is clinically well as assessed by a nurse or doctor and their blood levels are within normal limits prior to the blood draw.

Your child/ward will be asked questions about their sexual behavior, general health as well as other questions related to their lifestyle. He/ She will be asked questions about how often they take their antiretroviral treatment.

During the Study
The study nurse will notify your child/ward of their scheduled clinic visits. The number of times that he/she will be called in to the clinic will vary over time. He/she may leave the study at any time, but we would like to follow your child/ward up for a minimum of 5 years on therapy. After 5 years of follow-up he/she will be given the option to remain in the study for up to another 15 years with only yearly follow-up.

Your child/ward will be asked to come back after one month, 3 months and 6 months after starting therapy, and then every 6 months until he/she has reached 5 years on therapy. If it is more than 6 months since they started therapy, he/she will only come in every 6 months. At these visits, your child/ward will also be given a medical examination by one of the study doctors. He/she will be asked to give tubes of blood at each visit. The amount of blood will be around 90 – 100 mls (around 15 tubes). The blood will be used to see how their immune system responds while he/she is on treatment for HIV.

Once a year your child/ward will be tested for STIs. This will be done by testing their blood, and by using a vaginal swab sample and urine if they are female, or urine only if male. Your child/ward will be treated or referred for treatment for any STIs that they have. Your child/ward will be asked questions about their sexual behavior, general health as well as other questions related to their lifestyle.
People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity, even sometimes when they are on treatment. For any opportunistic infections that your child/ward may develop that are detected at the scheduled clinic visits or for any problems that they have with their medication, he/she will be managed at the clinic or referred to their treatment provider, at your public health clinic. Your child/ward will also be able to get counseling on how to stay as healthy as possible and how to get any services that he/she may be eligible to receive.

At each visit the study staff will update information on where your child/ward lives and how to keep in contact with him/her. They will use this information to remind your child/ward of their scheduled visits. If they miss a visit, the study staff will try to contact him/her to find out why they missed a visit. They may also visit your child/ward’s home to try and find him/her. They will try to reach your child/ward through the contact people that they list. If they talk to these people, they will not tell them why they are trying to reach your child/ward. If your child/ward has a cellular phone, study staff may ask if they can use this number to contact them.

Your child/ward’s visits should not last more than three hours. He/she will usually receive their results at their next scheduled visit. Should the study team feel that your child/ward needs to receive results sooner, he/she will be contacted to come to the clinic. For this reason, it is important to make sure that their contact details are up-to-date and that they have indicated how they would prefer to be contacted.

Some of the specimens collected may be stored. Your child/ward will be asked to sign a separate consent form to indicate whether they agree to allow the team to store their specimens.

**BENEFITS**
There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like vaccines.

However, your child/ward may benefit from the advice of the research team and/or the test results he/she will receive as a result of their participation in the study. For example, he/she will receive HIV/STI prevention education. This may help him/her protect themselves and others from sexually transmitted illnesses. If your child/ward contracts a treatable STI during the course of this study, he/she will be treated or referred to a relevant public health clinic for treatment. Your child/ward will receive free condoms and information on how to use condoms properly.

**PARTICIPANT RESPONSIBILITY**
By signing this informed consent form, you are allowing your child/ward to make themselves available to attend the scheduled clinic visits. You are not, however, giving up their right to freely withdraw from this study at any time. If your child/ward does decide to withdraw from the study, he/she needs to please tell the study nurse or doctor.

**REIMBURSEMENT**
Your child/ward will be compensated for their time, travel and inconvenience. If their visit is 3 hours or less he/she will be given R200. Clinic visits may take up most of their day. If your child/ward has to be at the clinic for the day he/she will be provided with food and refreshments for the time that they are at the clinic.

**RISKS/DISCOMFORT**
Your child/ward’s participation in this study is for research purposes. His/her involvement does not mean that they will receive special medicines or treatments that other people cannot get from clinics or hospitals.

Your child/ward may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. He/she may have bruising at the site of the
blood draw. There is a small chance that your child/ward may get an infection in the site of the specimen draws. The risks of collection of genital specimens (swab from vagina) are pain and discomfort.

Some genetic testing may be done on your child/ward’s samples. The greatest risk is to their privacy. It is possible that if others found out information about your child/ward that is learned from these tests (such as information about their genes), it could cause problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify your child/ward by name and they do not become part of your medical records.

NEW FINDINGS
Your child/ward will be told of any new information learned during this study that might cause him/her to change their mind about staying in the study. Your child/ward will be told when the results of the study may be available, and how to learn about them.

REASONS WHY YOUR CHILD/WARD MAY BE WITHDRAWN FROM THE STUDY WITHOUT THEIR CONSENT
Your child/ward may be removed from the study without their consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to your child/ward.
- Your child/ward is not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION
There may be other HIV research studies going on at the clinic or in your community that your child/ward may be eligible for. We will tell him/her about the other studies that we know about.

COSTS OF THE STUDY
There will be no financial cost to you or your child/ward for participation in this study.

CONFIDENTIALITY
Research records of your child/ward’s participation in the study will be kept confidential and we will not give this information to anyone, unless require by law to do so.

We will make every effort to protect their privacy and confidentiality. Your child/ward’s visits will take place in private. However, it is possible that others may learn that your child/ward has been here and assume that he/she is HIV-infected. Because of this, others may treat them differently or discriminate against them.

Medical records that identify your child/ward by name may be inspected by the research team who are responsible for keeping information for this study.

In order to protect your child/ward’s right to confidentiality, he/she will be assigned a code number. This will be used to identify them, rather than their name. Your child/ward’s personal information will not be released without their permission. No publications based on this research will contain your child/ward’s name. However, in some instances we may request additional consent from you both, if there is a possibility that your child/ward may be identified in a publication by description of their characteristics. Only the project investigators and clinic coordinators will know both their name and their identification number. This is necessary so that the coordinators can ensure that your child/ward is called in to the correct clinics. These investigators will not release your child/ward’s number and name to anyone else on the research team.

RESEARCH RELATED INJURIES
It is very unlikely that your child/ward will become injured as a result of involvement in this study. However, in the case of a research related injury, he/she will be referred for treatment. The cost of
this treatment will be free. There is, however, no compensation provided for research related injuries. Your child/ward do not give up any legal rights by signing this consent form. In the event of a research related injury, contact during office hours (8am-5pm):

Fathima Sayed (eThekwini Site) 031 2601611,
Hlengiwe Shozi (eThekwini Site) 031 2601943,
Duduzile Nkosi (Vulindlela Site) 033 2606863, or
Dr Nigel Garrett (Project Manager) 031 2604453

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS
If you have any questions about your child/ward’s involvement in this study, or would like to know more about the study, please call either of the following during office hours (8am-5pm):

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Dr Nigel Garrett (Project Manager) 031 2604453

You are not giving up your child’s/ward’s legal rights by signing the informed consent document. If you have any questions about your rights as a parent of research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

SIGNATURE:
If you have read the informed consent (or if you have had it read to you) and understand the information, and you voluntarily agree for your child/ward to join this study please sign your name on the informed consent form signature page. This will assist us to verify that the participant has your permission to participate in this study.

