

Study Number: CAPRISA 085

Identifying sources of HIV infection in adolescent girls in rural South Africa

Protocol

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List of acronyms and abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
BREC	Biomedical Research Ethics Committee
CD4 Cell count	Cluster of differentiation four cell count
CI	Confidence Interval
CAPRISA	Centre for the AIDS Programme of Research in South Africa
EIA	Enzyme Immuno-Assay
EDTA	Ethylenediaminetetraacetic acid
GCLP	Good Clinical Laboratory Practices
GIS	Geographic Information Systems
GPS	Global Positioning Systems
KZN	KwaZulu-Natal
HCT	HIV Counselling and Testing
HIPSS	HIV Incidence Provincial Surveillance System
HIV	Human Immunodeficiency Virus
HSV-2	Herpes simplex virus type 2
Lag	Limiting Antigen-Avidity
MMC	Medical Male Circumcision
NGO	Non-governmental organisation
pNAAT	Pooled nucleic acid amplification tests
PB	Peripheral blood
PCR	Polymerase chain reaction
PDA	Personal digital assistant
NIH	National Institutes of Health
PEP	Post-exposure prophylaxis
PrEP	Pre-exposure prophylaxis
PMTCT	Prevention of Mother To Child Transmission
QA/QC	Quality Assurance/Quality Control
RCT	Randomized Clinical Trial
SAG	South African Government
SOP	Standard Operating Procedure
STI	Sexually transmitted Infection
UNAIDS	United Nations Joint Programme on HIV/AIDS

1.0 STUDY SCHEMA

Study Number: CAPRISA 085 Identifying sources of HIV infection in adolescent girls in rural South Africa	
Background	Adolescent girls in South Africa remain disproportionately affected by the HIV epidemic, despite the availability of several behavioral interventions to reduce the sexual transmission of HIV. Strategies to reduce HIV transmission in this key population would benefit greatly from a better understanding of the sexual networks that drive HIV transmission in adolescent girls. Furthermore, if HIV infection rates in adolescent girls can be reduced, this could break critical chains of transmission and decrease the spread of HIV in the general population.
Purpose	The purpose of the study is to understand the HIV transmission dynamics and sexual networks that adolescent may belong to, potentially increasing their risk for HIV infection.
Study Site	Vulindlela in the uMgungundlovu municipality of KwaZulu-Natal, South Africa.
Study population	<ul style="list-style-type: none"> • School-based surveillance to recruit in-school adolescents • Respondent driven sampling to recruit out-of-school adolescents • Facility-based (PHC clinics) to recruit pregnant adolescent girls • Household sampling to recruit community members (in progress under BREC approval BF269/13)
Study Design	Cross sectional design study to include high school learners and prenatal clinic attendees. Respondent-driven sampling design to include older adolescents. Population based household survey.
Study size	The study will include individuals from <ul style="list-style-type: none"> • Schools-learners (n=8,976), • Respondent-driven sample of out-of-school adolescents (n=2,000), • Facility-based (PHC clinic) sample (n=850) • HIV Incidence Provincial Surveillance System (HIPSS) (n=20,000)
Study Time Lines	Approximately 5 years in total
Study Hypothesis	We hypothesize that complex sexual networks, including “mixing” between adolescents and community members as sexual partners, drive high HIV incidence in adolescent girls in rural KZN
Primary Objective	To identify HIV transmission linkages that contribute to the source of infection in adolescent girls in rural KZN
Secondary Objectives	<ul style="list-style-type: none"> • To identify HIV transmission linkages by age, gender and directionality • To identify transmission linkages constituting HIV-1 clusters

	<ul style="list-style-type: none"> • To characterize the genetic diversity of HIV-1 viruses in these populations • To assess the contribution and extent of HIV transmission linkages to sexual networks in adolescent girls in rural KZN. • To identify the likelihood of recent infection (identified with the Limiting antigen (LAg) Avidity EIA) and its linkage to the riskiest sexual relationships • To identify the behavioral and biological risk factors contributing to viral spread in rural KZN. • To geospatially map the clusters contributing to viral spread • To determine if any differences in viral sequences of girls who could possibly be survivors from mother to child transmission of HIV infection and therefore identifying vertical and horizontal HIV transmission.
Study measurements	<p>Behavioural measurements</p> <p>Validated, standardized, structured questionnaires will be administered to obtain detailed information on:</p> <ul style="list-style-type: none"> • Demographic characteristics • Psychosocial characteristics • Epidemiologic characteristics • Behavioural information including partnership characteristics • Knowledge of HIV status and risk factors • HIV testing history and behaviour • Sexual behaviour history • Exposure to information, education, prevention and treatment programmes for HIV • Male circumcision status (medical versus traditional) <p>The questionnaires will include age, gender, marital status, occupation, employment, educational status; epidemiologic information, HIV testing history, date of last test, HIV results, current HIV treatment, exposure to treatment for STI's, contraceptive use, male circumcision status, current location information, rural or urban, proximity to national roads, socioeconomic status. Behavioural information including sexual debut, practice of anal sex, contraceptive use (including partnership characteristics), number, type (regular/casual), concurrency of sex partners (i.e. information on last three months and or last three partners), frequency of partner change, condom use including knowledge of own and partner(s) HIV status, engagement in transactional sex, exposure to partner violence and exposure to antiretrovirals either as therapy or as pre-exposure prophylaxis.</p> <p>Laboratory measurements</p> <ul style="list-style-type: none"> • Testing for HIV antibodies • Testing for HIV-1 RNA viral load • Limiting antigen (LAg) Avidity EIA for recent HIV infection • Pooled nucleic acid amplification tests (pNAAT) for acutely HIV-infected individuals in the absence of HIV antibodies • Antiretroviral drug detection to address misclassification of LAG) and exposure to antiretrovirals. • Testing for HIV-1 viral sequences • Testing for pregnancy, sexually transmitted infections

Statistical considerations	Cross sectional study in a geographically defined region to maximise on population coverage to reveal sexual networks of the local epidemic.																																
	The Inadi ward having a population of approximately 90,000 individuals and at a population HIV prevalence of 20% we expect to have an estimated 18,000 HIV seropositive individuals. Sampling of 31826 participants for population coverage to identify HIV seropositive individuals. Through our sampling strategy we will identify approximately 5,145 HIV seropositive individuals to undertake HIV-1 viral sequencing on samples, resulting in a population coverage of almost 30% of seropositive samples. The sample sizes for the study will be as follows:																																
	<table><tr><th colspan="4">Sample size calculations</th></tr><tr><th>Population</th><th>HIV prevalence (%)</th><th>Sample size</th><th>Expected number of HIV seropositive individuals</th></tr><tr><td>School-based surveillance (females)</td><td>6</td><td>4,488</td><td>224</td></tr><tr><td>School-based surveillance (males)</td><td>2</td><td>4,488</td><td>90</td></tr><tr><td>Respondent driven sampling</td><td>30.1</td><td>2,000</td><td>602</td></tr><tr><td>Facility-based surveillance</td><td>27</td><td>850</td><td>229</td></tr><tr><td>Community sequences (HIPSS)</td><td>20</td><td>20,000</td><td>4,000</td></tr><tr><td>Total</td><td></td><td>31826</td><td>5,145</td></tr></table>	Sample size calculations				Population	HIV prevalence (%)	Sample size	Expected number of HIV seropositive individuals	School-based surveillance (females)	6	4,488	224	School-based surveillance (males)	2	4,488	90	Respondent driven sampling	30.1	2,000	602	Facility-based surveillance	27	850	229	Community sequences (HIPSS)	20	20,000	4,000	Total		31826	5,145
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Total		31826	5,145																														
Primary endpoint																																	
The primary endpoint will be HIV infection in adolescent girls phylogenetically linked to other male and female adolescents and older members in the community.																																	
Secondary endpoints																																	
The secondary endpoints will assess the contribution of the linked HIV-1 viral sequences to sexual networks and the extent of these networks.																																	
Using a combination of laboratory tests to assess the biological risk factors and their contribution to enhance sexual networks																																	
The responses from the administered questionnaires will assess the factors associated with linked HIV viral sequences and sexual networks																																	
Data Analysis																																	
Descriptive analyses																																	
Descriptive analyses will include: a description of all enrolled participants.																																	
Analysis of primary endpoint																																	

	<p>The primary endpoint will be measured through screening of <i>pol</i> gene sequences for transmission clusters and viral linkage between and amongst adolescents and community member sequences.</p> <p><i>Analysis of secondary endpoints</i></p> <p>Frequencies will be compared using T-tests and Chi-squared analyses of HSV-2, pregnancy, frequency of unprotected sex, numbers of lifetime partners, sex with older partners (≥ 5 years), and contraceptive use. Each variable will also be analysed to determine clustering of behaviours and/or HIV risk factors within social networks of individuals linked in RDS. We will analyse the length and structure of RDS recruitment chains, including the centrality of recruiters and the geographic dispersion of chains. We will identify potential bottlenecks in recruitment by comparing the prevalence of HIV-1 and sexually transmitted infections across different recruitment chains. For the facility- and school-based surveillance, frequencies and proportions will be measured for HIV serostatus in association with demographic, behavioural and biological using T- tests and Chi-squared analyses.</p>
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2.0 INTRODUCTION

2.1 Literature review and background

2.1.1 HIV epidemic in Africa

The Joint United Nations Program of HIV and AIDS estimates that globally there are a total of 35 million people living with HIV/AIDS (1). Recent trends in HIV infection also show that there have been substantial declines in AIDS related deaths largely attributable to the survival benefits of the large scale availability of antiretroviral therapy (ART) (1). Despite these gains, Africa remains the worst-affected region with Sub-Saharan Africa, home to only 12% of the global population yet carries a disproportionate burden and accounts for 71% of the global burden of HIV infection. Although the region has made substantial progress over the last several years in reducing the number of new HIV infections among adults and children with epidemiological trends showing some stabilization. However, these trends mask the continued spread of HIV and of the estimated 6000 new infections that occur globally each day (2), 60% occur in Sub-Saharan Africa (3). In this region adolescent girls aged 15-24 years bear a higher burden and have up to eight fold higher rates of HIV infection compared to their male peers (Figure 1) (3, 4). When compared to other African countries, South Africa has the largest epidemic. Despite South Africa having made impressive strides in the implementation of HIV programmes and the impact of which has begun to show substantial declines in numbers of new HIV infections in the general population (5), adolescent girls continue to acquire HIV infections at least 5 to 7 years earlier than their male peers regardless of the currently known and implemented interventions (Figure 2) (1, 4, 6).

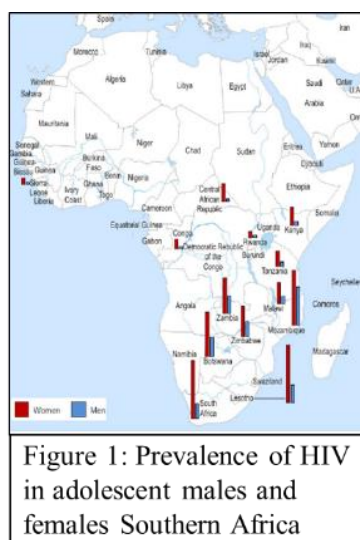


Figure 1: Prevalence of HIV in adolescent males and females Southern Africa

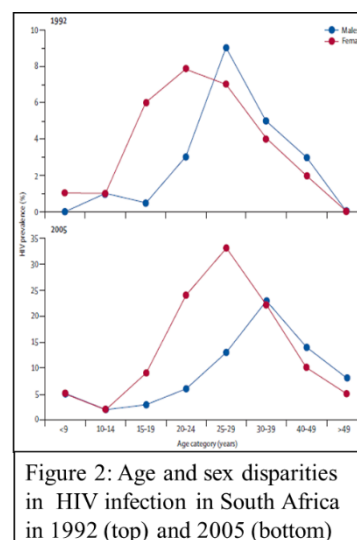


Figure 2: Age and sex disparities in HIV infection in South Africa in 1992 (top) and 2005 (bottom)

2.1.2 HIV in adolescent girls in KwaZulu-Natal

The province of KwaZulu-Natal (KZN) consistently experiences the most severe HIV epidemic. Representative sampling in several urban and rural districts of KZN show that approximately 40% of pregnant women are HIV-infected (7, 8), whilst the 2012 South African Household Survey report an HIV prevalence of 25.8% in KZN in the 15-49 year age group compared to 16.9% nationally (9).

HIV prevalence in pregnant adolescent girls in rural KZN: The primary health care (PHC) facility-based HIV surveillance system established in Vulindlela, rural KZN in 2002 to monitor the epidemic in pregnant women has been important to understand the evolving epidemic in this region. These surveys conducted annually over a 14 year period showed that i.) at least 30% of pregnant women were less 20 years in age, ii.) The overall HIV prevalence increased from 35.3% (95% CI 32.3-38.3) in the period 2001-2003 to 39.0% (95% CI 36.8-41.1) in the period 2004-2008 to 39.3% (95% CI 37.2-41.4) in the period 2010-2013; iii) HIV prevalence in the <20 year age group was 22.5% (95% CI 17.5-27.5) in the period 2001-2003 and declined slightly to 20.7% (95% CI 17.5- 23.8) in the period 2004-2008 to 17.2% (95% CI 14.3-20.2) in the period 2009-2013. Whilst these data show slight declines in HIV prevalence in the <20 year age group, prevalence remains exceptionally high among pregnant adolescent girls.

HIV incidence in adolescent girls in rural KZN: The spread and changes over time of HIV is tracked through measuring the rate and distribution of new HIV infections (incidence) in a population and is a

key measure of the impact of HIV prevention programmes. The gold standard method for estimating HIV incidence is through prospective cohort studies that measure the rate of new infections in a well-defined group of at-risk individuals followed over time. A key prospective cohort study among young women 14 to 18 years of age recruited from rural Vulindlela showed the HIV incidence rate to be 4.7 (95% CI 1.5–10.9)/100 women-years (wy) (10). HIV incidence remains exceptionally high in this rural (Vulindlela) setting and amongst sexually active women in the CAPRISA 004 tenofovir gel trial, the overall HIV incidence rates in the placebo arm in women 18 to 40 year of age was 9.1/100wy (95% CI 6.6-12.3) (11) and in women 18-19 and 20-24 years of age, the HIV incidence rates were 6.4/100wy (95% CI 2.6-13.1) and 12.1/100 wy (95% CI 7.9-17.7) respectively (unpublished data from the CAPRISA 004 trial). Thus, it is clear from several data sources that HIV incidence rates remain high in this rural setting of KZN. Even with higher access to HIV treatment, which has been shown to prevent HIV transmission in randomized clinical trials (12) and observational cohorts (13), high HIV incidence persists in KZN (10, 11). Thus, adolescent girls in KZN, are at high risk of being infected with HIV.

HIV prevalence in adolescents in schools in rural KZN: Young people commence secondary school around age (14) years, however, majority of learners even above 20 years are still in school (15) due to underperformance, despite educational legislation and reforms (14). As majority of young people <24 years of age are still in schools, limits our ability to recognize any developing trends in HIV infection rates over time. Expanding HIV surveillance to young at-risk populations would provide an opportunity on emerging trends of prevalence in younger age groups. Furthermore, poor school completion rates (16) and high pregnancy rates serve as a risk for HIV acquisition (17). South Africa is one of few countries that has a supportive legal framework to enable young children to access sexual reproductive health (SRH) services including HIV counseling and testing (HCT) autonomously from age 12 (18). This is in recognition of the fact that many children are sexually active at a very young age and the risk of HIV acquisition increases through changes in individual behavior within risky sexual networks and political, economic and social factors. Though there are many ethical and programmatic challenges on the implementation of these services, both Departments of Health and Education have made strategic investments to reduce HIV acquisition in this target group; a high priority and have taken SRH and HCT campaigns to young people at high schools (19). Within this context, the surveillance in schools' was undertaken to understand whether the high HIV prevalence in pregnant girls in PHC clinics was similar to that in non-pregnant girls. The results showed that gender disparity in HIV prevalence in learners was clearly apparent across several schools, with substantially lower rates in boys (range, 1.4% to 2.5%) compared to girls (range, 3.2% vs. 7.7%) (17). Surveillance in schools has found variable but high HIV prevalence in learners and higher at 6.8% in girls compared to 2.7% in boys (Table 1) (20).

Table 1: Age Specific HIV prevalence in high school girls and boys in Vulindlela, 2012		
Age group years	Girls N=1698 % (95% CI)	Boys N=1543 % (95% CI)
12-15 years	4.6 (1.9-7.3)	2.7 (0.6-4.9)
16-17 years	5.3 (0.8-9.9)	1.9 (1.2-2.6)
18-19 years	8.7 (0-17.7)	3.4 (0-8.6)
≥ 20 years	23.1 (7.7-38.5)	11.1 (2.7-19.4)
Total	6.8 (3.9-9.8)	2.7 (1.6-3.8)

Cumulatively, data from several levels of HIV surveillance suggest that the HIV infection risk before the age of 24 in women is substantial, with two-thirds of women who eventually acquire HIV doing so before the age of 25 (5, 6, 21). However, there is emerging evidence that not all girls are at equal risk and given the scale of the epidemic in this region, the need for more targeted HIV prevention interventions is a pressing need to decrease HIV rates.