PARENT/GUARDIAN SIGNATURE PAGE:
If you have read the informed consent (or if you have had it read to you) and understand the information, and you voluntarily agree for your child/ward to join this study please sign your name below.

____________________________________  __________________________  _________________
Parent/Guardian name
(print name as it appears in ID book/Birth certificate)

____________________________________  __________________________  _________________
Name of staff member who administered consent (print)

____________________________________  __________________________  _________________
Witness’ name (print)
For illiterate subjects:

Mark with an ‘X’: _______________________________ Date: __________

Independent Witness: ___________________________ Date: __________

Title and Name: ________________________________

Was a copy of the signed copy given to the parent/guardian:  □ Yes  □ No

If no, why not:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
INTRODUCTION

You have agreed for your child/ward to take part in a study named above. There may be some remaining blood and biological specimens taken from your child/ward during this study that might be useful for future research. You are being asked to agree to the storage of your child/ward’s blood, vaginal specimens and urine for possible future research that will include additional testing. This is research that will be conducted in the future that may or may not be related to the study.

This consent form gives you information about the collection, storage, and use of your child/ward’s blood and urine for possible future research. The study staff will talk to you about this information. Please ask if you have any questions. If you agree to the storage of your child/ward’s blood and urine for possible future research, you will be asked to note this on this consent form. You will get a copy of this form to keep. It is important that you know the following:

- Your child/ward does not have to agree to have their specimens stored if you don’t want them to OR if they do not want to.
- Please note that since your child/ward is younger than 18 years of age, we would like your permission to allow for your child/ward’s specimens to be stored. Your child/ward has been given a similar assent form to read and sign. If you agree for your child/ward’s specimens to be stored we will still require their agreement for storage.

HOW WILL YOU GET THE BLOOD AND URINE FROM MY CHILD/WARD?

The CAPRISA 002 study staff will collect your child/ward’s blood and other biological samples (vaginal swabs and urine) as part of the CAPRISA 002 study that you have consented your child/ward to participate in. This blood, urine and vaginal specimens is needed to carry out the regular tests for the CAPRISA 002 research study. If you agree to have your child/ward’s specimens stored for possible future research, we will store the remainder of this blood, vaginal and urine specimens after the tests for the CAPRISA 002 have been completed.

As with your child/ward’s other samples, only a confidential Participant Identification (PID) number and not your child/ward’s name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.
HOW WILL YOU USE MY CHILD/WARD’S STORED BLOOD AND URINE?
The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your child/ward’s cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your child/ward’s samples may be analyzed in laboratories outside of South Africa. Your child/ward’s samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.

The researchers do not plan to contact you or your child/ward’s regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your child/ward’s health, the researchers will notify the study doctor who will try to contact your child/ward or your child’s/ward’s regular doctor. If you or your child/ward wish to be notified of this type of test result, you or your child/ward need to make sure that you contact the study nurse or doctor with any changes to your child’s/ward’s phone number or address. If you want your child/ward’s regular doctor to be told about this kind of test result, you or your child/ward need to provide the study team with the contact details of your regular doctor.

WHERE WILL MY CHILD/WARD’S BLOOD AND URINE BE STORED?
If you agree to have your child/ward’s samples stored they will be stored with your child/ward’s confidential PID number at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is a possibility that your child/ward’s stored samples may be shipped and analysed overseas at specialized laboratories if a test is unavailable locally. There is no time limit on how long your samples may be stored.

HOW LONG WILL YOU KEEP MY CHILD/WARD’S BLOOD AND URINE?
There is no time limit on how long your child/ward’s blood and urine will be stored.

DOES STORAGE OF MY CHILD/WARD’S BLOOD, VAGINAL AND URINE SPECIMENS BENEFIT MY CHILD/WARD?
There is no direct benefit to you or child/ward through having his/her samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

WHAT ARE THE RISKS FOR MY CHILD/WARD?
There is very little risk to your child/ward when he/she has his/her samples stored. There is a small risk that others may find out information about his/her HIV status from stored samples. This risk is reduced as his/her sample is stored under a confidential PID number, and not his/her name. He/she is entitled to the same protections of confidentiality and privacy for stored samples as he/she is for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your child/ward’s health we cannot currently test for. Results from these tests may cause distress to your child/ward.

Some genetic testing may be done on your child/ward’s stored samples. The greatest risk is to your child/ward’s privacy. It is possible that if others found out information about your child/ward’s that is learned from these tests (such as information about your genes), it could cause him/her problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job
or insurance. This risk is extremely small, because the test results do not identify your child/ward by name and they do not become part of your child’s/ward’ medical records.

WHAT ABOUT CONFIDENTIALITY?
The results of future tests of your child/ward’s samples will not go onto your child’s/ward’s medical record. Although every effort is made to make sure that your child/ward’s samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your child’s/ward’s personal information may be disclosed. Medical records that identify your child/ward by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.

WHAT ARE MY CHILD/WARD’S RIGHTS?
The decision to allow your child/ward’s samples to be stored is completely voluntary. You may decide not to allow your child/ward’s samples to be stored after the tests that are needed for this study have been done or your child/ward decides to stop participating in the study. If you do decide to allow your child/ward’s samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your child/ward’s samples to be stored any more. If you decide to do this, your child/ward’s samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, “Withdrawal of Consent” so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your child’s/ward’s samples.

WHAT DO I DO IF I HAVE QUESTIONS?
If you have any questions about the storage of your child’s/ward’s samples for this study, or would like to know more about the storage of blood, please call either of the following during office hours 8am-5pm:

<table>
<thead>
<tr>
<th>Site</th>
<th>Contact Name</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>eThekwini Site</td>
<td>Fathima Sayed</td>
<td>031 260 1611</td>
</tr>
<tr>
<td></td>
<td>Hlengi Shozi</td>
<td>031 260 1943</td>
</tr>
<tr>
<td>Vulindlela Site</td>
<td>Duduzile Nkosi</td>
<td>033 260 6863</td>
</tr>
<tr>
<td>Project Director</td>
<td>Nigel Garrett</td>
<td>031 260 4453</td>
</tr>
</tbody>
</table>

You are not giving up your child’s/ward’s legal rights by signing the informed consent document. If you have any questions about your rights as a parent of research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za
PARENT/GUARDIAN SIGNATURE PAGE:
Please read the statement below and think about your choice. No matter what you decide, it will not affect your child's/ward's care or participation in the CAPRISA 002 Study.