2.1.3 Adolescent girls vulnerability to HIV

Young people are at the center of the global HIV/AIDS epidemic and they are also the greatest hope in the struggle against HIV. Several factors contribute to the increased vulnerability of young people and in particular adolescent girls to acquiring HIV. Biological factors play an important role in HIV acquisition in adolescents. The risk of acquiring HIV during unprotected vaginal intercourse is always

greater for women than men; and the risk for adolescent girls is further heightened because their vaginal tracts are immature and tissue tears occur far more easily (2). Several studies have consistently shown the association between early sexual debut and HIV (3, 22, 23) and in many of these studies, the increase in risk did not seem to be due to specific behavioural risk characteristics of the individuals or their sexual partners, suggesting that the risk may relate more to the potential of genital trauma. Early sexual debut is further associated with future risk taking behaviour of potentially having multiple and/ or older partners and associated exposure to sexual violence (2).

More recent data suggests that inflammation in the female genital tract is emerging as an important risk factor for acquiring HIV. In the CAPRISA 004 trial, women with genital inflammation had an almost three-fold higher risk of HIV acquisition (24). The risk of acquiring HIV also increases in the presence of sexually transmitted infections (STIs) (25). Majority of STIs occur in young people under 25 years of age. The presence of STIs, particularly those that cause genital ulceration or inflammation, has been shown to play an important role in the transmission and acquisition of HIV by increasing the infectiousness of HIV-infected individuals and the susceptibility of HIV-uninfected individuals facilitating and enhancing HIV transmission between sexual partners. Therefore, interventions to treat and prevent STIs are an important step in breaking the chains of HIV transmission. Whilst repeated exposure and ulcerative STIs increase HIV transmission almost seven fold (2), majority of STIs either do not produce any symptoms or signs, especially in women, or produce symptoms so mild that they are often disregarded. Some STIs may disappear over time creating the false impression that the disease has disappeared. Importantly, young people may not be able to differentiate between normal and abnormal conditions, or may be embarrassed to seek treatment, or there may be deficiencies in the services for young people and therefore fail to seek adequate care and treatment (2).

Structural factors such as poverty and lack of schooling opportunities increase adolescent girls risk in HIV acquisition (2). Many adolescent girls living in poverty are often coerced into sex with older partners in order to stay in school to support themselves and their families. Because of circumstances around themselves, adolescent girls lack power to negotiate safer sex practices making them vulnerable to HIV and other STIs. Educating young people about HIV, and teaching them skills in abstinence and safe sex negotiation potentially improves their self-confidence and decision-making with informed choices on postponing sex until they are mature enough and consequently protect themselves against HIV, STIs and pregnancy.

When HIV spreads to the wider population, the number of infections rise rapidly. The epidemic in southern Africa where, despite HIV being rare prior to 1990, the prevalence is at an unprecedented high level—in excess of 15% in the general population—and new infection rates higher than 5% per year occur despite high and increasing morbidity and mortality rates (26). The province of KZN experiences this special and unique typology of generalized hyper endemic epidemic, with all sexually active persons having an elevated risk of acquiring HIV in these settings. The consequence of this epidemic typology is the higher burden of HIV in young mothers with a large number of children orphaned by AIDS. These children have been at a greater risk sexual exploitation increasing their vulnerability to HIV acquisition. These children experience further stigma and discrimination and consequently denied further education, work and basic needs. Adolescent girls are likely to drop out of school to care for siblings or face further discrimination as they are less likely to afford school fees.

Overall, many adolescent girls remain vulnerable to HIV through multiple complex reasons and require exceptional support to mitigate their vulnerability at multiple levels.

2.1.4 HIV prevention options for adolescent girls

Whilst the HIV prevention field has evolved rapidly over the last five years with numerous interventions to prevent HIV acquisition available, these are not necessarily designed and readily accessible for young girls in relation to the magnitude to the HIV burden in this key population. Several behavioral interventions have been implemented to reduce the sexual transmission of HIV, including programs aimed at delaying sexual debut, preventing age disparate and intergenerational sex, promoting medical male circumcision, and condom use within the structured ABC guidelines (Abstinence, Being faithful

to one's partner, and Condom use) (5). HIV prevalence remains persistently high in adolescent girls. Despite the testing of these programmes through several innovative models to enhance knowledge of HIV status, access HIV prevention and treatment programs and minimize stigma and discrimination in association with HIV, knowledge of HIV status remains low to prevent onward transmission (1, 5).

Recent breakthroughs from several randomized clinical trials of testing of ARV-based vaginal microbicides, oral pre-exposure prophylaxis (PrEP) and early ART initiation have transformed the HIV prevention agenda and provide hope in reducing the risk of acquiring HIV. Clinical trials on newer ARVs with alternate delivery mechanisms are currently underway (27) and the role of potent broadly neutralizing monoclonal antibodies are being explored as newer HIV prevention interventions (28-30). These interventions would fill an important gap as HIV prevention options for young women and impact on new HIV infections. The major challenge of these promising interventions is that they are not yet licensed in Sub-Saharan Africa for public sector use. Whilst vaginal microbicides and oral PrEP are urgently needed as behaviors are difficult to modify, effect and sustain, their effectiveness is largely dependent on risk perception, uptake of interventions and adherence to interventions, further complicated by genital inflammation with increased concentrations of HIV target cell-recruiting chemokines and a genital inflammatory profile contributing to HIV acquisition. More importantly these interventions have not been tested in adolescent girls <18 years of age who are highly vulnerable and at high risk of HIV.

Due to the limited, well tested biomedical interventions for adolescent girls; and prevention being key to reducing HIV infection rates, interventions should be locally relevant and tailored to this specific age group. As majority of young people are still in schools, schools are an important venue and provide an opportunity for the provision of good quality education which includes life skills training, essential information and skills to protect themselves against HIV, STIs and unintended pregnancy such that young people receive the tools and incentives to adopt safe behaviour and are capable of making responsible choices to protect themselves.

Despite the availability of school based programmes, many adolescent girls remain at high risk for HIV acquisition and as such, it is important to understand why and with whom they are having sex with to better understand HIV transmission dynamics.

2.1.5 Need to understand HIV transmission dynamics in adolescent girls

HIV transmission is driven in part by individual level high risk behaviors which are well recognized; though these explain only a fraction of the variation in HIV infection risk, and recent studies have increasingly emphasized the role of sexual networks and HIV infection chains as important determinants of individual and group-specific variation in HIV infection risks (20, 31). Despite this emphasis on sexual networks in recent studies and HIV prevention policies, the risks revolving around sexual networks continue to be somewhat poorly understood in KZN and other regions for various reasons. Among these include mis- and under-reporting of sexual relationships, and social desirability bias (31). Adding to this, evidence on sexual networks is often controversial; the interactions between network characteristics and HIV risks is incompletely documented; and many studies being affected by problems of measurement. For example, similar sexual risk taking behavior could lead to different levels of HIV risk, depending on the nature of sexual partnerships of the partners of adolescent girls, including sequential and/or concurrent sexual relationships (32, 33). Therefore, if the risk of HIV is reduced; infection rates in women under 24 years can be reduced and this could break the critical chains of transmission and decrease the spread of HIV in the general population.

2.1.6 Phylogenetic analyses of HIV-1 sequences to understand HIV transmission dynamics in adolescents

Sexual networks are poorly understood, the transient nature of risky relationships, and various cultural factors are other determinants. Traditional methods to map sexual networks are labor-intensive and require extensive engagement with participants and verification of relationships (31). One alternative is to use HIV-1 sequence data for phylogenetic analysis as a tool to gain information on networks that is

otherwise difficult to obtain. The concept of phylogenetic methods has been useful in understanding HIV-1 viral diversity (34, 35) and viral transmission (36-38). This technique takes advantage of the high error rate and rapid pace of HIV replication (39), which leads to a predictable rate of evolution over long periods of time, or a ‘molecular clock’. Phylogenetic trees can be generated and used to determine the most recent common ancestor in a given set of sequences, revealing linkages that define their transmission history. These methods have been used to determine the emergence of HIV globally from the non-human primate reservoirs, estimate the age of Simian Immunodeficiency Virus (SIV) (40, 41) and map the spread of HIV between continents early in the epidemic (42). Phylogenetic methods have been important for studying HIV-1 transmission between individuals (43) and phylogenetic linkages have empirically been assessed in HIV-1 clinical trials as in the HPTN 05221 and Partners in Prevention HSV/HIV Transmission trials (36). The analysis of HIV-1 *pol* sequences from HIV-1 seroconversions showed that at least a quarter of these occurring within stable partnerships were unlinked and demonstrate the magnitude of sexual networks that potentially sustain epidemics. In addition, several forensic cases have utilized phylogenetics as a method to establish both intentional and unintentional HIV transmission (44-46).

Recently, phylogenetic methods have been applied to the understanding of HIV transmission more generally, especially in Western countries among men having sex with men (MSM) to detect transmission clusters leading to outbreaks (47-50). Fewer studies have been carried out in Africa (38, 51). However, based on the phylogenetic analyses of the *gag* and *env* genes, a major study conducted in the Rakai district of Uganda provided some indication that viral transmissions occurred beyond household partnerships (38) and were common from outside the communities. In South Africa, phylogenetics has been used by our research group to identify circulating HIV-1 subtypes among the MSM population in Cape Town (52). Applying phylogenetics to understand HIV transmission in the areas most affected by the pandemic has rarely been done successfully, and could assist with optimal direction for targeted interventions to interrupt viral transmission.

Phylogenetic analysis of HIV sequences from five schools in Vulindlela:

During our school-based work, we carried out pilot studies to characterize HIV-1 sequences from 5 schools in relatively close geographical proximity. During HIV surveillance of both boys and girls (n=3,242), a response rate of 82% was obtained for HIV testing. Of these, 148 individuals were HIV-infected, and 106 (72%) of the samples obtained from these individuals could be characterized virologically. The reconstructed phylogeny showed significant groupings (figure 3) with support values >90%. Five clusters were observed. Only two of these consisted of a male and a female, while the remaining three were male-male (1 cluster) and female-female (2 clusters). There was little evidence of widespread within-school transmission (2 clusters), and some evidence for linked sequences between schools (3 clusters). It should be highlighted that for each dyad, the direction of infection, and/or an upstream source of infection, remains unknown, as it would require an expanded knowledge of the wider network. One interpretation of these pilot data is that transmission dynamics will be better understood by sequencing HIV from individuals in the community, and that networks that contain individuals who are outside of school are high risk adolescent girls in school. Expansion of the HIV-1 sequence phylogenetic analyses may reveal linkages indicative of the size and nature of sexual transmission networks and improve understanding of the epidemiological attributes of individuals in the networks.

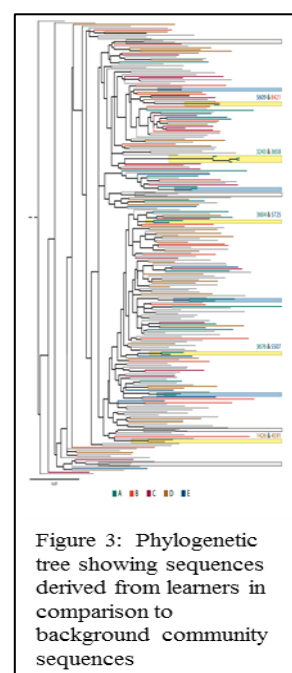


Figure 3: Phylogenetic tree showing sequences derived from learners in comparison to background community sequences

2.1.7 Behavioural and Biological risk factors to understand HIV transmission dynamics in adolescents

Understanding structural, behavioral and biological risk factors of HIV acquisition are key to design of HIV intervention programmes. However, it is imperative to understand the structural features of risk in HIV transmission in sexual networks as very little work has been undertaken on characterizing these

structures. Large complex studies are required to understand these risks and therefore there is no data for a clearer understanding on these complex interactions of risks. Furthermore, the intersection of these risks with phylogenetic linkages is also not known. Underlying epidemiological and social determinants driving the high prevalence in young girls is critical to understand the extent and complexity of the sexual networks as primary mechanisms through which HIV is likely to be spread. In Vulindlela, a large proportion of women report to be in monogamous relationships and therefore underestimate their perceived risk even though many of them are aware that their current partner has other partners (53). Moreover, the interaction of early sexual debut, low condom use, multiple sex partners either concurrently or sequentially, age disparate relationships enhance the transmissibility and propagation of HIV in such heterosexual relationships. However, paucity of in depth data on the contribution and the interaction of the drivers of the epidemic may not well be measured and therefore fail to accurately determine association and temporality. Key HIV transmission chains have been studied in large-scale population-based studies, but current evidence about the sources of HIV infection for girls remains incomplete. The geographically defined region of Vulindlela, the highest HIV burden region and with a high proportion of the target adolescent population permits us to extend our assessments on the relevance and the proportion of HIV attributable to the behavioral and biological components to understand the complexity of HIV transmission dynamics.

It is clear that adolescent girls are a key population for HIV prevention (54, 55) and understanding of HIV transmission dynamics in this population is a major gap in the knowledge of HIV epidemiology.

2.2 Study Justification

It is well recognized that adolescent girls remain disproportionately affected by the HIV epidemic. Despite of our understanding of the high disease burden, risk factors for HIV acquisition and our experience in testing HIV prevention interventions, and the successful roll out of antiretroviral treatment, HIV incidence rates remain disturbingly high in adolescent girls in this area.

The study will make the following contributions to understanding the HIV transmission dynamics in adolescents towards the development of targeted HIV prevention intervention in this important group.

- (i) The study will be the first study to understand HIV transmission dynamics in a geographically defined high HIV burden setting
- (ii) The study will improve on prior phylogenetic studies of HIV-1 transmission networks in several ways.
 - a. First, whereas health facility-based studies have been affected by large selection biases (i.e. only sampling individuals who attend the facility), we propose to enhance the representativeness of the phylogenetic analyses by combining novel recruitment strategies including clinic- and school-based recruitment, and respondent-driven sampling for hard-to-reach populations.
 - b. Second, prior genetic studies of HIV-1 transmission networks have rarely included information on the behaviors and other risk factors of cluster members. Here we propose to supplement the phylogenetic data with detailed additional data collection on the behavioral and biological risk providing unique new information.
- (iii) The study design will allow us to characterize the transmission clusters and their members, describe the network characteristics of these transmission chains, and analyse the contribution of HIV clusters to the new infections occurring among adolescent girls.
- (iv) The data we obtain on HIV transmission will be used in future work to develop and test a focused combination intervention package aimed at reducing HIV transmission in adolescent girls, the highest risk group in this region.

2.3 Audience and Stakeholder Participation

Stakeholders include the KZN Departments of Health, Education and Social Services, schools learners, educators, principals, schools governing boards (SGB), including parents and guardians. HIV and AIDS Directorate from the Office of the Premier, the district offices of the PEPFAR and its implementation partners. Local non-governmental implementing partners in the district involved in the

implementation of prevention programmes, other donors and the population of the district will also participate.

2.4 Hypotheses

We hypothesize that complex sexual networks, including mixing between adolescents and community members, drive high HIV incidence in adolescent girls in rural KZN

3.0 STUDY OBJECTIVES

3.1 Primary objective

To identify HIV transmission linkages that contribute to the source of infection in adolescent girls in rural KZN

3.2 Secondary objectives

- To identify HIV transmission linkages by age, gender and directionality
- To identify transmission linkages constituting HIV-1 clusters
- To characterize the genetic diversity of HIV-1 viruses in these populations
- To assess the contribution and extent of HIV transmission linkages to sexual networks in adolescent girls in rural KZN.
- To identify the likelihood of recent infection (identified with the Limiting antigen (LAG) Avidity EIA) and its linkage to riskiest sexual relationships
- To identify the behavioral and biological risk factors contributing to viral spread in rural KZN.
- To geospatially map the clusters contributing to viral spread
- To determine if any differences in viral sequences of girls who could possibly be survivors from mother to child transmission of HIV infection and therefore identifying vertical and horizontal HIV transmission.

4.0 METHODS

4.1 Study Design

The study design is cross sectional and will utilize an innovative approach to identify networks of HIV transmission that combines extensive epidemiologic sampling with phylogenetic analyses of HIV-1 sequence data and traditional sexual networking methods. Phylogenetic analysis have recently emerged as powerful and informative methods to use viral diversity to examine the underlying dynamics of HIV-1 transmission in affected populations. Due to the underrepresentation of transmission pairs in large populations of HIV infected individuals; studies have often failed to find linkages in endemic settings. This study will identify HIV-1 clusters and viral linkages in adolescents and link these to the community sequences by extensively sampling HIV infected individuals from a defined geographic area, using several epidemiological approaches to achieve a very high population coverage. This area is uniquely suited for this research as the study area is rural, geographically well-defined and is one of the highest HIV burdened districts in South Africa.

4.2 Study Setting

Vulindlela is a rural community situated to the west of Pietermaritzburg and northwest of the Greater Edendale area within the boundaries of uMsunduzi and uMgeni municipalities in the uMgungundlovu District. The sub district is approximately 28 000 hectares in extent. The majority of the land belongs to the traditional authority through the iNgonyama Trust and is made up of 9 wards, of which 5 are under the traditional leadership of the Amakhosi and

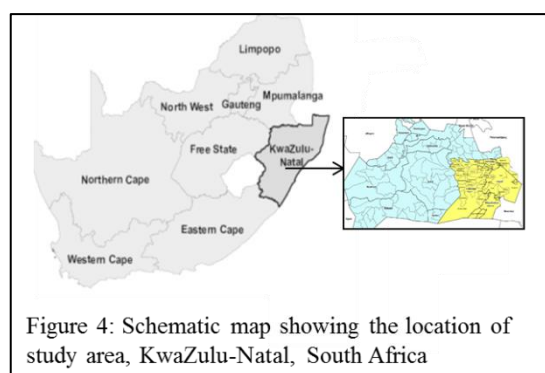


Figure 4: Schematic map showing the location of study area, KwaZulu-Natal, South Africa

4 are under the ward counsellors of the local government municipal system. The study area is the Inadi Ward (Ward 9) within Vulindlela which has a population of just over 90 000 and is predominantly Zulu speaking. There are six primary health care (PHC) clinics in the Vulindlela and trained nurses provide comprehensive primary health care, including family planning services, voluntary HIV counselling and testing, sexually transmitted infection (STI) treatment, antenatal care, treatment of opportunistic infections and minor ailments. The PHCs are linked by ambulance to the regional referral hospitals, Grey's Hospital (about 30 minutes away) providing optimal tertiary level of health care to people of the Western area of KZN and Edendale Hospital (about 20 minutes away) a Regional and District level hospital providing comprehensive services. In addition, there are several community based organizations in the district representing a variety of civic interests such as youth, women, religion, politics, and housing. Several of these organizations are currently providing HIV prevention and home-based care services to these communities and have links with the CAPRISA Vulindlela Clinical Research Site.

As part of the on-going epidemiological studies, CAPRISA has monitored the HIV prevalence in pregnant women in Vulindlela. The prevalence increased from 32.4% (95%CI 27.6-37.6) in 2001 to 40.0% (95% CI 35.2-44.8) in 2010. About a third of pregnant women surveyed were less than 20 years of age and about 20% were already HIV infected. The HIV epidemic in this district is being fueled by high incidence rates, estimated at 11.2% per annum in young women under the age of 20 years. Between March 2004 and February 2005 we assessed the feasibility of establishing sexually experienced cohorts in Vulindlela. Results indicate that of the 981 volunteers, 14-30 years of age, 35.7% (95% CI 32.7–38.8) were already HIV positive and the HIV incidence rate was 6.5 (95% CI 4.4–9.2) /100pyo. These data underscore the persistently high HIV incidence rates in young women. The impact of HIV infection in this community was apparent by the disproportionately high AIDS related mortality rate of 5.2 (95% CI 2.9-7.5) /100pyo in young women 20-24 years of age (56)

4.3 Study Population

The study population will be drawn from the Inadi ward, a geographically defined region of Vulindlela through:-

- School-based surveillance to recruit in-school adolescents
- Respondent driven sampling to recruit out-of-school adolescents
- Facility based (PHC clinics) to recruit pregnant adolescent girls
- Household sampling to recruit community members (in progress under BREC approval BF269/13)

4.3.1 School-based study population

As majority of adolescent girls are still in schools, schools provide an ideal opportunity to undertake HIV surveillance to understand emerging trends and to re-inforce HIV information, education and prevention messaging. Although some concerns have been raised about cognitive ability of adolescent to provide first person consent (57), many young people are sexually active and access health services through school based programmes to obtain contraceptives, manage STI and access HCT (18, 58). We have over years enhanced informed consent processes by having separate HIV and study related information sessions and allowing sufficient time for participants to consider their study participation. The school-based surveillance will be conducted strategically in the ten schools in the geographically defined Inadi ward.

Inclusion criteria:

- All learners 12 years and older in the selected secondary schools
- Willing and able to provide informed consent and/or assent to participate in the study
- Willing to participate in this study
- Willing to complete all study procedures
- Willing to provide clinical samples

Exclusion Criteria

- Refusal by the learner and/or parent or legal guardian to participate in the study.

- Unable to provide necessary informed consents
- Cognitively challenged learners

4.3.2 Respondent-driven sampling (RDS)

RDS is an established recruitment method that increases the representativeness of sampling by providing primary and secondary incentives for participation/recruitment, while limiting the number recruited by each ‘seed’, and linking individuals in recruitment waves. This technique is particularly useful for recruiting hard-to-reach populations that are best known by similar peers.

Two separate RDS will be conducted in

Adolescent girls: The following specific considerations to this approach include: Seeds (n=8) will be carefully selected through HIV counseling and testing (HCT) facilities, and will act as the initial recruiters for the study. Seeds will vary in age, geography, school/occupational status, and pregnancy history. The RDS will target out of school adolescents girls.

Community men: Seeds (n=8) will be recruited through a HCT facility targeting men at Taxi ranks and outside of Shebeens (a makeshift tavern for socializing over alcoholic drinks). RDS theory suggests that after a certain number of recruitment waves, the sample becomes independent (i.e. no longer related) of the initial choice of seeds. These will be given to participants at enrollment (primary) and for each successful recruit who qualifies for the study (secondary). The nature of the incentives is essential to encourage appropriate recruitment, and therefore the amount and format (shopping voucher, airtime, other gift, etc.) will be piloted prior to commencing the full study. The recruitment message will be carefully considered to prevent recruiter-participants from seeking the incentive by ‘coaching’ recruits to meet the perceived study criteria. Therefore, we will use some amount of ‘direction’ (i.e. sexually active and 12 years and older), while ‘mentioning’ out-of-school girls, if they know any. Each participant will be asked to recruit eight other participants.

Inclusion criteria:

- Referred through the RDS recruitment strategy
- 12 years and older not attending school
- Have ever had penetrative sex (peno-vaginal and / or peno-anal)
- Willing and able to provide informed consent to participate in the study
- Willing to participate in this study
- Willing to complete all study procedures
- Willing to provide clinical samples

Exclusion criteria:

- Refusal by individual to participate in the study.
- Unable to provide necessary informed consents
- Cognitively challenged individual

4.3.3 Facility-based study population

Given the high HIV prevalence observed in pregnant adolescent girls, we will continue our existing facility-based surveillance activities conducted annually amongst all first visit prenatal clinic attendees at the PHC clinics in the study area.

Inclusion criteria:

- Pregnant adolescent girls ≥ 12 years ≤ 24 years
- Attending any of the 6 PHC clinics in the district
- Willing to participate in this study
- Willing and able to provide informed consent and/or assent to participate in the study
- Willing to complete all study procedures
- Willing to provide clinical samples

Exclusion criteria:

- Refusal by individual to participate in the study.
- Unable to provide necessary informed consents
- Cognitively challenged individual

4.3.4 Community-based study population

In addition to the above, the study will utilize data from the HIV Incidence Provincial Surveillance System (HIPSS), a study which is currently on going, (Study number, CAPRISA 251 – BREC approval number BF269/13). HIPSS is a longitudinal study that is monitoring HIV incidence trends in a rural KwaZulu-Natal, South Africa to enhance surveillance efforts in high prevalence areas. HIPSS platform has been established in two adjacent sub-districts of Vulindlela and Greater Edendale in the uMgungundlovu municipality. This study obtains a household-based representative sample of both men and women, with the primary objective of estimating HIV incidence. Laboratory based methods measure HIV incidence from the cross sectional surveys whereas the cohort method establishes HIV incidence from individuals sampled one year later. The study estimates changes in the rate of new HIV infections from year to year. Other secondary objectives of this study include measuring levels of transmitted HIV drug resistance and carrying out HIV-1 phylogenetic sequencing to determine whether there is empirical evidence for the localized clustering of HIV infections of varying intensity. The study also determines the association of transmission clusters and viral linkages in HIV infected individuals with early or chronic stages of HIV infection diagnosed on the Limiting antigen (LAG) Avidity EIA. The phylogenetic sequence analyses from HIPSS will provide a robust number of community based HIV-1 sequences to effectively merge, triangulate and link to sequences from adolescents in the same area.

Inclusion criteria:

- All available HIV-1 sequences from HIV positive participants in HIPSS.
- Participants who have provided written consent for sample storage and future testing.

Exclusion criteria:

- Participants who have not provided written consent for sample storage and future testing.

4.4 Participant Eligibility

Volunteers from the study area will be eligible for inclusion in this study and must meet the inclusion and exclusion criteria defined in the respective sections 4.3.1 to 4.3.4

4.5 Study Procedures

4.5.1 Recruitment procedures

School based recruitment: Prior to study commencement, study staff will collaborate with the Departments of Health and Education to obtain all the require approvals. Consultative meetings will be held with all the principals, educators and learners from each of the selected schools. In addition members of the schools governing boards (SGB) and parents or guardian of learners will be provided with information on the background and epidemiology of the HIV epidemic in this community, the sustained high HIV incidence, pregnancy and STI rates among adolescents, the impact of these on individuals and communities. Specific information in relation to the study will be provided.

Each of the 10 schools will be visited over a three week period by a team of trained nurses and counsellors. In each of the classrooms learners will be provided with general information on HIV/AIDS risk, prevention and treatment and specifically on the purpose of the study, the implications of participation, the informed consent process, study and sample collection procedures, use of the results and confidentiality of data will be provided to learners. Should learners require additional information, this will be provided in small groups of 10-15 learners or on a one on one basis. Learners willing to participate will provide individual informed consent and continue with study related procedures.

Respondent-driven sampling recruitment: out of school adolescent girls and community men will be recruited through the RDS sampling strategy (**Appendix 1:RDS recruitment tools**). The first eight participants will be recruited through and HCT facility or through word of mouth. These eight individuals once enrolled will serve as seeds and asked to identify, recruit and refer volunteers whom they are aware of being sexually active. The RDS will weight the sample to compensate for the fact that the sample is collected in a non-random way. Key information will be collected on

- Personal Network Size (Degree) - Number of people the respondent knows within the target population.
- Respondent's Serial Number - Serial number of the coupon the respondent was recruited with.
- Respondent's Recruiting Serial Numbers - Serial numbers from the coupons the respondent is given to recruit others.

Facility-based recruitment: PHC clinic staff will be trained to identify pregnant adolescent girls ≥ 12 and ≤ 24 years who are accessing antenatal care for the first time for the current pregnancy. These individuals will identify and refer volunteers whom they are aware of being at high risk for HIV.

Community-based recruitment: Community members have been recruited through the HIPSS protocol (BREC approval number BF 269/13).

All volunteers through each of the recruitment strategies will be provided with information on the background and epidemiology of the HIV epidemic in this community, the sustained high HIV incidence, pregnancy and STI rates among adolescents, the impact of these on individuals and communities. Specific information in relation to the purpose of the study, the implications of participation, the informed consent process, and study and sample collection procedures, use of the results and confidentiality of data will be provided to learners. Volunteers willing to participate will provide individual informed consent and / or assent (**Appendix 2: Informed consent and Assent forms**) and enrolled into the study.

4.5.2 Schedule of assessments

The schedule of study assessments and procedures are shown in Table 2.

Table 2: Schedule of study assessments and procedures	
Measurement	Cross Sectional study
Informed consent/ Assent	X
Demographic data and locator information and fingerprinting	X
Questionnaire administration	X
Sample collection <ul style="list-style-type: none"> • Dried blood spot samples (males and females), • first-pass urine (10-20 mls) (males) • self-collected vulvo-vaginal swab samples (females) 	School based study population RDS Facility based sample Community based sample (ongoing)

4.5.3 Enrolment procedures

Following written consent, all study related enrolment procedures will be undertaken. Enrolled participants will be reassured that no personal identifiers will be documented on any study related data collection instruments. Each participant will be assigned a unique study number that will be linked to the structured questionnaire to be administered by study staff.

We will use biometric fingerprint scanning and all eligibly enrolled participants will be asked to provide a finger-print to identify individuals who could potentially be included through the multiple sampling strategies to be identified and analyzed accordingly. The participants will be informed on the purpose of the collection of the finger-print and that these will not be used for any other purposes. Finger-prints will be stored with an encrypted code to maintain confidentiality.

Trained staff will

- Use a handheld personal digital assistant (PDA) to administer the questionnaire.
- Collect DBS samples

- Male participants will be guided to collect 10-20 mls of first-pass urine sample
- Female participants will be guided to self-collected vulvo-vaginal swab samples.

4.5.4 Biological sample collection and processing procedures

Collection and shipping of specimens

All samples collected will ensure proper collection, processing, labelling, and transport of specimens to the laboratories.

Sample Storage and Possible Future Research Testing

All remaining DBS sample collections cards collected from each study participant at the time of study entry will be stored. In addition, study participants will be asked to provide written informed consent and assent for their samples to be stored beyond the end of the study for possible future research testing. Any residual samples 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 and quality assurance testing has been completed.

Quality Assurance of Laboratory testing

All testing will follow laboratory specified protocols which include quality checks and assurance programmes. Procedures for sample management (e.g. chain of custody, handling, labelling and transport), assay procedures, proficiency testing and quality assurance procedures and sample storage procedures and good laboratory practices (GLP) will be followed for all laboratory testing. Procedures will be in place for performing and documenting the quality of a sample, including storage under appropriate temperature conditions and transport conditions, monitoring of equipment and temperatures, and function indicators. Each stored sample will have a unique identifier which will be unlinked from the study participant's name. The samples will be stored indefinitely.

Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all study staff in the collection of samples, shipping and handling of all specimens for this study, as currently recommended by South African guidelines on biohazard containment.

4.6 Study Measurements

4.6.1 Laboratory measurements

As described in section 4.5.1 samples collected from participants will have the following measurements (**Appendix 3: HIV antibody testing algorithms and Appendix 4: HIV incidence testing algorithms**)

HIV antibody measurement: All samples will be tested with 4th generation HIV enzyme immunoassays (EIAs) to test for HIV antibodies and antigens.

HIV-1 RNA viral load measurement: Participants who test HIV seropositive will have individual HIV-1 RNA viral load measurements.

Recent infection HIV infection measurement: Participants who test HIV seropositive will be tested for recency by the Limiting antigen (LAg) Avidity EIA.

Pooled nucleic acid amplification tests (pNAAT) measurement for acutely HIV-infected individuals in the absence of HIV antibodies: Participants who test HIV EIA negative will have the pNAAT assay performed following pooling of 10 samples. Any sample pools testing positive will be disaggregated for individual quantitative testing of HIV-1 RNA. pNAAT will be used in this study to identify individuals with acute HIV-infection who have not seroconverted and therefore do not have detectable antibody. The use of pNAAT for HIV RNA detection is to account for acutely HIV-infected individuals in the absence of HIV antibodies.

Antiretroviral drug detection: testing will be performed on all HIV antibody positive samples irrespective ART use, by means of High Performance Liquid Chromatography (HPLC) coupled to Tandem Mass Spectrometry for the qualitative detection of Efavirenz, Emtricitabine, Lamivudine, Lopinavir, Nevirapine, Tenofovir and Zidovudine Results were reported as positive or negative.

Testing for pregnancy: Pregnancy testing will be conducted for female participants, using a sensitive blood BHCG quantitative assay (Siemens Centaur XP, USA)

Testing for sexually transmitted infections: will be conducted retrospectively on stored samples on first-pass urine sample from males and on self-collected vulvo-vaginal swab samples from females. DBS samples will be screened for active syphilis using the qualitative Rapid Plasma Reagin (RPR) test. All reactive samples will undergo testing with the quantitative RPR and further testing with the *Treponema pallidum* haemagglutination test (TPHA). HSV-2 antibodies will be measured using the Kalon HSV-2 ELISA test.

Testing for HIV-1 viral sequences

DNA extraction: Nucleic acids will be extracted from 3 mm-diameter punched out DBS of HIV positive samples using the QIAamp® DNA Mini kit (Qiagen).