I agree to allow some of my child/ward's biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_______ Yes
_______ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: ____________________________________________  
Parent/Guardian

Signed: ____________________________________________  Date:______________  
Parent/Guardian

Name: ____________________________________________  
Researcher

Signed: ____________________________________________  Date:______________  
Researcher

Name: ____________________________________________  Date:______________  
Witness

Signed: ____________________________________________  Date:______________  
Witness

For illiterate subjects:

Mark with an 'X':___________________________________  Date:______________

Independent Witness:______________________________  Date:______________

Title and Name:____________________________________

Was a copy of the signed copy given to the parent/guardian:  Yes ☐  No ☐
If no, why not:

_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________
Withdrawal of Consent

I hereby withdraw my consent for the storage of my child's/ward's biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed: ___________________________ Date: __________
Volunteer

Signed: ___________________________ Date: __________
Researcher

For illiterate subjects:

Mark with an 'X': ___________________________ Date: __________

Independent Witness: ___________________________ Date: __________

Title and Name: ___________________________
Appendix 6: Screening and Diagnostic Algorithm (Recruitment and follow-up in Phase I has been completed)

Diagnostic Process:
Adapted from the UNAIDS and WHO HIV testing strategies (WHO, 1997).

600 Female Sex Workers

A1
(First Rapid Assay)

A1+
(Positive)

A1- Report HIV-

A2
(Second Rapid Assay)

A1+A2+ Both Positive: report HIV+

Refer for HIV confirmation\(^2\) care and withdraw

A1+A2-
Consider indeterminate

Confirmatory ELISA

\(^1\)A1 and A2 refer to two different assays.

\(^2\)For newly diagnosed individuals, a positive result should be confirmed on a second sample (done at the appropriate primary health care clinics).

Enroll and Monthly Monitoring
Appendix 7: HIV Diagnostic Algorithm for Phase I (HIV Negative FSW Cohort) (Recruitment and follow-up in Phase I has been completed)

* HIV RNA >5,000 copies/ml in the absence of a positive HIV antibody test, diagnosis of acute HIV infection. (Hecht et al. 2002, Walker and Altfeld, 2003). Follow up testing to confirm subsequent antibody seroconversion will be done to provide final confirmation of the diagnosis (adapted from Marcus Altfeld & Bruce D. Walker, in Hoff & Kamps (Eds), 2003, p.50).

** Where one test is negative and the other positive, or one of the tests is indeterminate and the other positive
15.8 Appendix 8: Specimen Procurement, Processing Methodology, Labeling, Storage and Shipping

The major goal of the collection of specimens is for routine laboratory assessment, as well as to provide access to cells, plasma and genital specimens, which will be useful for the current research studies as well as future studies. Samples will be stored for future use to address issues not currently available or understood, or apply technologies not currently available.

On collection, specimens will be labeled with a preprinted Patient Identification (PID) number as described in section A8.4 below. All samples collected will be transported to the laboratory. Participants’ samples will be received and registered into the LIMS by laboratory personnel. Those specimens for local analysis will be duly processed. Specimens for storage will be centrifuged, aliquoted and stored as described in the relevant SOPs. Shipment of specimens to collaborating laboratories is referred to in section A8.6 below.

15.8.1 Specimen Procurement

The study schedule (Appendix 4) outlines the tubes and specimen type to be taken at each study visit. The table below describes the tubes to be drawn and volume required for assays and for the repository. Section A8.2 described laboratory where the assay will be performed. Specimen collection kits will be pre-packaged for each visit. At every clinic visit, these will be available according to the scheduled participant’s visits. From the clinic, all tubes should be sent to the CAPRISA laboratory where they will be either processed or transported to their respective laboratories as summarized below and outlined in the Standard of Procedures (SOP). All samples should be kept at room temperature.

<table>
<thead>
<tr>
<th>Phase I (Recruitment and follow-up in Phase I has been completed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood Draw/specimen</strong></td>
</tr>
<tr>
<td>Plain SST</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>EDTA</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Na/Fi</td>
</tr>
<tr>
<td>Urine</td>
</tr>
<tr>
<td>FCU from males or 2 VVS, CVL/Tear Flo strip (or equivalent) from females</td>
</tr>
<tr>
<td>ACD</td>
</tr>
</tbody>
</table>
### PHASE II - IV

<table>
<thead>
<tr>
<th>Blood Draw</th>
<th>Assay</th>
<th>Remainder of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>FBC</td>
<td>Global</td>
</tr>
<tr>
<td></td>
<td>CD4/CD8 , RT-PCR/VL</td>
<td>CAPRISA</td>
</tr>
<tr>
<td></td>
<td>Sequencing (provirus)</td>
<td>UCT</td>
</tr>
<tr>
<td></td>
<td>Virus isolation</td>
<td>NICD</td>
</tr>
<tr>
<td>Plain SST</td>
<td>LFT, U&amp;E, chol, , TG, Fe, B12, Folate, Ca, Mg, Phosphate</td>
<td>Global</td>
</tr>
<tr>
<td></td>
<td>Neutralization assays</td>
<td>NICD</td>
</tr>
<tr>
<td></td>
<td>Antibody binding assay</td>
<td>NICD</td>
</tr>
<tr>
<td></td>
<td>RPR, HSV &amp; HBV</td>
<td>Global</td>
</tr>
<tr>
<td>Na/Fl</td>
<td>Glucose</td>
<td>Global</td>
</tr>
<tr>
<td>FCU from males; urine and 2 VVS from females</td>
<td>STI diagnosis</td>
<td>Global &amp; CAPRISA CRS Labs</td>
</tr>
<tr>
<td>ACD</td>
<td>Elispot screen and confirmation, ICC flow, MHC tetramer, HMA, HTA Sequencing</td>
<td>Store</td>
</tr>
<tr>
<td>Softcup (or equivalent)</td>
<td>Viral sequencing</td>
<td>UCT</td>
</tr>
<tr>
<td></td>
<td>HSV-2, HPV</td>
<td>Global</td>
</tr>
<tr>
<td></td>
<td>determining the potential impact of tenofovir levels on viral and host dynamics at acute infection</td>
<td>NICD UCT CAPRISA</td>
</tr>
</tbody>
</table>