HIV-1 viral sequencing: The *pol* gene region has been used to investigate transmission linkages and has the added advantage as it provides information on the prevalence of drug resistance mutations. We will generate ~1700 bp *pol* gene sequences, and confirm linkages on partial *env* sequences. Nucleic acids will be extracted from 3 mm-diameter punched out DBS and or using plasma of HIV positive samples using the QIAamp® DNA Mini kit (Qiagen). Amplicons for sequencing will be generated using one-step reverse transcription-PCR and nested PCR. PCR products will be sequenced using BigDye Terminator V3.1 sequencing kit (Applied Biosystems) and sequencing primers. Six primers will be used to sequence *pol* and four for *env*. Chromatograms were assembled using Sequencher software (Gene Codes). As blood spots only contain a limited volume of blood (~75ul /spot) there are potential amplification problems with samples with low viral loads. Theoretically using this approach we should be able to amplify samples with viral loads greater than 3 333 copies/ml. In addition, published studies have shown an amplification success rate of 73% for samples below 10 000 copies/ml, and 54% for samples below 2000 copies/ml (reviewed by WHO protocol on DBS, March 2010, https://www.who.int/hiv/pub/drugresistance/dried_blood_spots/). The CAPRISA002 study recruits HIV acutely infected individuals from a similar geographical region and we have shown that approximately 10% of the CAPRISA 002 acutely infected study participants had viral loads below 4 000 copies within the first year of infection. We therefore anticipate poor amplification from approximately 10% of DBS using the standard protocol and have accounted for these in our sample size calculations.

4.6.2 Behavioural measurements

As described in section 4.5.1 study staff will administer validated, standardized, structured questionnaire (**Appendix 5: Questionnaires**) to obtain detailed information on:

- Demographic characteristics
- Psychosocial characteristics
- Epidemiologic characteristics
- Behavioural information including partnership characteristics
- Knowledge of HIV status and risk factors
- HIV testing history
- Sexual behaviour history
- Exposure to information, education, prevention and treatment programmes for HIV
- Male circumcision status (medical versus traditional)

The questionnaires will include age, gender, marital status, occupation, employment, educational status; epidemiologic information, HIV testing history, date of last test, HIV results, current HIV treatment, exposure to treatment for STI's, contraceptive use, male circumcision status, current location information, rural or urban, proximity to national roads, socioeconomic status. Behavioural information sexual debut, practice of anal sex, contraceptive use (including partnership characteristics), number, type (regular/casual), concurrency of sex partners (i.e. information on last three months and or last three partners), frequency of partner change, condom use including knowledge of own and partner(s) HIV status, engagement in transactional sex, exposure to intimate partner violence and exposure to antiretrovirals either as therapy or as pre-exposure prophylaxis.

4.7 Study Timelines

The timelines for the study are shown in Table 3.

Table 3: Schedule of timeline for the proposed study project.						
Year	2015	2016	2017	2018	2019	2020
Protocol development	X					
Submit protocol for review to UKZN BREC for Ethics review	X					
Translate study instruments	X					
Recruitment and training of staff	X					
Procurement of supplies and laboratory preparations	X					
Community preparation, consultation with DOE and DOH, preparation of schools and ongoing study updates	X	X	X	X	X	X
Field work data collection, school- and facility based surveillance		X	X			
Initiation of RDS		X	X	X		
Sequencing of HIV-1 strains		X	X	X	X	
Obtain community (HIPSS) sequence data				X	X	
Sequence alignment				X	X	X
Data analysis					X	X
Writing of reports (Annual)		X	X	X	X	X
Dissemination & discussion of results with stakeholders						X

5.0 STATISTICAL CONSIDERATIONS

5.1 Review of study design

This study is cross sectional in design, being conducted in a very specific geographic region of KZN, South Africa. This study specifically focusses on sampling high-risk groups that have been poorly studied using traditional epidemiologic approaches. Our goal is to include these key risk groups to maximise on our approach to identify HIV seropositive individuals through several sampling strategies, these are adolescent non-pregnant and pregnant girls, out of school adolescents and young adult men in the community. The goal of the study design and selected populations is to maximise on population coverage to reveal sexual networks of the local epidemic.

5.2 Sample size

The goal of the sample size is to utilise a sampling strategy in a geographically defined region that will maximize on population coverage to identify HIV seropositive individuals. The Inadi ward having a population of approximately 90,000 individuals and at a population HIV prevalence of 20% we expect to have an estimated 18,000 HIV seropositive individuals. Through our sampling strategy we will undertake HIV-1 viral sequencing on approximately 5,145 HIV seropositive samples, resulting in a population coverage of almost 30% of seropositive samples. The sample sizes for the study will be as follows:

Samples size for school-based study population

Based on the schools census data, there are 10 high schools (Figure 5) with a learner population of 8,977. With approximately 4,488 (50%) female learners we expect a total of 224 HIV infections for a prevalence of 6% and 90 infections in male learners for a prevalence of 2%, resulting in 314 HIV-1 sequences for alignment.

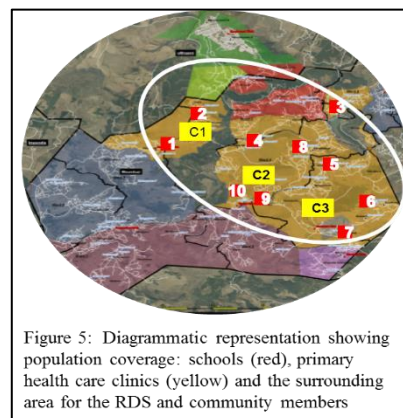


Figure 5: Diagrammatic representation showing population coverage: schools (red), primary health care clinics (yellow) and the surrounding area for the RDS and community members

Sample size for Respondent-driven sampling (RDS)

The RDS will aim to reach 1,000 out of school adolescent girls and 1,000 older adult men. Based on a population prevalence of ~30.1% in young women <24 years of age and in men in the age group 25 years and older for KZN, we expect a total of 602 HIV infections. These sequences will be aligned with sequences from the schools, prenatal clinic attendees and from the community.

Sample size for Facility-based study population

Given the high HIV prevalence observed in pregnant adolescent girls we will recruit those attending PHC clinics for the first time for the current pregnancy. Approximately 50% of pregnant women attending these clinics are <24 years of age and the overall HIV prevalence in this group is around 27%. Our sample will be drawn from 1,700 first visit attendees, the numbers who attend clinics on average per year. We therefore expect 850 girls to be under 24 years of age of whom approximately 229 will be HIV seropositive. The sequencing of these HIV-1 isolates will contribute to the phylogenetic analyses.

Sample size for Community-based study population

The community based study population surveillance platform has been established in greater Vulindlela and the Greater Edendale areas and the Inadi ward is rooted within. HIPSS is designed to capture two sequential cross sectional surveys conducted one year apart, each sampling 10,000 individuals selected randomly in the age group of 15-49 years. Within the cross-sectional surveys two sequential observational cohorts of approximately 6400 HIV uninfected individuals in the age group 15-35 years will be selected to participate in the longitudinal follow-up. The study is powered on a community adult HIV prevalence of 20% and an HIV incidence of 3%. With a sample size of 10,000 at two time points; 4,000 HIV-1 isolates are to be sequenced, 2,000 from each time point from the cross sectional surveys and a total of 277 isolates from HIV incident infections; 163 and 114 from the first and second year of follow-up respectively. All positive samples will have *pol* region sequenced to establish community transmission linkages and spatial clustering of these sequences and linkages.

Through CAPRISA's data sharing agreement all HIPSS *pol* region sequence data will be obtained and included in the HIV-1 transmission cluster and linkage analyses.

A key limitation to the HIPSS study is that based on the random selection criteria a large proportion of young adolescents might not be selected to participate and therefore limit the representativeness of data from adolescent girls. However, the HIPSS study provides an extensive number of community sequences as it is clear that although this community is rural, it is not completely isolated, with many individuals traveling for work, school, or other reasons. Furthermore, some individuals may prefer to form sexual partnerships with individuals outside of their immediate area, for reasons of social acceptability or chance meetings, thus will allow us to determine how much more HIV transmission can be mapped by panning out to areas beyond the concentrated area.

Table 4: Sample size calculations			
Population	HIV prevalence (%)	Sample size	Expected number of HIV seropositive individuals
School-based surveillance (females)	6	4,488	224
School-based surveillance (males)	2	4,488	90
RDS	30.1	2,000	602
Facility-based surveillance	27	850	229
HIPSS	20	20,000	4,000
Total		31826	5,145

5.3 Endpoints

5.3.1 *Primary endpoint*

- The primary endpoint will be HIV infection of adolescent girls phylogenetically linked to other male and female adolescents and adults in the community.

5.3.2 *Secondary endpoints*

- The secondary endpoints will assess the contribution of the linked HIV-1 viral sequences to sexual networks and the extent of these networks.
- Using a combination of laboratory tests to assess the biological risk factors and their contribution to enhance sexual networks
- The responses from the administered questionnaires will assess the factors associated with linked HIV viral sequences and sexual networks

5.4 **Data Management**

All the data collection instruments for measurements will be developed, piloted and study staff will be trained in the correct completion of data collection forms. The use of PDA allows for data collection and real time data entry. Data from the PDAs will be uploaded to the server daily. This server will be housed in a secure data center.

Data quality on PDAs will be assured in the following ways:

- PDA-based questionnaires will ensure that study staff completes certain fields with legitimate range of values in each field. Skip patterns are enforced by the PDA. “Illegal” data entries (e.g. vaginal infections being documented for a man) are automatically rejected by the PDA and study staff is prompted about the problem.
- Quality control and validation checks will be undertaken for recruitment, enrolment, informed consent, interviews, data collection, data handling, forms processing, data management and other study operations will be on going. Study staff on a weekly basis will review the key indicators for each of the procedures. Following this any areas of concern will be defined, assessed and the areas of improvement will be verified. The possible solutions will be considered and action plans developed for improvement, including implementation, communication, and measuring/monitoring.

Database files will be password-protected and access to the files will be limited to authorised study staff members only. All study related documents will be stored securely both during and after the completion of the study. All data will be backed up at regular intervals, and backups will be stored in secure areas with limited access. All HIV-related laboratory data will be stored in a dedicated spreadsheet and will be merged in the database using the participant identifying number only without any names or any other identifying information. All participant information from the questionnaire and HIV viral sequences will be linked through a bar code and stored in databases to protect participant’s privacy. Once the study is complete a back-up of the data excluding the identifying information will be archived and the identifying information deleted from server of the service provider.

5.5 **Data Analysis**

5.5.1 *Descriptive analyses*

Descriptive analyses will include: a description of all enrolled participants.

5.5.2 *Analysis of primary endpoint*

The primary endpoint of HIV transmission linkages that contribute to the source of infection in adolescent girls in rural KZN will have HIV -1 viral sequencing and included in this analysis.

Screening for transmission clusters: *Pol* gene sequences will be used for the identification of transmission clusters. Phylogenetic trees will be generated using Bayesian and maximum likelihood methods. Nucleotide sequences obtained from surveillance, from HIPSS and existing HIV-1 sequences in the database as background data will be included in the reconstruction of transmission networks. In addition, sequences recently generated from the laboratory to screen for contamination will be included. For *pol* sequences that are linked with high bootstrap support, we will then amplify and sequence partial *env* to confirm linkage. This will also confirm the identity of the sequence. We will use the Los Alamos

sequencing quality analysis tool (www.Hiv.Lanl.gov) to check for common problems and to format data for submission to GenBank.

Computational Analysis: Phylogenetic reconstruction will be carried out using both Bayesian and maximum likelihood approaches implemented in MrBayes (www.mrbayes.csit.fsu.edu) and RAxML (icwww.epfl.ch/~stamatak/index-Dateien) respectively. Using a relaxed clock model implemented in BEAST, the time to most recent common ancestor (tMRCA) will be calculated for each internal node in the phylogeny. Transmission networks will be defined as phylogenetic clades strongly supported by reconstruction approaches. Matching networks between the *env* and *gag* datasets, coupled with correlation with the epidemiological data, will provide a high degree of confidence in the observed transmission networks. Including epidemiological data such as age, time of seroconversion (if known), geographical location and sex into subsequent BEAST analysis will allow us to investigate whether transmission is localized within, or shared between, schools and to potentially identify the presence of individuals either in schools, community or outside of the community that appear to be in sexual networks and how they may be linked to multiple infections within a transmission cluster. Cluster and linkage analysis will be performed using generated viral sequences from individuals from aim 1. The optimal evolutionary model will be identified for the aligned set of query sequences using jModelTest. Viral linkage between and amongst adolescents and community sequences will be made based on above analyses.

5.5.3 Analysis of secondary endpoints

All enrolled participants will contribute to the secondary objectives. Frequencies will be compared using T-tests and Chi-squared analyses. Secondary outcome variables will include HSV-2, and pregnancy prevalence, frequency of unprotected sex, numbers of lifetime partners, sex with older partners (≥ 5 years), and contraceptive use. Each variable will also be analysed to determine clustering of behaviours and/or HIV risk factors within social networks of individuals linked in RDS. We will analyse the length and structure of RDS recruitment chains, including the centrality of recruiters and the geographic dispersion of chains. We will identify potential bottlenecks in recruitment by comparing the prevalence of HIV-1 and HSV-2 across different recruitment chains. For the facility- and school-based surveillance, frequencies and proportions will be measured for HIV status in association with demographic, behavioural and biological using T- tests and Chi-squared analyses.

The phylogenetic data collected during this project will allow identifying HIV-1 transmission networks, i.e., clusters of relationships within which HIV-1 has been transmitted. We will characterize the size of these clusters, their socio-demographic composition, whether they extend within and between social settings (e.g., schools). We will also measure the extent of overlap between the HIV transmission clusters reconstructed through phylogenetic analysis and the RDS recruitment chains. This will permit identifying whether the new infections among adolescents also cluster within social networks. Finally, we will use the sexual partnership histories collected during the RDS data collection to ascertain how HIV transmission networks emerge within broader population networks. To do so, we will use the following methodology, derived from case-control approaches in epidemiological studies. We will 1) identify HIV transmission clusters (“cases”) and characterize their size and socio-demographic composition. For each transmission cluster of size n , we will 2) select - among the non-infected population – a set of n individuals with matching socio-demographic characteristics (“controls”). We will use conditional logit models to describe the characteristics of HIV transmission clusters that differ from the rest of the population networks.

6.0 STUDY MONITORING

6.1 Monitoring and evaluation of study performance

A steering Committee consisting of the Principal Investigators (PIs) (Kharsany and Kohler) and the protocol team members will ensure that the study’s implementation and follow-up is scientifically sound, ethical, and of high quality. The members of the CAPRISA Research Support Group will contribute to the informed consent procedures, questionnaire design and administration, play a key role in monitoring a random number of informed consent procedures and will monitor the assent procedures

for participants <18 years of age. The committee will meet through face to face meeting or through teleconference to review the study and updated information. Any necessary adjustments to the study will be at the discretion of the Principal Investigators and the protocol team. Study team meetings will be held weekly to monitor progress and to resolve any operational challenges.

6.2 Quality Assurance

The study will be monitored by the Study Quality Assurance Team. The monitoring will be undertaken according to the Study Quality Assurance Plan and will consist of on-going monitoring of study progress and safety of study participants by the Protocol Team in accordance with ICH, GCP guidelines. The Investigators will allow NIH study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents and data collection instruments eg PDAs), as well as observe the performance of study procedures. The Investigators will also allow inspection of all study-related documentation by study sponsors. Study monitoring will be conducted by staff adequately trained on this study. Monitoring shall commence shortly after enrolment of the first participant and at regular intervals thereafter. Any issues or findings related to participants' safety or any compromise on scientific integrity will be reported immediately to the PIs or designee.

6.3 Limitations of study

We anticipate a 10 % non-response rate as volunteers may not wish for study staff to know their HIV status or that they may be mobile population with insufficient time. There is a possibility that those who do not wish to disclose or migrate and those who do are different from each other in meaningful ways related to HIV infection risk. Data on sexual behaviours (such as past/current number of partners, condom use, etc.) and history of STI symptoms will be self-reported and are thus subject to potential recall and social desirability bias. However, efforts will be made to ensure that the study staff recruiting, enrolling, interviewing and performing the clinical sample collection will not be from within the community to minimise reporting bias, concerns about stigma and disclosing personal information during data collection. There is also a possibility that self-reported exposure to prevention programmes may be incorrect.

6.4 Multiple PI/PD Leadership Plan

This research operates at the intersection of epidemiology and the social and behavioral sciences, and therefore requires in-depth knowledge in all of these fields. Given their training and experience, Prof. Ayesha BM Kharsany of Centre for the AIDS Programme of Research in South Africa (CAPRISA), (South Africa) and Professor Hans-Peter Kohler, University of Pennsylvania, (United States of America), have extensive experience in running large projects (often with NIH funding) of the type proposed herein. Their leadership by Kharsany and Kohler enables a natural and seamless division of scientific direction. The two PIs will jointly provide oversight of the programme, development of policies and procedures and will have oversight responsibilities for implementation and monitoring. Both PIs will jointly be responsible for systems to be in place to guarantee the requirements for institutional regulatory and administrative compliance in relation to the laws governing research in South Africa and the United States of America.

7.0 HUMAN SUBJECTS CONSIDERATIONS

7.1 Ethical considerations

The study will be conducted under the oversight of the University of KwaZulu-Natal's Biomedical Research Ethics Committee (BREC) and University of Pennsylvania, Institutional Review Board. No study activities will begin until all approvals have been obtained. Subsequent to the initial review and approval, BREC will review the protocol at least annually. The study protocol, informed consent forms, participant recruitment materials, and other requested documents will be reviewed and approved by BREC. Any future amendments will be conducted in full compliance of BREC requirements prior to implementation.