### PHASE V

<table>
<thead>
<tr>
<th>Blood Draw</th>
<th>Assay</th>
<th>Remainder of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>FBC</td>
<td>Global</td>
</tr>
<tr>
<td></td>
<td>CD4/CD8 , RT-PCR/VL</td>
<td>CAPRISA</td>
</tr>
<tr>
<td></td>
<td>Sequencing (provirus)</td>
<td>UCT</td>
</tr>
<tr>
<td></td>
<td>Virus isolation</td>
<td>NICD</td>
</tr>
<tr>
<td>Plain SST</td>
<td>LFT, U&amp;E, Chol, TG, Ca, Mg, Phosphate</td>
<td>Global</td>
</tr>
<tr>
<td></td>
<td>Neutralization assays</td>
<td>NICD</td>
</tr>
<tr>
<td></td>
<td>Antibody binding assay</td>
<td>NICD</td>
</tr>
<tr>
<td></td>
<td>RPR, HSV, HBV</td>
<td>Global</td>
</tr>
<tr>
<td>Na/Fl</td>
<td>Glucose</td>
<td>Global</td>
</tr>
<tr>
<td>FCU from males; urine and 2 VVS from females</td>
<td>STI diagnosis</td>
<td>Global &amp; CAPRISA CRS Labs</td>
</tr>
<tr>
<td>ACD</td>
<td>Elispot screen and confirmation, ICC flow, MHC tetramer, HMA, HTA Sequencing</td>
<td>Store</td>
</tr>
<tr>
<td></td>
<td>determining viral resistance and the potential impact of tenofovir levels on viral and host dynamics after ART initiation</td>
<td>NICD UCT CAPRISA</td>
</tr>
</tbody>
</table>

UriCheck 10: Leukocytes, Nitrite, Urobilinogen, Protein, pH, Blood, S.G., Ketone, Bilirubin, Glucose.
FCU: first catch urine VVS: vulvovaginal swab
15.8.2 Specimen Flow

The flow chart below summarized the flow of samples throughout the study.

---

**Clinic**

On site testing:
- Urine Pregnancy test
- Haemoglobin
- Urine dipstick
- Point-of-care Viral load and STI testing
- HIV rapid test (Phase I only)

---

**Preparation and shipping**

CAPRISA laboratory

---

Collection by Global couriers

---

Sent to Global

---

Shipped to NICD

---

Shipped to UCT

---

Global Lab
- FBC
- U&E & LFT
- Creatinine clearance
- Glucose/HB A1C
- Ca, Mg, PO4
- Fe studies
- B12, & Folate
- Cholesterol, TG, HDL, LDL

---

Global Lab
- Syphilis, HBV serology
- HSV-2 serology

---

CAPRISA Lab
- PCR pooling
- Viral Loads
- CD4/CD8 counts
- HIV Elisa
- Process whole blood
- Cryopreservation
- Plasma and serum storage
- Long-term storage
- Softcup (or equivalent) for:
  - HPV typing and viral load
  - Genital Tract tissue biopsies

---

NICD
- Neutralizing Ab
- Binding Ab
- MHC/Tetramer analysis
- Virus Isolation
- Tenofovir resistance

---

UCT
- PCR
- HMA
- HTA
- Sequencing
- Host genetics
- CD4/CD8 phenotype
- CD4/CD8 function
- Plasma cytokines
- HLA
- IFNg Elispot
- CD4 Function

---

**Clinical**

On site testing:
- Urine Pregnancy test
- Haemoglobin
- Urine dipstick
- Point-of-care Viral load and STI testing
- HIV rapid test (Phase I only)

---

Collection by Global couriers

---

Sent to Global

---

Shipped to NICD

---

Shipped to UCT

---

Global Lab
- FBC
- U&E & LFT
- Creatinine clearance
- Glucose/HB A1C
- Ca, Mg, PO4
- Fe studies
- B12, & Folate
- Cholesterol, TG, HDL, LDL

---

Global Lab
- Syphilis, HBV serology
- HSV-2 serology

---

CAPRISA Lab
- PCR pooling
- Viral Loads
- CD4/CD8 counts
- HIV Elisa
- Process whole blood
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- Plasma and serum storage
- Long-term storage
- Softcup (or equivalent) for:
  - HPV typing and viral load
  - Genital Tract tissue biopsies

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NICD
- Neutralizing Ab
- Binding Ab
- MHC/Tetramer analysis
- Virus Isolation
- Tenofovir resistance

---

UCT
- PCR
- HMA
- HTA
- Sequencing
- Host genetics
- CD4/CD8 phenotype
- CD4/CD8 function
- Plasma cytokines
- HLA
- IFNg Elispot
- CD4 Function

---

**FBC**: full-blood count; **FCU**: first catch urine; **VVS**: vulvovaginal swab; **HMA**: heteroduplex mobility assay; **HTA**: heteroduplex screening assay, **HSV**: Herpes simplex virus; **IFNg**: interferon gamma, **HBV**: Hepatitis B virus.
15.8.3 Specimen Processing

Processing of samples for research purposes described below. Routine laboratory assays will be performed at the Global Laboratories including: FBC, U&E & LFT, Glucose, Ca, Mg, PO4, Fe studies, B12, & Folate, Cholesterol, LDL, TG. These bloods will not be processed by the CAPRISA laboratory but will be transported directly to Global Laboratories. All other specimens will either be processed by CAPRISA laboratory, or where indicated below, transported to the National Institute for Communicable Diseases (NICD), Johannesburg, or University of Cape Town.

Yellow top tube (ACD)

Tubes for PBMC isolation will be transported to the CAPRISA Repository and processed to separate cells and plasma within 24 hours of collection. Tubes must be transported at room temperature for immunological assays. Specimens will be transported and delivered to NICD when requested by NICD collaborators. PBMC will be isolated and cryopreservation of the PBMC’s will be stored in a vapor-phase liquid nitrogen freezer to ensure maximum viability and recovery post thaw. Plasma will be stored in an ultrafreezer where temperatures range between -70 to -85 degrees celcius. PBMCs will be used for immunological assays. In addition, DNA will be isolated from PBMCs for HLA typing and for host genetic studies. Lastly, DNA from cells and RNA from plasma will be used for HIV sequence analysis.

EDTA blood

Virus Isolation

One 5 ml EDTA tube for virus isolation will be shipped, at room temperature, to the Virology laboratory at the NICD when requested by NICD collaborators. Whole blood will be transferred to a 15 ml plastic Falcon tube and spun at 1,500 rpm for 10 minutes. Plasma will be collected and stored in an ultrafreezer where temperatures range between -70 to -85 degrees celcius. The mononuclear cell layer will be collected and stored in liquid Nitrogen. Frozen cells will be thawed later for virus isolation.

HIV RT-PCR

One 5 ml EDTA tube will be transported to the CAPRISA laboratory for HIV RNA testing. Tubes must be transported at room temperature. The ROCHE COBAS Ampliprep-COBAS TaqMan version 2.0 assay will be used to measure viral loads. The test quantifies HIV-1 RNA over a range of 20 – 10 000 000 cp/ml. The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test uses reverse transcription and PCR amplification primers that define a sequence within the highly conserved region of the HIV-1 gag gene32. The gag region encodes the group-specific antigens orcore structural proteins of the virion. The nucleotide sequence of the primers has been optimized to yield comparable amplification of group M subtypes of HIV-1. Alternatively, the point-of-care Xpert HIV-1 Viral Load (Cepheid) assay will be used at clinic level to facilitate clinic visits.