7.2 Justification for including minors 12-<18 years of age

This study will include children. Children aged 12-18 years attending schools and living in the study area (Vulindlela) will be included. Based on current knowledge on HIV incidence, more than 80% of new HIV infections are acquired in young people less than 24 years of age (59, 60). Thus the study sample to include younger population is intended to enhance the efficiency of the study, rather than the inclusion of an older population where fewer incident infections are expected to occur and therefore reducing the power of the study. Even though the burden of HIV infection is in young girls and adolescent women, young boys will not be excluded from the study. Parallel to the high HIV prevalence, girls become pregnant at a very young age and pregnant girls will not be excluded from study participation. Previous CAPRISA studies have demonstrated that HIV prevalence and incidence is low in children 12 to 13 years, but increases rapidly in the 14 to 18 year age groups. All children participating in the study will be required to provide informed consent and assent in accordance with current Ethics guidelines. All children lacking cognitive maturity to provide consent will provide assent with consent from a parent/guardian/adult community proxy. Children 12 years and older can access relevant health services, including HIV counseling and testing, contraceptive counseling and provision and treatment for sexually transmitted infections according to applicable South African National Department of Health guidelines.

7.3 Informed consent process

Each potential study volunteer will be informed about the study and complete the English or isiZulu consent form prior to enrolment, in accordance with 21 CFR Part 50 and ICH GCP guidelines. The study volunteer will be informed that he/she will be compensated with an item to the approximate value of R25 for their time should they wish to continue with study participation and for responding to the demographic, behavioural questionnaire and for the collection of biological samples. The consent / assent forms for study participation and sample storage that will be used in this study are:

- Individual consent form for participants 18 years and older
- Parental/guardian consent for participants 12-<18 years of age
- Individual assent form for participants 12-<18 years of age
- Sample storage consent form

All consent forms and data collection forms will be translated from English into isiZulu. Back translations will also be completed and reviewed by a bilingual independent source in order to ensure accuracy of translated information. The informed consent discussion will take place in either English or isiZulu. Participants will be given the opportunity to choose their preferred language. Prior to initiation of any study procedures, all potential volunteers will be given a printed copy of the consent form in either English or isiZulu depending upon their preference. A staff member will then read the consent form aloud to the participant. At this time, potential participants will be informed that their participation in the study is voluntary and that they may withdraw at any time. Withdrawal from the study will have no effect on the participant's access to health facilities or HIV related care in the district. Further, participants will be informed that they do not have to answer questions that make them uncomfortable and that any information that they disclose during the course of the study will be considered confidential (i.e., no personal identifiers will be used and only summary information across all participants will be reported). Participants will have the potential risks and benefits of the study explained to them as well. After the consent form has been read aloud, potential volunteers will be invited to ask questions about any aspect of the study and their participation. If they agree to participate in the study, literate participants will document their provision of informed consent by signing their name on the consent document. Non-literate volunteers will be asked to identify a person that they would be comfortable to serve as an impartial witness to support them through the consent process after which the volunteer could provide a fingerprint to indicate consent and to be signed by the witness. Volunteers will be provided with a copy of their informed consent form, should they wish to receive it.

The study team will involve the community in establishing recruitment procedures through its community engagement strategies. The study team will draw on its prior experience with this community through its Community Research Support Group (CRSG) and will work with the members to obtain assents / consent in culturally and linguistically appropriate formats. As per South African

Laws and Guidelines, the team will seek parental / guardian consent and individual assent from all individuals who are <18 years of age. We estimate that about 20% of the individuals will be <18 years of age, and will require parental /guardian consent. It is anticipated that some <18 year olds will not have a parent, or legal guardian, available to provide consent, e.g. migrant, sick hospitalised or deceased parents and in such instances we will identify a care giver / “guardian” of the child, who will then provide consent for that child’s participation in the research.

7.4 Finger-print Scanning

All eligible participants will be asked to provide a finger-print. The finger- print device will be attached to the mobile data collection device. The finger-print will be scanned and stored on the data collection server with the identifying information. We will be integrating biometric verification capabilities (via fingerprint scanning) into our Android mobile application. The finger print devices will be configured to support a USB fingerprint scanner will be connected directly to the study staff device. Software installed on the device will handle fingerprint extraction and matching. The participants will be informed on the purpose of the collection of the finger print to identify individuals who could potentially be included through the multiple sampling strategies to be identified and analyzed accordingly and that these fingerprints will not be used for any other purposes.

7.5 Potential risks

The study protocol involves minimal risk to participants ie the collection of DBS or peripheral blood samples. As part of this study, participants will be asked questions on personal information and sensitive topics, including sexual behaviour, HIV status, access to care and treatment for HIV and male circumcision. It is possible that some individuals may experience discomfort from taking part in these study activities. Study staff will be trained to address any potential stress or discomfort that may result from study participation and to help make participants feel comfortable. As part of the informed consent procedure, all potential participants will be informed that they do not have to disclose personal information which they are uncomfortable sharing and that they can withdraw from the study at any time. There is a potential risk for participants to be “presumed” to be HIV positive by community members as study staff undertake the study. We plan on minimising these misconceptions through extensive and on-going community engagement process. Volunteers who may be HIV-seropositive may not have disclosed to family members and therefore feel distressed in responding to questions related to HIV. However, study staff will support study participants assuring them that all responses and information will remain confidential

There could also be a slight risk of discomfort to participants associated with blood collection. Feelings of discomfort could include feeling ill and/or having complications such as slight bruising or tenderness from the site of sample collection. Study staff will be trained in how to deal with these complications and will refer participants to local health facilities for additional care, as needed. Although every effort will be made to keep volunteer information confidential, complete confidentiality cannot be guaranteed. Participants will be informed of this potential breach of confidentiality as part of the informed consent process. Study staff will be trained in maintaining confidentiality of study participants and of any information collected.

7.6 Potential benefits

Participants would benefit from the study through receiving information on HIV and getting a broader understanding of HIV in the community, information on accessing general health care and possibly for early referral to HIV counselling and testing services. In addition study staff would refer participants for management of conditions, if necessary and therefore access care and treatment much earlier.

Societal benefits of this study include gaining a better understanding of the methods to minimize HIV acquisition. In addition information from study participants will help refine projections of HIV infections that may be averted from prevention programs and the potential costs savings realized, compared to HIV care and treatment costs. The participant will receive a gift to the approximate value of R25 to compensate for their time in study participation.

7.7 Confidentiality

All study staff will receive training on procedures to protect participant confidentiality and Good Clinical Practices (GCP). In order to protect confidentiality, each participant will be assigned a unique study participant identification number (PID) so that their name is not linked to any of their personal data or laboratory results. The PID will be written on all data collection forms, HIV test results and will be matched only by this identification number, not by participants' names or other identifying information. A master list with each participant's name and their assigned identification number will be created and will be accessible to the Study Coordinator or designee.

The master list will be securely maintained in password protected file at the local data management centre. All study data, including laboratory results, will be stored securely in the study offices. All databases will be encrypted and password protected. Study data will be accessible only to study staff directly involved in this study. Personal locating information, including participant's name, address and phone numbers, will be stored separately from study data in a filing cabinet in a secure room in the office.

All study consent forms will include the contact information of Principal Investigators and BREC if participants have questions about the study; if they wish to withdraw themselves as a participant; if they have concerns about their rights as a study participant; or if they believe that have been harmed by the study. All staff that through the course of their work have knowledge of or access to personal information about participants will be required to sign a confidentiality agreement as part of their employee contract.

For this study extensive information will be collected from study participants, these include personally identifying or potentially identifying information such as, address, first names, sensitive sexual and behavioural information. Given the sensitive nature of all these data, study staff will be trained so as not to divulge any study related information to any person/s outside of the study team. In addition study related information will be delinked and stored with staff having limited access to such information.

7.8 Identifying, managing, and reporting adverse events

As this is a cross sectional study, standard adverse event (AE) reporting will not be undertaken; there are no anticipated adverse events. All unanticipated problems will be documented and immediately reported to the Principle Investigator. These unanticipated problems will be discussed and a verbal and/or written action plan will be devised and implemented within 48 hours of the initial report. The study team will maintain written documentation on all events, including details of the action plan and event resolution. If necessary, a formal report will be sent to BREC. Reporting of unanticipated problems will be the responsibility of the Principal Investigators of this study and all procedures will be included in staff training.

7.9 Linkage to care services and referrals

As this study is determining the HIV seropositive status in young people, study staff will remind participants on where they could access health care and any other social support services.

Participants who would like to know their HIV status will be offered a choice of being tested by qualified staff using HIV rapid testing kits and algorithms approved by the SA DoH or being referred to a SA DoH accredited parallel HCT facility or being provided with information on where to access HCT for themselves, partners and family members through public sector facilities or other NGOs providing the service in the district. Referral systems will be set up to ensure that participants and family members who tests HIV positive can access treatment and care services through the local health care facilities. As per Department of Health guidelines participants with signs and symptoms of STIs and / or TB will be referred to PHC services in the district to access care and services.

7.10 Community partnership

CAPRISA has established its presence in the area since 2001 and has created strong community programmes. Through the teams consultative and advocacy engagements, a strong Community Research Support Group (CRSG) has been established. The CRSG membership includes local community leaders, traditional leaders, leadership of local HIV/AIDS organisations, previous study participants, local health service provider representatives and HIV positive local community members.

The CAPRISA community programme in partnership with the CRSG's are involved in creating and awareness on HIV, STIs, and HIV related research studies, HIV treatment and impact of HIV treatment at a community level. Similarly the CRSG members are actively involved in reviewing all study documentation; inform the community and other community organisation in the districts on CAPRISA related studies and will do the same for HIPSS. Thus the CRSG members play a key role in being the interface between the researchers and community members serving as advocates for the community's best interests and ensuring that the researchers are aware of any concerns within the community about the research being conducted. The CRSG also plays an important role in reviewing study educational materials, consent forms and Zulu translations of documents to be shared with study participants.

7.11 Study Records

Complete, accurate, and current study records will be maintained and stored in a secure manner, throughout the study. All study records will be maintained for a period as required by the funders.

7.12 Protocol Deviations/New and Unexpected findings/Changes to the study environment

All protocol deviations, new/unexpected findings and changes to the study environment will be documented and immediately reported to PIs and or designate. If necessary, a formal report will be sent to the appropriate IRBs. Reporting of such incidents will be the responsibility of the PIs of this study. Any discussions, issues, and complaints related to the study will be reviewed promptly to ensure close monitoring of the impact of the study on participants. Appropriate action will be taken to resolve or deal with all issues accordingly.

8.0 DISSEMINATION, NOTIFICATION, AND REPORTING OF RESULTS

8.1 Use of Information and Publications

All abstracts and manuscripts developed in line with the study's primary and secondary objectives for presentation at conferences and publication in peer-reviewed scientific journals will be in collaboration with investigators from the study. Analysis of data to answer novel research questions will be governed by CAPRISA policies.

Written material summarizing the findings from this study will be made available to participants and study staff upon completion of the study.

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10.0 APPENDICES

APPENDIX 1:RDS RECRUITMENT TOOLS

APPENDIX 2:INFORMED CONSENT AND ASSENT FORMS

APPENDIX 3:HIV ANTIBODY TESTING ALGORITHM

APPENDIX 4:HIV INCIDENCE TESTING ALGORITHM

APPENDIX 5:QUESTIONNAIRES

APPENDIX 1: RDS RECRUITMENT TOOLS

Recruitment guidelines for respondent driven sampling study participants.

Instructions to participants: “Here are three coupons for you to use to recruit other people like yourself. Please do not give the coupons to any strangers but make sure that you give these coupons to your friends and peers who are like yourself and you have known them over the last three months and are:

- i) 12 years of age or above
- ii) sexually active
- iii) out of school
- iv) have not received this coupon from someone else (i.e., has not participated in this study before).

Please inform the person(s) you recruit that this study is anonymous and confidential and to explain to them that the information they provide is used to understand the spread of HIV and for developing HIV/AIDS prevention programs for adolescents. If that person accepts the coupon, show him/her the address where he/she can go to be interviewed and inform him/her that he/she can call or send a please call me to the number on the coupon to make an appointment. Also, explain to him/her that the interview and other study related procedures will take at least 30 minutes if they agree to be part of the study.

Please look at the coupon. Each coupon has two parts with a unique numbers. Please give the top part of the coupon to the person you are recruiting. The top part with the unique number will allow us track if the person you gave the coupon has made contact with the research team and has completed the interview. The linking bottom part second is for you to keep in order for you to claim your reimbursement for recruiting one of your peers

“For each person you recruit who is eligible and completes an interview, you will be given an airtime voucher of R25 to thank you for your time taken to inform and recruiting your friends/ peers.”

Once you give a coupon to one of your peers, and that peer enrolls into the study and completes the interview, you can come back to the interview site to claim your compensation (your airtime voucher). You can also call the number provided on the bottom portion of the coupon to check your incentive status (whether the person you recruited has enrolled and completed his/her interview).

Only you can recruit peers with your coupons. If you have another person recruit peers for you, you will become ineligible to receive your incentive. Remember to keep the bottom part of the coupon because you will not be able to claim your incentive without it.

Thank you for your participation. Do you have any questions?

Recruitment and payment coupons




**Front
1**

CAPRISA 085 – RDS survey Referral Coupon

Coupon number _____

Address _____

Telephone _____

You can call to make an appointment in advance
Opening hours: Mon- Thurs 9.00am-5.00pm

Expiration date _____

Contact Details : CAPRISA Vulindlela Clinic
 Next to Mafakhatini Clinic
 Ms Nonzwakazi Ntombela on (031) 260 6851




**Back
1**

CAPRISA 085 – RDS survey

Come with this coupon and if eligible for the study, you can receive

A compensation to the value of R25 for completing study related procedures

The coupon will not be accepted if:-

- The project has reached its target numbers
- The coupon is torn, not readable or lost
- The coupon holder has already been enrolled in the study
- The coupon has expired




**Front
2**

CAPRISA 085 – RDS survey Payment Coupon

Coupon No _____

Telephone _____

You can call to make an appointment in advance

You will receive _____ for each recruitee you refer and enroll in the study (up to 3 recruits)
Please call us in advance and present this coupon for payment

Contact Details : CAPRISA Vulindlela Clinic
 Next to Mafakhatini Clinic
 Ms Nonzwakazi Ntombela on (031) 260 6851




**Back
2**

CAPRISA 085 – RDS survey

This coupon will not be accepted in the following conditions:

- The project has reached the target sample size
- This coupon is torn up, tampered with or unreadable
- This coupon has passed the expiration date

You will receive your incentive if each person you recruited

- Is eligible for the study
- Has not participated in the study with a similar coupon
- Completes all steps of the study

Client checklist form			
Date			
Coupon number			
	Yes	No	Signature
1. The participant is eligible to join the study.			
2. Informed consent signed			
3. The participant has completed questionnaire			
4. Blood samples taken			
5. Urine samples taken (males)			
6. Self-collected vulvoVaginal swabs taken (female only)			
7. Recruitment coupon released			
8. Primary incentive paid			
9. Secondary incentive paid			
9.1. First			
9.2. Second			
9.3. Third			
10. Notes			
If no to 1, Please fill in ineligibility criteria form			
If no to 2, Please fill in refusal form			

Ineligibility form					
Please complete a row on this form for each person you contact who does NOT meet the inclusion criteria to participate in the study.					
Ineligibility Codes					
1	2	3	4	5	
Under 12 years	Not sexually active	Not from the geographic area	Attends school	Other	
Number	Coupon number	Date	Reason for Ineligibility (use code from above)	If other, specify	Signature
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Refusal form					
<i>Please complete a row on this form for each person who meets the inclusion criteria but refuses to participate in the study</i>					
Refusal Codes					
1	2	3	4	5	
Did not want to sign consent	Did not want to answer questions	Did not want to give clinical samples	No time*	Other	
Number	Coupon number (take away coupon)	Date	Reason for refusal (write code)	If other, specify	Signature
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Financial reporting form

To be completed by coupon manager each day for each seed. The date primary incentive was given (first column) is the same date the participant was interviewed.