CD4/CD8 COUNT

For CD4+ T-cell Count whole blood will be stained using the Beckton Dickinson Multitest (Becton Dickinson 342447). The reagent contains a monoclonal antibody cocktail composed of CD4, CD8, CD3 and CD45. When a known volume of whole blood is added to the staining reagent, the monoclonal antibodies bind to specific cell markers. When these pass through the flow cell of the FACSCalibur flow cytometer, they are excited by the laser beam and fluoresce. Staining takes place
directly in a TruCOUNT tube containing a lyophilized pellet. The pellet dissolves liberating a known concentration of fluorescent beads. The absolute number of positive cells is determined automatically by the software (Multiset) by comparing the number of cellular events with the number of bead events. The CAPRISA laboratory will register with the Immunology Quality Assessment Program and participate in the UKNEQAS for Leucocyte Immunophenotyping.

Clotted blood

Clotted blood will also be used for HIV and STI serology as well as HIV binding and neutralizing antibody. Serum is collected at the CAPRISA laboratory and stored in aliquots in ultrafreezers where temperatures range between -70 to -85 degrees celcius. The clot will be discarded in biohazardous containers.

Cervicovaginal lavage (CVL) (This specimen is no longer collected)

CVL specimens, collected for viral diversity and immunology assays, should be transported to the laboratory within one hour. Samples should be kept at 4°C and should be processed within 6 hours to ensure the stability of the RNA. Samples will be processed and stored by the CAPRISA laboratory. CVL should be fractionated into supernatant and cellular fractions by centrifugation at 400 x g for 10 minutes. The supernatant should then be carefully aliquoited into 1.5 ml cryovials and stored in an untrafreezer where temperatures range between -70 to -85 degrees celcius. The pellet should be re-suspended in 10mL of 1x PBS followed by centrifugation at 400 x g for 10 minutes with the discarding of the PBS supernatant. The cells need to be re-suspended in 1ml of PBS with the removal of 20μl for counting. Re-suspended cells should then be centrifuged at 400 x g for 10 minutes with the supernatant PBS aspirated from cell pellet. The cells should then be re-suspended with cold cryopreservation media to a concentration of 1 x 10^7 /ml. Aliquots of the cell suspension should be then be separated into cryovials and stored in liquid nitrogen.

Tear Flo strip (or equivalent high quality blotting paper) (This specimen will no longer be collected from January 2015)

Cervical secretions collected for viral diversity and immunology assays, should be transported to the laboratory within one hour. This specimen should be collected before any other cervical or vaginal samples, including the CVL. Each Tear-Flo™ strip adsorbs approximately 12μl of specimen. Adsorption usually takes approximately one minute, but may take a little longer. The round end of the two strips should be held over and slightly inside one labeled plastic transport tube (1.5 or 2.0-mL cryovial) containing 500 μL of NASBA 1x Nucleic Acid Sequence Based Amplification (NASBA) lysis buffer. The strips should be cut at the “15” mark with scissors, allowing the round end to fall into the cryovial tube containing buffer. The specimen should be capped and inverted or vortexed for 5 seconds.

Note: If the Tear-Flo™ strips are to be stored in NASBA lysis buffer, the buffer must be crystal free before you begin. Most crystals will dissolve by placing the lysis buffer tube at room temperature for a few hours. If crystals remain in the tube, it should be vortexed until the crystals are gone. Tear-Flo™ strips in NASBA are stable for 24 hours at room temperature (~25°C), for 14 days at 2-8°C, and then in an untrafreezer where temperatures range between -70 to -85 degrees Celsius. Keep the sample refrigerated or on ice until it is frozen at -70°C. DO NOT store specimens in lysis buffer at -20°C.

Cytobrush (This specimen is no longer collected)

Cytobrush specimens, for determining the potential impact of tenofovir levels on viral and host dynamics at acute infection, will be collected from the cervical os using two 360° rotations. The cytobrush will immediately be placed into a 15ml conical centrifuged tube filled with 5 ml of
collection RPMI medium (supplemented with 10% FCS, L-glutamine, streptomycin, and penicillin) and placed into an ice bucket with ice for shipping to the CAPRISA laboratory for storage. Cytobrush specimens should be processed within 6 hours of sampling. A Pasteur Pipette will be used to pipette up/down approximately 20 times to dislodge mucous and cells from the cytobrush in the transport medium. Transfer the transport medium to a fresh 15mL conical centrifuge tube and centrifuge at 320g (approx 1300rpm) for 10 minutes. Supernatant will be removed using a pastuer piette and sample will be stored in an ultrafreezer. 2.1mL of 10% FCS RPMI will be added to the cells and pellet will be resuspended by flicking.

For lysate, immediately upon receipt of the sample in the laboratory a wet preparation should be performed looking for the presence or absence of sperm by placing a drop of the specimen on a slide and cover slip the slide. Slide will be viewed under the microscope using the 10x and 40x magnification with no oil. Presence or absence of sperm on the CVL/Cytobrush worksheet should be noted. The cytobrush should be removed from the tube and the falcon tube spun at 4 °C at 800g for 10 minutes. Supernatant should be poured off and discarded being careful not to disturb the cell pellet and resuspend with 1ml PBS. Viable mononuclear cells will be counted using either the manual counting technique or hemocytometer. The remainder of the 1ml sample at 4 degrees celcius at 800g for 10 minutes should be spun and carefully poured off. The supernatant should be discarded being careful not to disturb the cell pellet. 0.5 ml of well-mixed ice cold 70% methanol solution (prepared by separately measuring with graduated cylinders 70 ml of MeOH with 30 ml of H2O) will be added to the 15 ml falcon tube containing the cell pellet. Solution should be vortexed lightly to lyse the cells and kept on ice for 15 minutes. Sample will be spun at 800g for 10 minutes to pellet cell debris then supernatant poured into a labelled cryovial and stored at -85 degree celcius.

Aspirate (This specimen is no longer collected)
Vaginal aspirates, for determining the potential impact of tenofovir levels on viral and host dynamics at acute infection, will be collected using the UNC CFAR Vaginal Specimen Aspirators. Vaginal fluid from the aspirator will be placed into a labeled cryovial and immediately placed into an ice bucket with ice and transported to the CAPRISA laboratory and stored in an untrafreezer where temperatures range between -70 to -85 degrees celcius.

Biopsies (This specimen is no longer collected)

For pharmacokinetic study
Biopsies will be collected from the cervix and vagina at the enrollment visit and will be stored under specified conditions pending shipment in batches on dry ice to the central laboratory. For each biopsy sample the following procedure applies: Weigh labeled cryogenic vial prior to specimen placement. Place biopsy specimen in vial and weigh biopsy and vial. Snap freeze either in a liquid nitrogen bath or in an ethanol or acetone bath containing dry ice. Stored in an untrafreezer where temperatures range between -70 to -85 degrees celcius. The specimens will be stored at the site. Tenofovir levels will be quantified using the high performance liquid chromatography with tandem mass spectrometry HP-LC/MS assay. Other tests or procedures may also be conducted on the samples in order to answer the primary or secondary study objectives.

For viral identification study
Genital tract biopsy specimens (vagina and cervix) will be taken from each participant at enrolment into the study by means of a Tischler biopsy forceps. Alternate specimens will be distributed for transmission electron microscopy (TEM) or immuno-fluorescence (IMF) staining and viewing with confocal laser scanning microscopy (CLSM). Specimens will be stored and transported in appropriate fixatives viz. Karnovsky’s fixative for TEM or in a cryovial to be snap frozen in liquid nitrogen for IMF-CLFM.
STI Diagnosis

STI diagnosis will be performed in the CAPRISA CRS and Global or NICD Laboratories. Participants will provide a urine sample and/or blind vaginal swabs for on-site point-of-care STI testing. For serology testing, aliquots for serum will be provided by the CAPRISA laboratory. All other specimens will be processed on-site or directly transported to the Global or NICD Laboratory.

Bacterial vaginosis. Vaginal secretion smears prepared on glass slides will be Gram stained and the vaginal flora interpreted using Nugent’s criteria for the diagnosis of bacterial vaginosis.

Gonorrhea, chlamydial infection, and trichomoniasis. Vaginal swab specimens and a urine specimen will be tested for these STIs using point-of-care assays.

Syphilis serology. RPR, which will be performed on undiluted and 1:8, diluted serum. TPHA will be performed for all specimens. Using the results of the RPR and TPHA a diagnosis of active syphilis or a past history of syphilis will be made.

HSV serology. Serological tests for herpes simplex virus (HSV) type-1 and –2 will be directed against antibodies to HSV glycoproteins G-1 and G-2, which evoke a type-specific antibody response. HerpeSelect-1 and HerpeSelect-2 EIA will be used to confirm a genital herpes diagnosis, and identify asymptomatic carriers.

HBV serology. Previous hepatitis B viral infection will be excluded in all participants by detecting antibodies to Hepatitis B Virus Core Antigen using AxSYM CORE. All those seropositive for anti-HBc will then be screened for Hepatitis B Surface Antigen (HBsAg) using AxSYM HBsAg.

15.8.4 Specimen Labeling

A standardized labeling system shall be applied to all samples and appropriate forms for identification and tracking purposes. A Participant Identification (PID) number shall function as the unique, primary identifier. This shall be a randomly generated, pre-printed self-adhesive label to be affixed to samples, CRF and laboratory requisition form at the time of sample collection, according to the Study visit schedule. The Study visit schedule will be issued to the relevant study nurse in advance, and indicate which participants are due and on which dates. The PID will be a 10 digit number in the format of XXX YY ZZZZ, where XXX = Study identifier, YY = Site identifier and ZZZZ is a unique participant identifier. Samples will be forwarded to the CAPRISA laboratory and upon receipt will be entered into the Laboratory Information Management System (LIMS). The CRFs will not be forwarded to the laboratory. Samples shall be tracked by paper-trail or by management reports on the LIMS.

15.8.5 Specimen Inventory and Storage

Samples shall be shipped between sites using either a manually entered Shipping Manifest form (for any remote site which may not be connected electronically), or by a LIMS generated Shipping Manifest. The manifest will indicate date, shipper, number of specimens, specimen type and tests requested, an electronic copy (disc) of this will be included with shipments between the CAPRISA laboratory and remote laboratory sites. The shipper is required to sign the manifest on dispatch, as is the receiver. Acknowledgement on the LIMS of a received shipment will facilitate tracking. Tracking Management reports will facilitate timely intervention of identified errors.
The Repository will accommodate long-term storage of samples. PBMCs are to be stored in vapor-phase liquid nitrogen, at -170°C. Plasma and serum samples will be Stored in an ultrafreezer where temperatures range between -70 to -85 degrees celcius in 500µl aliquots. A tracking point (workstation and hand-held barcode scanner) will be sited in the Repository. All samples stored will be scanned, the information being uploaded to the freezer management module of the LIMS. This information will reflect the user id of the person performing the storing, time, date, freezer, compartment, shelf, rack, position, sample type and volume. Removal of samples will be by predetermined permission protocols, signed for, with the records adjusted accordingly (i.e. volume), indicating the recipient and by whose authority. This will be applicable for ad-hoc removals as well as batch shipments.

15.8.6 Sample Shipment

All samples will be packed and shipped according to IATA guidelines. Shipment of large batches of samples will be by means of Shipping Manifests. Recipients will receive pre-notification of all shipments, and will also be able to query the LIMS. There will be no shipments before weekends or Public Holidays. Shipments will be scheduled to ensure they will be received during operating hours. Samples stored at -70°C will be shipped on dry-ice. Samples stored at -170°C will be shipped in liquid nitrogen dry shippers.

Appendix 9a: Algorithm for measuring cellular immune responses in the AI project

Peripheral blood → PBMC

Subtype C peptide pools corresponding to 9 gene regions of HIV Gag; Nef; Pol; gp160; Rev; Tat; Vif; Vpr; Vpu

From fresh PBMC

response in any of 1-9 pools

No response to any pool (< threshold)

Narrow down to likely peptide

Responses in pool

Confirm peptide responses using single peptides in ELISPOT (by CD8 depletions) and FACS (ICC) assays

Confirm CD4 response

No confirmation

No further analysis

Confirm CD8 response

Peptide stimulation in vitro to generate CD8+ cell line (expanded cells)

Use truncated peptides to confirm optimal CD8 epitope and identify HLA restriction using partially mis-matched BLCL

From cryopreserved PBMC

Vial 1

Vial 2

Vial 3

Vial 4

Phenotype and tetramer analysis

CD4 epitope response

Method 1: IFN-γ ELISPOT

Method 2: EBV-transformation of B cells

Method 3: ICC flow cytometry

Method 4: 51Cr-release CTL function

Method 5: surface and ICC flow cytometry

Method 6: MHC tetramer analysis

Method 7: MHC tetramer analysis
Appendix 9b: Algorithm for measuring virological changes associated with immune responses*

*Whole length genome sequencing will be performed on at least five individuals with well defined clinical information, viral phenotype, neutralization, HLA and CTL data. Individuals with broad CTL responses in early infection, with evidence of escape, will be targeted to investigate co-variation (compensatory changes).

Appendix 9c: Algorithm for monitoring for viral evolution and dual infection

Direct population sequencing of HIV-1 (plasma, DNA, CVL*) at transmission (partial gag and env)

HTA screen (env, gag, nef) to determine intraperson diversity and dual infection

HMA Screen for diversification or super infection (plasma) (env, gag, nef) (6 monthly intervals)

Low diversity: Clone and sequence; Or HTA and direct PCR sequencing of variants

High diversity: Clone and sequence; Or HTA and direct PCR sequencing of variants

Low diversity, no further analysis

High diversity, no further analysis

Dual Infection, repeat screen of earlier timepoints to identify timing of superinfection

Low diversity, no further analysis

Direct PCR sequence of gene regions targeted by immune response.