Seed number :

Date:

Date of primary incentive given	Coupon number	RDS coupons given	Date secondary incentive given	Expiration date (two weeks)	Running total for primary incentive	Running total for secondary incentive
Total						

Coupon Tracking Form					
To be completed for each seed each day by screener					
Seed number					
Serial	Referral coupon numbers				
	Date	Coupon number	Coupon 1	Coupon 2	Coupon 3
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Coupon Rejecter Form			
<i>Collect this information face-to-face from returning recruiters each time they come to collect their compensation</i>			
Coupon number/identification number			
Name of interviewer			
Date of interview			
1. Is this the first time you have been here to collect compensation?		Yes (Continue)	No (Answer questions for the period of time between when the participant was last here and filled out this same questionnaire and now)
2. How many coupons did you give out? (Between the last time you came here to receive compensation and now. If > zero, complete coupon rejecter questionnaire.			
3. How many people refused to accept coupons? _____ (If zero, do not complete the rest of this questionnaire. If > zero, continue.)			
Ask These Questions for Each Individual Who Refused to Accept a Coupon			
Questions	Response codes	Response for each person who refused to accept coupon	
1. What is your relationship to this person? (check only 1)	1. A stranger, someone you met for the first time. 2. Someone you knew, but nit closely. 3. A close friend. 4. A family member. 5. Other	Person 1____. Person 2____. Person 3____. Person 4____. Person 5____. Person 6____. Person 7____.	
2. How long have you known this person?	1. Less than six months 2. 6 months to a year 3. 1-2 years 4. 3-6 years 5. more than 6 years	Person 1____. Person 2____. Person 3____. Person 4____. Person 5____. Person 6____. Person 7____.	

3. Why do you think this person refused to accept the coupon? (Do not read, ask for each individual who refused to accept the coupon)	1. Too busy 2. Already had a coupon/ already participated in the study 3. Younger than 18 years 4. is not sexually active/ does not have a girlfriend/boyfriend 5. Site is too far 6. Not interested 7. Incentive is not worth the time	Person 1____. Person 2____. Person 3____. Person 4____. Person 5____. Person 6____. Person 7____.
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APPENDIX 2: INFORMED CONSENT AND ASSENT FORMS

Title of Study

IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN RURAL SOUTH AFRICA

Version 1.0 – 1 Dec 2015

INFORMATION TO VOLUNTEERS / PARENTS/ GUARDIAN/ CARE GIVERS/EDUCATORS

I am from a research team working with CAPRISA, University of KwaZulu-Natal.

For the last several years CAPRISA has been testing for HIV infection in pregnant women to understand the burden of HIV in this community. However, this information is limited to pregnant women and does not accurately reflect the spread (Transmission) of HIV across the community. We are undertaking a study which seeks to better understand the spread and the factors that contribute to this spread of HIV amongst young people, specifically young girls. This is important as it would allow us to design programmes to prevent the further spread of HIV specifically in young girls who are highly vulnerable (in danger) to HIV. We have limited information on HIV in young girls for example which young girls are becoming infected and why. However, we do know that young girls acquire HIV at a very young age and are also three to five time more likely to be HIV positive compared to boys in the same age group. We also have some information that HIV in school learners is not always acquired through sexual intercourse from other learners within and across schools, but possibly from people who are not in schools, that is, from community members. Knowing more about the spread of HIV and factors linked with getting HIV will help us improve programmes on preventing and treating HIV with the aim of breaking the chains of spread of HIV and reducing the overall number of new HIV infections specifically in young girls and in the general population.

The study is expected to enroll a learners from high schools, pregnant girls attending clinics and the community members from Vulindlela and Greater Edendale districts.

If you agree to be part of the study, we would like to you to provide your finger prints which will be scanned and stored securely, ask a few questions about your health and your experiences and request you to give us a small amount of your blood, possibly a few drops or about 3 teaspoons, which the nurse will collect and ask males to give some urine and females to give some samples from the vagina. You will receive a number, so no names will be written on any of the forms and samples so no-one will know who the information comes from. If you do not agree to your samples being collected then you will not be able to participate in the study. There is no limit on how long the blood, urine and vaginal samples will be stored and may be tested for other infections. If you do not want us to store the sample then we will destroy the sample as soon as the tests are completed for this study.

You are free to decide on participation, but we will be happy if you agree and are enrolled in the study. All the information we collect from you will kept securely and restricted to study staff only. Once you have completed all the procedures, you will be compensated with an item to the approximate value of R25 for their time. Your results will not be available to you, but we encourage you to go to your local clinic for health care.

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (Approval number XXXXXX) and the University of Pennsylvanis . (Approval number XXXXXX)

PERSONS TO CONTACT

For any concerns/questions you may contact,

Dr Ayesha Kharsany on (031) 260 4555/4558 or

the Study Co-ordinator Ms Nonzwakazi Ntombela on (031) 260 4494, at CAPRISA, Second Floor Doris Duke Medical Research Institute, Durban or

The UKZN Biomedical Research Ethics Administration,

Research Office, Westville Campus, Govan Mbeki Building

Private Bag X 54001, Durban, 4000, KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

Thank you for your time and we will be happy to answer any questions about the study?

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA

Version 1.0 – 1 Dec 2015

Informed Consent form for enrolment of volunteers 18 years and older

If the volunteer cannot read, this form must be read to the volunteer exactly as written, in the volunteer's language of choice, and a witness must sign this form to confirm that the correct information was given to the volunteer who freely consents to be in this study.

INTRODUCTION

Good day, my name is _____ (Consenting staff name), I am a member of a research team working with CAPRISA, University of KwaZulu-Natal.

The Principal and Co-Principal Investigators of the study are

Dr Ayesha BM Kharsany 2nd Floor Doris Duke Medical Research Institute Nelson R Mandela School of Medicine Private Bag 7, Congella 4013, Durban, South Africa Tel: +27 031-260 4555 Email address: Ayesha.kharsany@caprisa.org	Dr Hans Peter Kohler Population Studies Center 3718 Walnut Street, 272 McNeil Bldg, Philadelphia, USA PA 19104-6298 Tel: +1-215.898.7686 Email address: hpkohler@pop.upenn.edu
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BACKGROUND

You are being invited to participate in a research study that seeks to understand the spread (Transmission) of HIV amongst young people, specifically young girls, so that we better understand how HIV spreads and the factors that contribute to this spread. This is important as it would allow us to design programmes to prevent the further spread of HIV specifically in young girls who are highly vulnerable (in danger) to HIV. To date, we have limited information on HIV in young girls, however, we do know that young girls acquire HIV at a very young age and are also three to five times more likely to be HIV positive compared to boys in the same age group. We also have some information that HIV in school learners is not always acquired through sexual intercourse from other learners within and across schools, but possibly from people who are not in schools, that is, from community members. Knowing more about the spread of HIV and factors linked with getting HIV will help us improve programmes on preventing and treating HIV with the aim of breaking the chains of spread of HIV and reducing the overall number of new HIV infections in the general population.

The study is expected to enroll a large number of individuals from Vulindlela and Greater Edendale districts. We will include about

- 8,977 high school learners, 12 years and older from high schools in Vulindlela
- 2,000 adolescent girls and boys, 12 years and older who are not in schools
- 850 adolescent pregnant girls, any age but up to 24 years attending primary health care clinics.
- 20,000 community members 15-49 years of age who have participated in the HIV Incidence Provincial Surveillance System (HIPSS) (BREC approval number BF 269/13), an ongoing study.

PURPOSE OF THE STUDY

The purpose of this research is to identify and understand more about the spread of HIV and factors linked with getting HIV.

YOUR PARTICIPATION IS VOLUNTARY

Your participation in this study is voluntary and you should not hesitate to ask about anything you are not clear about. Please read (or have someone to read to you) this Consent Form in the language of your choice (English or Isizulu) in order to make sure that you are given enough information about taking part in this research study. If you agree and you qualify to take part in this study, you will be asked to sign this consent form (or make your mark on the form in the presence of a witness). The study staff will then enroll you in this research. They will also give you a copy of this signed consent form to

keep. Once you join the study we hope that you answer the questions truthfully and to the best of your ability. We want to reassure you that none of the information you provide will be shared with anyone in the community or any person outside the study.

PROCEDURES

After you have agreed (**CONSENTED**) and joined the study, staff will:

- Scan your fingerprints. This is done to ensure that we keep all the information in such a way that no one can access it. If you have participated from the school and a facility we will match the fingerprint so that you are counted once only. Should we need to make contact with you in the future, we will confirm your identity through your finger print by comparing it with the finger print taken during this visit.
- Use a tracking device called Global Positioning System (GPS) to determine the location of your school or facility. Should we need to contact you for a follow-up visit the study staff will use this information to assist in finding you?
- Ask you some general and behavioural questions on your age, who you live with, and what kind of work you do. Whether you have had an HIV test and know your HIV status and some information on your sex partners. We will not ask the name(s) of your sex partners. Whether you know of HIV related programmes that are offered in the district.
- Collect a small amount of blood using a “finger-prick” with a special device. This will be used for laboratory testing for HIV related and specialized testing to understand the spread of HIV, herpes simplex virus- type 2, syphilis and pregnancy in females.
- Ask females to self-collect vulvo vaginal swab and males to collect a first pass urine sample for testing for sexually transmitted infections.
- **If you do not agree to your samples being collected, then you will not be able to participate in the study.**

We will not write your name on any of the forms or samples, we will only use numbers, so there will be no way of knowing who the form or sample came from and all this information will have restricted to study staff only. There is no limit on how long the blood, urine and vaginal samples will be stored and may be tested for other infections. If you do not want us to store the sample, then we will destroy the sample as soon as the tests are completed for this study.

We will be happy if you take part in the study, but if you do not wish to, then please just say so and we will not continue. Also if you wish to stop at any time in the interview you will be free to do so and you will not lose your rights to other services.

The results from the testing will not be available to you, but we encourage you to attend any of the local health care clinics to access general health care.

For respondent driven sampling only: You may be asked to recruit other possible participants and you will be guided on how to recruit these individuals. You will be expected to be able to briefly describe the study and ask the potential respondent if he/she would be willing to come to the interview site to discuss the study and possible enrolment. You will receive an incentive for completing this interview and another incentive for recruiting your peers to participate into the study.

RISK AND/OR DISCOMFORTS

You may feel uncomfortable or anxious about some of the questions you are asked. You are allowed to refuse to answer any question that you do not want to answer. The risks of finger prick may be a little painful for you and may result in some bruising from the site of blood collection, but staff members will help you in coping with these.

BENEFITS

The benefit of your participation will help inform research to understand and learn more about the spread of HIV in the Vulindlela district, more importantly to understand why young girls are at high risk for HIV. We hope that designing interventions/programmes to prevent and control the spread of HIV would benefit this community in the long term. You could also benefit as it would be possible to know where to access health care. In addition, study staff would refer you for the management of HIV,

pregnancy or any other minor ailments, if necessary. We hope you benefit from these referrals as you would be able to access care and treatment much earlier.

CONFIDENTIALITY

The study staff will do everything they can to keep your participation in the study private. Access to any information that you provide, the location of your school, house or facility, your fingerprints and records will be restricted and limited to the study staff. You will be given a study number so that we do not use your name. This number and your name will only appear together on one form. This form will be kept in a locked file to which only certain study staff will have access to. All questionnaires, blood samples, blood samples in storage, laboratory result sheets will not contain your name or personal information and will remain confidential. It will not be possible for people looking at any of these forms to know that they belong to you. Any reports or work that will be written and shared with the public will not make it possible for you to be identified in these reports. We will keep all information from your study records private to the extent allowed by law.

COSTS FOR BEING IN THE STUDY AND COMPENSATION

There is no cost to you for being in the study. You will receive an item to the approximate value of R25 to thank you for your time and effort.

RIGHT TO REFUSE STUDY PARTICIPATION

It is your choice to be in this study. If you decide not to take part, you will need to inform the study staff and it will not affect your education, healthcare or any services you are entitled to in any way. If you choose to take part in the study and change your mind at any time, then you can stop being in the study. Should you withdraw from the study, the samples collected from you will be included for all the testing. Your participation is entirely voluntary.

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 investigator decides that continuing in the study would be harmful to you.
- The study is cancelled by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC).
- Other administrative reasons.

STUDY APPROVAL

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (Approval number: to be inserted once received) and the Institutional Review Board of University of Pennsylvania (Approval number: to be inserted once received).

PERSONS TO CONTACT

In the event of any problems / concerns / questions you may contact

Dr Ayesha Kharsany on (031) 260 4555/4558 or

The Study Co-ordinator Ms Nonzwakazi Ntombela on (031) 260 4494, at CAPRISA, Second Floor Doris Duke Medical Research Institute, Durban or

The UKZN Biomedical Research Ethics Administration,
Research Office, Westville Campus, Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 260 4769 - Fax: 27 31 260 4609
Email: BREC@ukzn.ac.za

The study staff will be happy to answer any question or concerns.
Thank you for your time.

**CONSENT STATEMENT AND SIGNATURE PAGE FOR ENROLMENT OF VOLUNTEERS
18 YEARS AND OLDER**

- ☐ I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.
- ☐ I declare that my participation in this study is entirely voluntary and can withdraw should I wish to and it would not affect any of my treatment or care.
- ☐ I have been made aware of the procedures and that this study has minimal risks.
- ☐ I have been informed as to who the Principal Investigators are and should I wish to, I could contact
- ☐ The Principal Investigator, Dr Ayesha Kharsany on (031) 260 4555/4558 or the study Co-ordinator Ms Nonzwakazi Ntombela on 031-260 4494.
- ☐ If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers, then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001

Durban

4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 260 4769 - Fax: 27 31 260 4609

Email: BREC@ukzn.ac.za

Volunteer
Name (print)

Volunteer
Signature

Date

Study staff member who
Administered consent (print)

Study staff
Signature

Date

Witness
Name (print)

Witness
Signature

Date

Was a copy of the signed copy given to the volunteer?

☐ Yes

☐ No :If no, why not: _____

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA
Version 1.0 – 1 Dec 2015

PARENT/GUARDIAN/CARE GIVER Consent form for enrolment of volunteers younger than 18 years

ADMINISTRATIVE PAGE

If the volunteer is younger than 18 years of age, this administrative section must be completed prior to completing the consent / assent forms for enrolment.

1. Has the volunteer's age been verified?

☐ Yes

☐ No

2. If yes, indicate below how the participant's age has been verified

☐ Birth Certificate

☐ Identification Document (ID)

☐ School records

☐ Other: Specify_____.

3. Who has provided consent for this volunteer, younger than 18 years to participate in this study?

☐ Parent

☐ Legal Guardian

☐ Care giver

☐ Other_____

Study staff member
(print)

Study Staff signature

Date

If you have indicated NO to Question 1 or in 3 above there is no adult consent, please do not proceed any further.

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

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA

Version 1.0 – 1 Dec 2015

PARENT/GUARDIAN/CARE GIVER Consent form for enrolment of volunteers younger than 18 years

If the child/ward volunteer's parent/guardian/care giver cannot read, this form must be read to the child volunteer's parent/guardian/care giver exactly as written, in their language of choice, and a witness must sign this form to confirm that the correct information was given and freely consents for the child/ward to be in this study.

INTRODUCTION

Good day, my name is _____ (Consenting staff name), I am a member of a research team working with CAPRISA, University of KwaZulu-Natal.

The Principal and Co-Principal Investigators of the study are

Dr Ayesha BM Kharsany 2nd Floor Doris Duke Medical Research Institute Nelson R Mandela School of Medicine Private Bag 7, Congella 4013, Durban, South Africa Tel: +27 031-260 4555 Email address: Ayesha.kharsany@caprisa.org	Dr Hans Peter Kohler Population Studies Center 3718 Walnut Street, 272 McNeil Bldg, Philadelphia, USA PA 19104-6298 Tel: +1-215.898.7686 Email address: hpkohler@pop.upenn.edu
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Since your child/ward is younger than 18 years of age, you as the parent/guardian/caregiver may provide consent for your child/ward to participate in this study. This does not mean that your child/ward has to agree to be in the study.

- If you as the parent/guardian/caregiver agrees to your child/ward's participation in the study, we will still require your child/ward to agree to participate in this study.
- **Consent** is the permission given by you as the parent / guardian / caregiver for your child to participate in the study.
- **Assent** is a term used to describe your child/wards agreement to participate in this study because your child/ward is under 18 years of age.
- We would like to receive both **consent from you** and **assent from your child/ward** to participate in this study since your child/ward is under 18 years of age.
- The consent form will describe to you the purpose of the study, study procedures, the type of information that we will be collecting, the risks, benefits and your child/wards rights as a study participant.

BACKGROUND

Through you, your child/ward is being invited to participate in a research study that seeks to understand the spread (Transmission) of HIV amongst young people, specifically young girls, so that we better understand how HIV spreads and the factors that contribute to this spread. This is important as it would allow us to design programmes to prevent the further spread of HIV specifically in young girls who are highly vulnerable (in danger) to HIV. To date, we have limited information on HIV in young girls, however, we do know that young girls acquire HIV at a very young age and are also three to five times more likely to be HIV positive compared to boys in the same age group. We also have some information that HIV in school learners is not always acquired through sexual intercourse from other learners within and across schools, but possibly from people who are not in schools, that is, from community members. Knowing more about the spread of HIV and factors linked with getting HIV will help us improve programmes on preventing and treating HIV with the aim of breaking the chains of spread of HIV and reducing the overall number of new HIV infections in the general population.

The study is expected to enroll a large number of individuals from Vulindlela and Greater Edendale districts. We will include about

- 8,977 high school learners, 12 years and older from high schools in Vulindlela
- 2,000 adolescent girls and boys, 12 years and older who are not in schools
- 850 adolescent pregnant girls, any age but up to 24 years attending primary health care clinics.
- 20,000 community members 15-49 years of age who have participated in the HIV Incidence Provincial Surveillance System (HIPSS) (BREC approval number BF 269/13), an ongoing study.