Change in epitope recognition (pos to neg, or neg to pos)

No change in epitope recognition

No response to any pool (< threshold)

No sequence analysis

Direct PCR sequence of early and late isolates. Correlate genetic changes with virus escape

Direct PCR sequencing of gp160 from early and late isolates.

Kinetics of autologous neutralization and virus escape

Potency and breadth of serum neutralization

Autologous neutralization (using early and late pseudotyped viruses)

Heterologous neutralization. Sera tested against a panel of neutralization-sensitive and resistant pseudotyped viruses.

~50 individuals

~50 individuals

Subtype C peptide pools corresponding to 9 gene regions of HIV Gag; Nef; Pol; gp160; Rev; Tat; Vif; Vpr; Vpu

Monitor over time

Response in any of 1 - 9 pools

Low diversity:

Direct PCR sequencing

High diversity:

Clone and sequence;
Or HTA and direct PCR sequencing of variants
15.10 Appendix 10: Management of HIV Infection in Pregnancy

South African Guidelines for the Management of pregnant women with HIV will be followed. This will include:

- referral to antenatal services
- immediate initiation of ART irrespective of CD4 count and lifelong continuation of ART following pregnancy.

15.11 Appendix 11: CAPRISA Scientific Review Sub-Committee (Publication Policy)

CAPRISA Scientific Review Sub-Committee (CSRC)

Chair: Andy Gray, University of KwaZulu-Natal
Members: Derseree Archary, CAPRISA, University of KwaZulu-Natal
          Wendy Burgers, University of Cape Town
          Hoosen Coovadia, MATCH
          Halima Dawood, CAPRISA, University of KwaZulu-Natal
          Nigel Garrett, CAPRISA, University of KwaZulu-Natal
          Tanuja Gengiah, CAPRISA, University of KwaZulu-Natal
          Anneke Grobler, CAPRISA, University of KwaZulu-Natal
          Ayesha Kharsany, CAPRISA, University of KwaZulu-Natal
          Kerry Leask, CAPRISA, University of KwaZulu-Natal
          Lenine Liebenberg, CAPRISA, University of KwaZulu-Natal
          Lyle McKinnon, CAPRISA, University of KwaZulu-Natal
          Nono Mhkize, National Institute for Communicable Diseases
          Nivashnee Naicker, CAPRISA, University of KwaZulu-Natal
          Kogieleum Naidoo, CAPRISA, University of KwaZulu-Natal
          Vivek Naranbhai CAPRISA, University of KwaZulu-Natal

Administrator: Cheryl Baxter

This committee will serve three roles: a) delineation of guidelines for authorship on future publications emanating from CAPRISA; b) delineation of guidelines for ancillary studies using material stored or gleaned from CAPRISA and c) reviewing that these guidelines have been met prior to publication and that ancillary studies meet the requirements as laid out.

The committee is divided into four areas of expertise:
1) Social sciences, Epidemiology, Ethics (HC, ALG, AG, KN, NG, HD, TG)
2) Clinical sciences (ALG, HC, VN, KN, NG, HD, TG, NN)
3) Laboratory sciences (LM, NM, VN, DA, WB, LL)
4) Statistics (AG, KL)

Procedures applicable to this policy are outlined in the document entitled: CAPRISA SCIENTIFIC REVIEW SUB-COMMITTEE (CSRC) PROCEDURE attached hereto.

A. Guidelines For Authorship On Publications Emanating from CAPRISA projects.

Reference is made to The Danish Committees on Scientific Dishonesty (http://www.forsk.dk/eng/uvvu/publ/guidelines98/kap5.htm); Harvard University Authorship guidelines (http://www.hms.Harvard.edu/integrity/authorship.htm) and Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org).

Authorship of Manuscripts

1. Right to authorship

The following requirements need to be fulfilled to obtain the right to authorship:
a. To have contributed substantially to the creative process in more than one of the following areas: idea, planning, experimental work, collection of clinical or clinical-epidemiological data, analysis of data and interpretation.

b. To have contributed substantially to the preparation of the manuscript by helping to write drafts and through critical revision of the article.

c. To accept the final draft of the manuscript in writing to the principal author prior to submission. Additionally, a comprehensive co-author statement template will be circulated describing the nature and extent of each author’s contribution. This will be signed by all authors and should be kept by each author in case they need to be submitted to journals if asked to provide evidence of co-authorship.

d. To be able to present a detailed account of his or her contribution and to discuss principal aspects of other co-author’s contributions.

A creative effort results in a right to authorship when it fulfils the above-mentioned four conditions. Laboratory technicians or other co-workers who play no role, or a peripheral role in the generation of the manuscript, will not have a right to authorship.

2. Order of authors

The researcher who has made the most important work effort and who has prepared the first manuscript draft is listed as first author, which is line with common international practice. The most senior co-worker who has the ultimate responsibility for the project, but who also must fulfill the above-mentioned criteria for authorship, is usually listed last. The last author is usually a member of the team who guides and overseas the framework of the paper. The remaining authors are listed according to their estimated part in the work.

The following will be a requirement of the CSRC:

i) the authors should decide the order of authorship together

ii) the primary author should be able to justify the order of authorship and to outline the role of each author.

The CSRC are available to give advice on authorship issues, but the CAPRISA Director should be approached to resolve authorship problems. Where the Director is a co-author, the CSRC will be asked to deal with the matter.

3. Acknowledgements in the Manuscript

List of contributors who do not meet the criteria for authorship, such as a person who provided technical help, writing assistance, or a Head of Department who provided only general support. Financial and material support should also be acknowledged. Manuscripts arising from the CAPRISA 001 – START study and the CAPRISA 002 – Acute Infection study must acknowledge the NIH support as follows:

“CAPRISA is part of the Comprehensive International Program of Research on AIDS (CIPRA) and is supported by the National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health (NIH) and the US Department of Health and Human Services (DHHS) (grant# AI51794)”

If the research being submitted for publication is not supported by the NIH then the relevant funding body and or training programme needs to be acknowledged as follows:
This research was supported by [insert name of organization or training grant], grant # [insert grant number]

or

[researchers names] are supported by [insert name of organization] grant # [insert grant number] for example: Manuscripts arising from the TRAPS studies should include the following acknowledgement:

The parent trial (CAPRISA 004) was supported by the United States Agency for International Development (USAID), FHI360 [USAID co-operative agreement # GPO-A-00-05-00022-00, contract # 132119], and the Technology Innovation Agency (LIFElab) of the South African government’s Department of Science & Technology. Tenofovir was provided by Gilead Sciences and the gel was manufactured and supplied for the CAPRISA 004 trial by CONRAD. The current studies are part of the CAPRISA TRAPS (Tenofovir gel

Research for AIDS Prevention Science) Program, which is funded by CONRAD, Eastern Virginia Medical School [USAID co-operative grant #GP00-08-00005-00, subproject agreement # PPA-09-046]. The views expressed by the authors do not necessarily reflect the views of USAID, Gilead Sciences, Eastern Virginia Medical School or CONRAD.

Note: All grant numbers can be obtained from the CAPRISA finance department (swartm@ukzn.ac.za)

Groups of persons who have contributed materially to the paper, but whose contributions do not justify authorship may be listed under such as “clinical investigators” or “participating investigators” and their function or contribution should be described. All persons must have given written permission to be acknowledged.

**Some journals are now accepting brief descriptions for each author of how they contributed to the work and paper**

**4. Authorship to publications other than articles in journals**

Some of the scientific work in CAPRISA will sometimes be published in publications other than scientific journals, e.g. in reports or reviews. This may occur during report-backs to donor funders or for each individual institutional annual report

Scientific reports are sometimes intended to be published without the authors being mentioned. Participants fulfilling the above mentioned four requirements to authorship may be mentioned in other ways, e.g. by contributor descriptions. The acknowledgement of a creative contribution should take place according to the same general principles independent of how results are published.

**General Comments on Professionalism**

**Deviations from correct execution of right to and duty of authorship**

Gift authorship should be avoided. Examples may be Institute leaders or supervisors, who have not participated in the work underlying the publication. Authorship should not be used as a service for friends, a commodity or as compensation for services rendered.

Planted authorship should be avoided; which is a gift authorship supplied without the knowledge or acceptance of the person involved. It may be used to give a false guarantee of quality to a work by including an acknowledged scientist.
Relinquished authorship should be avoided, in which a person with a right to authorship has ceded the duty to accept this right, but has let other co-workers with or without the right to authorship appear as sole authors.

Ghost authorship in which a person with the right to authorship has declined to this right, but has induced another person to accept a gift authorship instead. This may make the work e.g. unwarrantedly appear to be done by an independent expert.

**Professional considerations in the preparation of publications**

The preparation of manuscripts for publications must take place within the framework of the research group, in full openness and in accordance with the agreements made between CAPRISA research team. Thus, members of the CAPRISA research group should not prepare separate publications without prior agreement with the other members of the group.

The use of results from a project for special sorts of publications, e.g. academic dissertations, which were not anticipated at the initiation of the project, requires information to and approval by the full research group.

**B. GUIDELINES FOR ANCILLARY STUDIES EMANATING FROM CAPRISA**

**Definition**

Ancillary studies refer to studies that are undertaken within CAPRISA and use the infrastructure and opportunities provided by the projects and cores but address issues and objectives that are different from stated primary and secondary objectives of the CAPRISA projects.

**Categories**

i. Ancillary studies occurring concurrently with projects

ii. Ancillary studies that use data and/or stored specimens collected for CAPRISA projects

iii. Ancillary studies that require collection of additional data and/or specimens

Note: Please also refer to the guidelines on acknowledgements provided in section 3 above

**C. GUIDELINES FOR ABSTRACT SUBMISSIONS FOR CONFERENCES AND MEETINGS.**

Copies of all abstracts that are submitted to conferences and meetings should be sent to the CSRC for approval not less than **five working days** prior to abstract deadline submissions. This will ensure that CAPRISA investigators are aware of the work and that authorship is appropriate.

Note: Please also refer to the guidelines on acknowledgements provided in section 3 above

**15.12 Appendix 12: Study Definitions**

**Clinical**

**HIV Negative Cohort (Phase I)**

HIV negative female sex workers, followed until diagnosis of HIV infection (refer to diagnostic algorithm).
Acute Infection (Phase II)
The first 3 months post enrollment, where enrollment refers to enrollment onto Phase II of the study.

Early Infection (Phase III)
3 months and ≤ 12 months post enrollment into Phase II.

Chronic Infection (Phase IV)
≥ 12 months until ART initiation

Time of HIV infection
Time of HIV infection will be estimated using the following algorithm:

- If a positive RNA is available on the same date as a negative HIV EIA, the HIV infection is estimated at 14 days prior to the negative EIA test date.
- HIV infection is estimated as the midpoint between the last documented HIV negative EIA and any of the first positive EIA, or RNA tests.

Post ARV Initiation (Phase V)
Participants initiated on ART will be transitioned to phase V and will be followed-up for 5 years

Immunological

Anti-HIV CD4+ or CD8+ T cell numbers: The frequency of CD4+ or CD8+ T cells in the peripheral blood from HIV-1 infected participants that recognize peptides derived from subtype C HIV-1.

CD4+ or CD8+ T cell function: the % proliferation (CD4+) or cell kill (CD8+).

Number of CD4+ or CD8+ T cell epitopes: the number of epitopes recognized by CD4+ or CD8+ T cells.

CD4+ trajectory: CD4+ T cell count trend over time

Viral

Viral set point: Viral load at one year, with measurements made at other time points to ascertain as a secondary objective if alternative time point can be used.

Viral load trajectory: Viral load trend over time

HIV-1 binding antibody titers: The titers of antibodies in plasma that are specific for HIV proteins.

HIV-1 neutralizing antibody titers: The reciprocal serum dilution resulting in 80% inhibition of p24 antigen production or the 50% reduction in cell killing.

Co-receptor usage: use of either CCR5 (R5 tropic), CXCR4 (X4 tropic) or both co-receptors (R5X4) for viral entry.

Dual Infection: Infection with two HIV strains which group on distinct branches on the phylogenetic trees where the two viruses are no closer to each other than another epidemiologically
unlinked sequence and/or the mean pairwise distances between the two viruses are at least as distant to each other as to a group of unlinked sequences in the HIV-1 database. Dual infection can be a consequence of either co-transmission (infection with two strains at or close to seroconversion) or superinfection (subsequent HIV-1 infection of an already infected individual).

**Viral variant**: Distinct banding patterns on either HMA or HTA. The sensitivity of the method distinguishes variants if the viral diversity is on average >1 % where diversity is the mean pairwise distance calculated between all viral sequences within an individual at a time point.

**Viral diversity**: Mean pairwise distance calculated between all viral sequences within an individual at a time point.

**Viral genetic changes**: Selective pressure will be measured by estimating rate of synonymous (dS) to non-synonymous (dN) mutations, as well as the frequency of changes at positions predicted or known to affect cellular and antibody recognition. An excess of dN over dS it would indicate selective pressure in a particular region. Synonymous mutations are changes in the nucleotide sequence that do not result in an amino acid change. Non-synonymous mutations are changes in the nucleotide sequence that result in an amino acid change.

**Behavioral**

**High risk**: Prevalence rates higher than 10% and/or incidence rates greater than 3%.