PURPOSE OF THE STUDY

The purpose of this research is to identify and understand more about the spread of HIV and factors linked with getting HIV.

YOUR CHILD/WARDS PARTICIPATION IS VOLUNTARY

Participation of your child/ward in this study is voluntary and you should not hesitate to ask about anything you are not clear about the study. Please read (or have someone to read to you) this Consent Form in the language of your choice (English or isiZulu) in order to make sure that you are given enough information about your child/ward taking part in this research study. If you agree and your child/ward qualifies to take part in this study, you will be asked to sign this consent form (or make your mark on the form in the presence of a witness). The study staff will then enroll your child/ward in this research. They will also give you a copy of this consent form to keep. Once your child/ward joins the study we hope that he/she will answer the questions truthfully and to the best of his/her ability. We want to reassure you that none of the information your child/ward provides will be shared with anyone in the community or any person outside the study.

PROCEDURES

After you have agreed (**CONSENTED**) for your child/ward to join the study and additionally he/she agrees (**ASSENTED**) to participate, the study staff will:

- Scan your child/ward's fingerprints. This is done to ensure that we keep all the information in such a way that no one can access it. If he/she has participated from the school and a facility, we will match the fingerprint so that he/she is counted once only. Should we need to make contact with him/her in the future, we will confirm his/her identity through the finger print by comparing it with the finger print taken during the first visit.
- Use a tracking device called Global Positioning System (GPS) to determine the location of his/her school or facility. Should we need to contact him/her for a follow-up visit the study staff will use this information to assist in finding him/her?
- Ask your child/ward some general and behavioural questions on their age, who they live with, and what kind of work you do. Whether they have had an HIV test and know their HIV status, some information on their sex partners. We will not ask the name(s) of their sex partners. Whether they know about the HIV related programmes that are offered in the district.
- Collect a small amount of blood using a "finger-prick". This will be used for laboratory testing for HIV related and specialized testing to understand the spread of HIV, herpes simplex virus-type 2, syphilis and pregnancy in females
- Ask females to self-collect vulvo vaginal swabs and males to collect a first pass urine sample for testing for sexually transmitted infections.
- **If you do not agree to your child/ward samples being collected, then he/she will not be able to participate in the study.**

We will not write your child/ward's name on any of the samples, we will only use numbers, so there will be no way of knowing who the sample came from and all this information is restricted to study staff. There is no limit on how long the blood, urine and vaginal samples will be stored and may be tested for other infections. If you do not want us to store your child/ward's sample, then we will destroy the sample as soon as the tests are completed for this study.

We will be happy if your child/ward takes part in the study, but if you do not wish for your child/ward to take part, then please just say so and we will not ask your child/ward to participate. Also if your

child/ward wishes to stop at any time in the interview they will be free to do so and will not lose their rights to other services. The results from the testing will not be available to your child/ward, but we encourage you and your child/ward to attend any of the local health care clinics to access general health care.

For respondent driven sampling only: Your child/ward may be asked to recruit other possible participants and he/she will be guided on how to recruit these individuals. Your child/ward will be expected to be able to briefly describe the study and ask the potential respondent if he/she would be willing to come to the interview site to discuss the study and possible enrolment. Your child/ward will receive an incentive for completing this interview and another incentive for recruiting peers to participate into the study.

RISK AND/OR DISCOMFORTS

Your child/ward may feel uncomfortable or anxious about some of the questions he/she is asked. Your child/ward is allowed to refuse to answer any question that he/she does not want to answer. The risks of finger-prick may be a little painful for him/her and may result in some bruising from the site of blood collection, but staff members will help him/her in coping with these.

BENEFITS

The benefit of your child/ward's participation will help inform research to understand and learn more about the spread of HIV in the Vulindlela district, more importantly to understand why young girls are at high risk for HIV. We hope that designing interventions/programmes to prevent and control the spread of HIV would benefit this community in the long term. Your child/ward could also benefit as it would be possible to know where to access health care. In addition, study staff would refer your child/ward for the management of HIV, pregnancy or any other minor ailments, if necessary. We hope your child/ward benefits from these referrals as he/she would be able to access care and treatment much earlier.

CONFIDENTIALITY

The study staff will do everything they can to keep your child/ward's participation in the study private. Access to any information that your child/ward provides, the location of his/her school, house or facility, his/her fingerprints and records will be restricted and limited to the study staff. Your child/ward will be given a study number so that we do not use his/her name. This number and his/her name will only appear together on one form. This form will be kept in a locked file to which only certain study staff will have access to. All questionnaires, blood samples, blood samples in storage, laboratory result sheets will not contain his/her name or personal information will remain confidential. It will not be possible for people looking at any of these forms to know that they belong to your child/ward. Any reports or work that will be written and shared with the public will not make it possible for any individual to be identified in these reports. We will keep all information from your child/ward study records private to the extent allowed by law. Any samples collected will remain in storage without your name but with a number, they will not be discarded and the results of the testing will be used in the analysis.

COSTS FOR BEING IN THE STUDY AND COMPENSATION

There is no cost to you or to your child/ward for being in the study. Your child/ward will receive an item to the approximate value of R25 to thank him/her for his/her time and effort.

RIGHT TO REFUSE STUDY PARTICIPATION

It is yours and your child/ward's choice for him/her to be in this study. If you decide that your child/ward should not take part, you should inform the study staff and it will not affect your child's/ward's education, healthcare or any services that they are entitled to in any way. If you choose for your child/ward to take part in the study and change your mind at any time, then your child/ward can stop being in the study. Should your child/ward withdraw from the study, the samples collected from him/her will be included for all the testing. Your child/wards participation is entirely voluntary.

REASONS WHY YOUR CHILD/WARD MAY BE WITHDRAWN FROM THE STUDY WITHOUT THEIR CONSENT

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

- The investigator decides that continuing in the study would be harmful to your child/ward.
- The study is cancelled by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC).
- Other administrative reasons.

STUDY APPROVAL

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (Approval number: to be inserted once received) and the Institutional Review Board of University of Pennsylvania (Approval number: to be inserted once received).

PERSONS TO CONTACT

In the event of any problems or concerns/questions, you and/or your child/ward may contact

Dr Ayesha Kharsany on (031) 260 4555/4558 or

The Study Co-ordinator Ms Nonzwakazi Ntombela on (031) 260 4494, at CAPRISA, Second Floor Doris Duke Medical Research Institute, Durban or

The UKZN Biomedical Research Ethics Administration,
Research Office, Westville Campus, Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

The study staff will be happy to answer any questions or concerns.

Thank you for your time.

**PARENT/GUARDIAN/CARE-GIVER CONSENT STATEMENT AND SIGNATURE PAGE
FOR ENROLMENT OF VOLUNTEERS YOUNGER THAN 18 YEARS**

- ☐ I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.
- ☐ I declare that my child/wards participation in this study is entirely voluntary and can withdraw should I or he/she wishes to and it would not affect any of their treatment or care.
- ☐ I have been made aware of the procedures and that this study has minimal risks.
- ☐ I have been informed as to who the Principal Investigators are and should I wish to, I could contact
- ☐ The Principal Investigator, Dr Ayesha Kharsany on (031) 260 4555/4558 or the study Co-ordinator Ms Nonzwakazi Ntombela on 031-260 4494.
- ☐ If I have any questions or concerns about my rights for my child/ward as a study participant, or if I am concerned about an aspect of the study or the researchers, then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Parent/Guardian/Care giver
Name (print)

Parent/Guardian/Care giver
Signature

Date

Study staff member who
Administered consent (print)

Study Staff
Signature

Date

Witness
Name (print)

Witness
Signature

Date

Was a copy of the signed copy given to the volunteer?

☐ Yes

☐ No :If no, why not: _____

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA
Version 1.0 – 1 Dec 2015

Assent form for enrolment of volunteers younger than 18 years

ADMINISTRATIVE PAGE

If the volunteer is younger than 18 years of age, this administrative section must be completed prior to completing the assent form for enrolment.

1. Has the volunteer's age been verified?
☐ Yes

☐ No
2. If yes, indicate below how the participant's age has been verified

☐ Birth Certificate
☐ Identification Document (ID)
☐ School records
☐ Other: Specify_____.

3. Who has provided consent for this volunteer to participate in this study?
☐ Parent
☐ Legal Guardian
☐ Care giver
☐ Other_____

Study staff member
(print)

Study staff signature

Date

If you have indicated NO to Question 1 or in 3 above there is no adult consent, please do not proceed any further.

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

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA
Version 1.0 – 1 Dec 2015

Assent form for enrolment of volunteers younger than 18 years

If the child volunteers cannot read, this form must be read to the volunteer exactly as written, in their language of choice and a witness must sign this form to confirm that the correct information was given and child has freely assented to be in this study.

INTRODUCTION

Good day, my name is _____ (Consenting staff name), I am a member of a research team working with CAPRISA, University of KwaZulu-Natal.

The Principal and Co-Principal Investigators of the study are

Dr Ayesha BM Kharsany 2nd Floor Doris Duke Medical Research Institute Nelson R Mandela School of Medicine Private Bag 7, Congella 4013, Durban, South Africa Tel: +27 031-260 4555 Email address: Ayesha.kharsany@caprisa.org	Dr Hans Peter Kohler Population Studies Center 3718 Walnut Street, 272 McNeil Bldg, Philadelphia, USA PA 19104-6298 Tel: +1-215.898.7686 Email address: hpkohler@pop.upenn.edu
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Since you are younger than 18 years of age, your parent/ guardian/caregiver may provide consent for you to participate in this study. This means that you as a child also has to agree (Assent) to be in the study.

- If your parent/guardian/caregiver agrees to your participation in the study, we will still require for you to agree to participate in this study.
- **Assent** is a term used to describe your agreement to participate in this study because you are under 18 years of age.
- **Consent** is the permission given by your parent/guardian/caregiver for you to participate in the study.
- We would like to receive both **assent from you** and **consent from your parent/guardian/caregiver** for you to participate in the study since you are under 18 years of age.
- The assent form will describe to you the purpose of the study, study procedures, the type of information that we will be collecting, the risks, benefits and your rights as a study participant.

I will give you and your parent/guardian/caregiver information about this study and talk to you today about what your participation would involve. If you and your parent / guardian /caregiver agrees for you to be part of this study and if you qualify to participate, I will further give you and your parent / guardian /caregiver information about the procedures that you will undergo, what we will expect from you and your rights as a participant.

BACKGROUND

You are being invited to participate in a research study that seeks to understand the spread (Transmission) of HIV amongst young people, specifically young girls, so that we better understand how HIV spreads and the factors that contribute to this spread. This is important as it would allow us to design programmes to prevent the further spread of HIV specifically in young girls who are highly vulnerable (in danger) to HIV. To date, we have limited information on HIV in young girls, however, we do know that young girls acquire HIV at a very young age and are also three to five time more likely

to be HIV positive compared to boys in the same age group. We also have some information that HIV in school learners is not always acquired through sexual intercourse from other learners within and across schools, but possibly from people who are not in schools, that is, from community members. Knowing more about the spread of HIV and factors linked with getting HIV will help us improve programmes on preventing and treating HIV with the aim of breaking the chains of spread of HIV and reducing the number of new HIV infections in the general population.

The study is expected to enroll a large number of individuals from Vulindlela and Greater Edendale districts. We will include about

- 8,977 high school learners, 12 years and older from high schools in Vulindlela
- 2,000 adolescent girls and boys, 12 years and older who are not in schools
- 850 adolescent pregnant girls, any age but up to 24 years attending primary health care clinics.
- 20,000 community members 15-49 years of age who have participated in the HIV Incidence Provincial Surveillance System (HIPSS) (BREC approval number BF 269/13), an ongoing study.

PURPOSE OF THE STUDY

The purpose of this research is to identify and understand more about the spread of HIV and factors linked with getting HIV.

YOUR PARTICIPATION IS VOLUNTARY

Your participation in this study is voluntary and you should not hesitate to ask about anything you are not clear about. Please read (or have someone to read to you) this Assent Form in the language of your choice (English or isiZulu) in order to make sure that you are given enough information about taking part in this research study. If you agree and you qualify to take part in this study, you will be asked to sign this assent form (or make your mark on the form in the presence of a witness). Your parent/guardian/caregiver will also have to give permission; that is consent. The study staff will then enroll you in this research. They will also give you a copy of this assent form to keep. Your parent / guardian /caregiver will also receive a copy of their form (consent) where he/she has given his/her permission. Once you agree to join the study we hope that you will answer the questions truthfully and to the best of your ability. We want to reassure you that none of the information you provide will be shared with anyone in the community or any person outside the study.

PROCEDURES

After you have agreed (**ASSENTED**) and your parent / guardian /caregiver has agreed (**CONSENTED**) for you to participate, the study staff will:

- Scan your fingerprints. This is done to ensure that we keep all the information in such a way that no one can access it and link it to you. If you participated from the school and a facility we will match the fingerprint so that you are counted once only. Should we need to make contact with you in the future, we will confirm your identity through the finger print by comparing it with the finger print taken during the first visit.
- Use a tracking device called Global Positioning System (GPS) to determine the location of your school or facility. Should we need to contact you for a follow-up visit the study staff will use this information to assist in finding you?
- Ask you some general and behavioural questions on your age, who you live with, and what kind of work you do. Whether you they have had an HIV test and know your HIV status and some information on your sex partners. We will not ask the name(s) of your sex partners. Whether you know of HIV related programmes that are offered in the district.
- Collect a small amount of blood using a “finger-prick” with a special device. This will be used for laboratory testing for HIV related and specialized testing to understand the spread of HIV, herpes simplex virus- type 2, syphilis and pregnancy in females.
- Ask females to self-collect vulvo vaginal swabs and males to collect a first pass urine sample for testing for sexually transmitted infections.
- **If you do not agree to your samples being collected then you will not be able to participate in the study.**

We will not write your name on any of the samples, we will only use numbers, so there will be no way of knowing who the sample came from and all this information will be restricted to study staff only. There is no limit on how long the blood, urine and vaginal samples will be stored and may be tested for other infections. If you do not want us to store the sample then we will destroy the sample as soon as the tests are completed for this study.

We will be happy if you take part in the study, but if you do not wish to take part, then please just say so and we will not ask you to participate. Also if you wish to stop at any time in the interview you will be free to do so and will not lose your rights to other services. The results from the testing will not be available to you, but we encourage you and your family members to attend any of the local health care clinics to access general health care.

For respondent driven sampling only: You may be asked to recruit other possible participants and you will be guided on how to recruit these individuals. You will be expected to be able to briefly describe the study and ask the potential respondent if he/she would be willing to come to the interview site to discuss the study and possible enrolment. You will receive an incentive for completing this interview and another incentive for recruiting your peers to participate into the study.

RISK AND/OR DISCOMFORTS

You may feel uncomfortable or anxious about some of the questions asked. You are allowed to refuse to answer any question that you do not want to answer. The risks of finger prick may be a little painful for you and may result in some bruising from the site of blood collection, but staff members will help you in coping with these.

BENEFITS

The benefit of your participation will help inform research to understand and learn more about the spread of HIV in the Vulindlela district, more importantly to understand why young girls are at high risk for HIV. We hope that designing interventions/programmes to prevent and control the spread of HIV would benefit this community in the long term. You could also benefit as it would be possible to know where to access health care. In addition study staff would refer you for the management of HIV, pregnancy or any other minor ailments, if necessary. We hope you benefit from these referrals as you would be able to access care and treatment much earlier.

CONFIDENTIALITY

The study staff will do everything they can to keep your participation in the study private. Access to any information that you provide, the location of your school, house or facility, your fingerprints and records will be restricted and limited to the study staff. You will be given a study number so that we do not use your name. This number and your name will only appear together on one form. This form will be kept in a locked file to which only certain study staff will have access to. All questionnaires, blood samples, blood samples in storage, laboratory result sheets will not contain your name or personal information, will remain confidential. It will not be possible for people looking at any of these forms to know that they belong to you. Any reports or work that will be written and shared with the public will not make it possible for any individual to be identified in these reports. We will keep all information from your study records private to the extent allowed by law. Any samples collected will remain in storage without your name but with a number, they will not be discarded and the results of the testing will be used in the analysis.

COSTS FOR BEING IN THE STUDY AND COMPENSATION

There is no cost to you for being in the study. You will receive an item to the approximate value of R25 to thank you for your time and effort.

RIGHT TO REFUSE STUDY PARTICIPATION

It is yours and your parent/guardian/caregiver choice for you to be in this study. If you decide that you should not take part, you should inform the study staff and it will not affect your education, healthcare or any services that you are entitled to in any way. If you choose to take part in the study and change

your mind at any time, then you can stop being in the study. Should you withdraw from the study, the samples collected from you will be included for all the testing. Your participation is entirely voluntary.

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 investigator decides that continuing in the study would be harmful to you.
- The study is cancelled by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC).
- Other administrative reasons.

STUDY APPROVAL

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (Approval number: to be inserted once received) and the Institutional Review Board of University of Pennsylvania (Approval number: to be inserted once received).

PERSONS TO CONTACT

In the event of any problems or concerns/questions you and /or your parent/ guardian/care giver may contact

Dr Ayesha Kharsany on (031) 260 4555/4558 or

The Study Co-ordinator Ms Nonzwakazi Ntombela on (031) 260 4494, at CAPRISA, Second Floor Doris Duke Medical Research Institute, Durban or

The UKZN Biomedical Research Ethics Administration,
Research Office, Westville Campus, Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

The study staff will be happy to answer any questions or concerns.

Thank you for your time.

**ASSENT STATEMENT AND SIGNATURE PAGE FOR ENROLMENT OF VOLUNTEERS
YOUNGER THAN 18 YEARS**

- ☐ I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.
- ☐ I declare that my participation in this study is entirely voluntary and can withdraw should I wish to and it would not affect any of my treatment or care.
- ☐ I have been made aware of the procedures and that this study has minimal risks.
- ☐ I have been informed as to who the Principal Investigators are and should I wish to, I could contact
- ☐ The Principal Investigator, Dr Ayesha Kharsany on (031) 260 4555/4558 or the study Co-ordinator Ms Nonzwakazi Ntombela on 031-260 4494,
- ☐ If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers, then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001

Durban

4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

Volunteer
Name (print)

Volunteer
Signature

Date

Study staff member who
administered assent (print)

Study staff
Signature

Date

Witness
Name (print)

Witness
Signature

Date

Was a copy of the signed copy given to the volunteer?

☐ Yes

☐ No :If no, why not: _____

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA
Version 1.0 – 1 Dec 2015

Informed Consent form for Biological Sample Storage for possible future research for
volunteers 18 years and older

The Principal and Co-Principal Investigators of the study are

Dr Ayesha BM Kharsany 2nd Floor Doris Duke Medical Research Institute Nelson R Mandela School of Medicine Private Bag 7, Congella 4013, Durban, South Africa Tel: +27 031-260 4555 Email address: Ayesha.kharsany@caprisa.org	Dr Hans Peter Kohler Population Studies Center 3718 Walnut Street, 272 McNeil Bldg, Philadelphia, USA PA 19104-6298 Tel: +1-215.898.7686 Email address: hpkohler@pop.upenn.edu
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INTRODUCTION

You have decided to be part of the study. There may be some remaining blood, urine and/or vaginal swabs (females) samples, taken from you during the study that might be useful for future research. You are being asked to agree to the storage of the left over samples for future research that will include additional testing and may or may not be linked to the current study.

This consent (agreement) form gives you information about the collection, storage and use of your samples 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 samples, you will be asked to sign this agreement form. You will get a copy to keep.

HOW WILL YOU GET THE SAMPLES FROM ME?

The study staff will collect your blood, and urine (males) and a vulvo-vaginal swab (females) samples as part of the study that you have consented to participate in. These samples are needed to carry out the regular tests for the research study. If you agree to have your samples stored for possible future research, we will store the left overs of the samples after all the tests that allow us to understand the spread of HIV for the current study have been completed.

HOW WILL YOU USE MY STORED SAMPLES?

Your samples will be used to confirm results with new tests when these become available or to look for other infections, or for damage caused by such infections, or the body's response to infection. Researchers may also look at your genes (DNA), since genes can affect the way the body responds to infections in important ways. Your genes might make you more or less likely to get infected, or make the responses to infection or to treatment stronger or weaker. If you become infected with HIV, your genes might also affect how fast or slowly you develop AIDS.

Your samples may be shared with colleagues both in and outside of South Africa. Your stored samples will be sent with only your number and will not be linked to any personal identifiers such as your name. Any future research using your samples will be reviewed first by the CAPRISA Scientific Review Committee and a special committee at the Nelson R Mandela School of Medicine Biomedical Research Ethics Committee.

It is important for you to know that your samples will not be sold or used in products that make money for the researchers.

WHERE WILL MY SAMPLES BE STORED?

Your samples will be stored with your number in special facilities that are safe and secure at the CAPRISA research Laboratory, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine and only approved researchers can have access to the samples.

HOW LONG WILL YOU KEEP MY SAMPLES?

There is no time limit on how long your samples will be stored for.

DOES STORAGE OF MY SAMPLES BENEFIT ME?

There are no direct benefit to you, but doing research on the stored samples may benefit the society in the future and include learning more about HIV infection.

WHAT ARE THE RISKS?

We do not anticipate any risks as the stored samples are not linked with name. But it is possible should genetic testing may be done, researchers will not have access to your personal information and it will not be possible for investigators to contact you or your family to inform you about the results.

WHAT ABOUT CONFIDENTIALITY?

All samples will be labelled with a number. Your personal information (name, address, phone number) will not be placed on the samples. When researchers are given your samples to study, they will not be given any personal information. We will make every effort to keep your personal information confidential, but may be disclosed if required by law.

WHAT ARE MY RIGHTS?

Allowing your samples to be stored is voluntary. You may decide not to have your samples stored other than what is needed to understand the spread of HIV has been completed, thereafter all remaining samples will be destroyed. Whatever you decide now, but change your mind in the future, you will need to let the study staff know about your decision.

STUDY APPROVAL

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (Reference number to be inserted) and the IRB of University of Pennsylvania, USA.

PERSONS TO CONTACT

For any questions on the storage of your samples you may contact

Dr Ayesha Kharsany on (031) 260 4555/4558 or

The Study Co-ordinator Ms Nonzwakazi Ntombela on (031) 260 4494, CAPRISA, Second Floor
Doris Duke Medical Research Institute, Durban or

The UKZN Biomedical Research Ethics Committee, contact details as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus, Govan Mbeki Building

Private Bag X 54001, Durban, 4000, KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

Thank you for your time.

CONSENT STATEMENT AND SIGNATURE PAGE FOR SAMPLE STORAGE FOR VOLUNTEERS 18 YEARS AND OLDER

- ☐ I have received the information and have had the chance to ask questions on the storage of my samples that have been collected from me for the research study titled '**IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN RURAL SOUTH AFRICA.**' I have been informed that any samples that are left over will be stored and possibly tested in the future if needed.
- ☐ Please carefully read the statements below and think about your choice. No matter what you decide it will not affect your participation in the research study.

I agree to have my samples stored for future research and possible testing related to HIV and other infections.

☐ Yes

☐ No

_____ Volunteer Name (print)	_____ Volunteer Signature	_____ Date
_____ Study staff member who Administered consent (print)	_____ Study staff Signature	_____ Date
_____ Witness Name (print)	_____ Witness Signature	_____ Date

Was a copy of the signed copy given to the volunteer?

☐ Yes

☐ No :If no, why not: _____

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA
Version 1.0 – 1 Dec 2015

PARENT/GUARDIAN/CARE GIVER Consent form for Biological Sample Storage for possible future research for volunteers younger than 18 years

The Principal and Co-Principal Investigators of the study are

Dr Ayesha BM Kharsany 2nd Floor Doris Duke Medical Research Institute Nelson R Mandela School of Medicine Private Bag 7, Congella 4013, Durban, South Africa Tel: +27 031-260 4555 Email address: Ayesha.kharsany@caprisa.org	Dr Hans Peter Kohler Population Studies Center 3718 Walnut Street, 272 McNeil Bldg, Philadelphia, USA PA 19104-6298 Tel: +1-215.898.7686 Email address: hpkohler@pop.upenn.edu
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INTRODUCTION

You have decided for your child/ward to be part of the study. There may be some remaining blood, urine and/or vaginal swabs (females) samples, taken from for your child/ward during the study that might be useful for future research. You are being asked to agree to the storage of your child/ward's left over samples for future research that will include additional testing and may or may not be linked to the current study.

This consent (agreement) form gives you information about the collection, storage and use of your child's/ward's samples 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's/ward's samples, you will be asked to sign an agreement form. You will get a copy to keep.

HOW WILL YOU GET THE SAMPLES FROM CHILD/WARD?

The study staff will collect your child/ward's blood, and urine (males) and vulvo-vaginal swab samples (females) as part of the study that you have consented for your child/ward to participate in. These samples are needed to carry out the regular tests for the research study. If you agree to have your child/ward's samples stored for possible future research, we will store the left overs of the samples after the tests for the current study have been completed.

HOW WILL YOU USE MY CHILD/WARD'S STORED SAMPLES?

Your child/ward's samples will be used to confirm results with new tests when these become available or to look for other infections, or damage caused by such infections, or the body's response to infection. Researchers may also look at your child/ward's genes (DNA), since genes can affect the way the body responds to infections in important ways. Your child/ward's genes might make him/her more or less likely to get infected, or make the responses to infection or to treatment stronger or weaker. If your child/ward become infected with HIV, his/her genes might also affect how fast or slowly he/she develop AIDS.

Your child/ward's samples may be shared with colleagues both in and outside of South Africa. Your child/ward's stored samples will be sent with only his/her number and will not be linked to any personal identifiers such as his/her name. Any future research studies using your child/ward's samples will be reviewed first by the CAPRISA Scientific Review Committee and a special committee at the Nelson R Mandela School of Medicine Biomedical Research Ethics Committee.

It is important for you to know that your child/ward's samples will not be sold or used in products that make money for the researchers.

WHERE WILL MY CHILD/WARD'S SAMPLES BE STORED?

Your child/ward's samples will be stored with your number in special facilities that are safe and secure at the CAPRISA research Laboratory, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine and only approved researchers can have access to the samples.

HOW LONG WILL YOU KEEP MY CHILD/WARD'S SAMPLES FOR?

There is no time limit on how long the samples will be stored for.

DOES STORAGE OF MY CHILD/WARD'S SAMPLES BENEFIT HIM/HER?

There are no direct benefit to you or your child/ward, but doing research on the stored samples may benefit the society in the future and include learning more about HIV infection.

WHAT ARE THE RISKS?

We do not anticipate any risks to you , your child/ward as the stored samples are not linked with names. But it is possible should genetic testing may be done, researchers will not have access to your child/ward's personal information and it will not be possible for investigators to contact you or your child/ward or family to inform you about the results.

WHAT ABOUT CONFIDENTIALITY?

All samples will be labelled with a number. Your child's/wards personal information (name, address, phone number) will not be placed on the samples. When researchers are given any samples to study, they will not be given any personal information. We will make every effort to keep your child's/ward's personal information confidential, but may be disclosed if required by law.

WHAT ARE MY, MY CHILD/WARD'S RIGHTS?

Allowing your child/ward's samples to be stored is voluntary. You may decide not to have your child/ward's samples stored other than what is needed to understand the spread of HIV has been completed, thereafter all remaining samples will be destroyed. Whatever you decide now, but change your mind in the future, you will need to let the study staff know about your decision.

STUDY APPROVAL

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Thank you for your time.

CONSENT STATEMENT AND SIGNATURE PAGE FOR PARENT/GUARDIAN/CARE-GIVER FOR SAMPLE STORAGE OF VOLUNTEERS YOUNGER THAN 18 YEARS

☐ I have received the information and have had the chance to ask questions on the storage of my child/ward's samples that have been collected for the research study titled '**IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN RURAL SOUTH AFRICA.**' I have been informed that any samples that are left over will be stored and possibly tested in the future if needed.

☐ Please carefully read the statements below and think about your choice. No matter what you decide it will not affect your child /ward's participation in the research study.

I agree to have my child/ward's samples stored for future research and possible testing related to HIV and other infections.

☐ Yes

☐ No

Parent/Guardian/Care giver
Name (print)

Parent/Guardian/Care giver
Signature

Date

Study staff member who
Administered consent (print)

Study Staff
Signature

Date

Witness
Name (print)

Witness
Signature

Date

Was a copy of the signed copy given to the volunteer?

☐ Yes

☐ No :If no, why not: _____

Title of Study
IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN
RURAL SOUTH AFRICA
Version 1.0 – 1 Dec 2015

ASSENT form for Biological Sample Storage for possible future research for volunteers
younger than 18 years

The Principal and Co-Principal Investigators of the study are

Dr Ayesha BM Kharsany 2nd Floor Doris Duke Medical Research Institute Nelson R Mandela School of Medicine Private Bag 7, Congella 4013, Durban, South Africa Tel: +27 031-260 4555 Email address: Ayesha.kharsany@caprisa.org	Dr Hans Peter Kohler Population Studies Center 3718 Walnut Street, 272 McNeil Bldg, Philadelphia, USA PA 19104-6298 Tel: +1-215.898.7686 Email address: hpkohler@pop.upenn.edu
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HOW WILL YOU USE MY STORED SAMPLES?

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Thank you for your time.

ASSENT STATEMENT AND SIGNATURE PAGE FOR SAMPLE STORAGE OF VOLUNTEERS YOUNGER THAN 18 YEARS

☐ I have received the information and have had the chance to ask questions on the storage of my samples that have been collected for the research study titled '**IDENTIFYING SOURCES OF HIV INFECTION IN ADOLESCENT GIRLS IN RURAL SOUTH AFRICA.**' I have been informed that any samples that are left over will be stored and possibly tested in the future if needed.

☐ Please carefully read the statements below and think about your choice. No matter what you decide it will not affect your participation in the research study.

I agree to have my samples stored for future research and possible testing related to HIV and other infections.

☐ Yes

☐ No

Volunteer
Name (print)

Volunteer
Signature

Date

Study staff member who
administered assent (print)

Study staff
Signature

Date

Witness
Name (print)

Witness
Signature

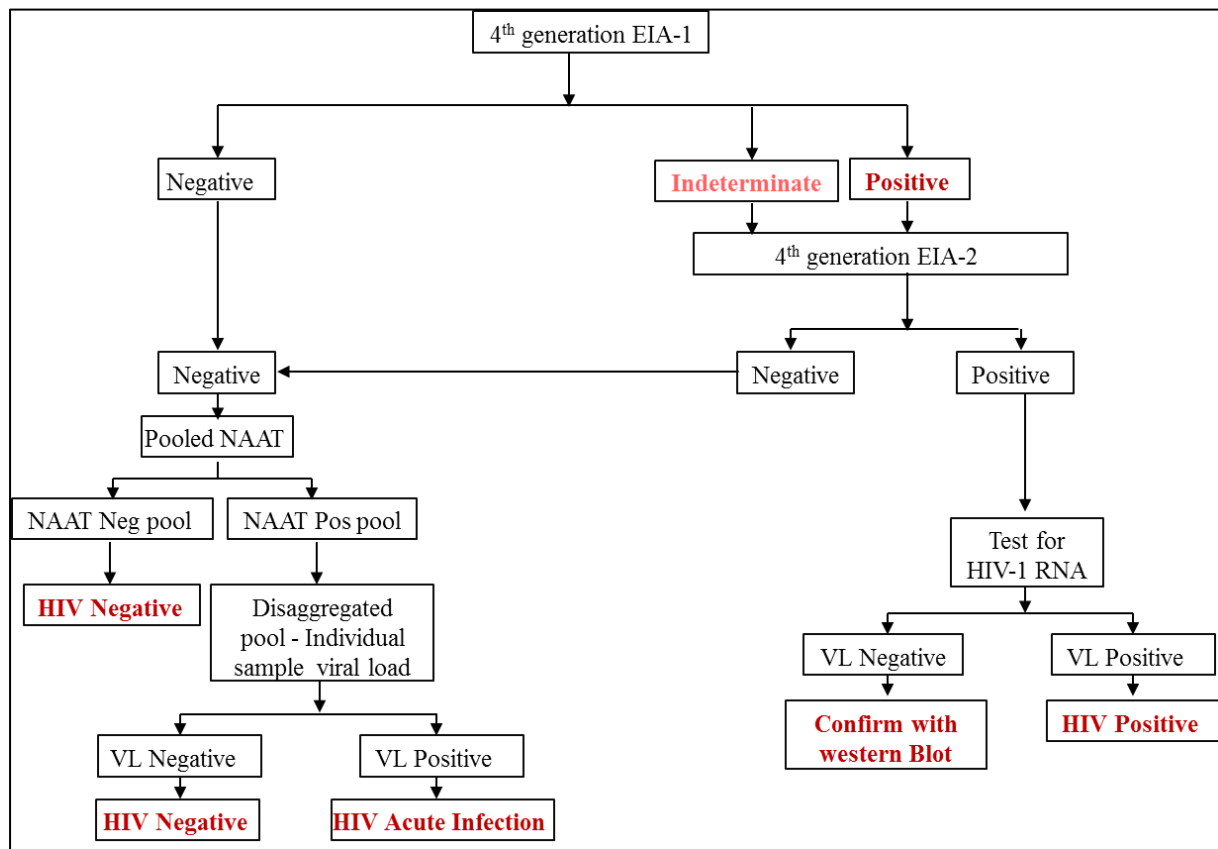
Date

Was a copy of the signed copy given to the volunteer?

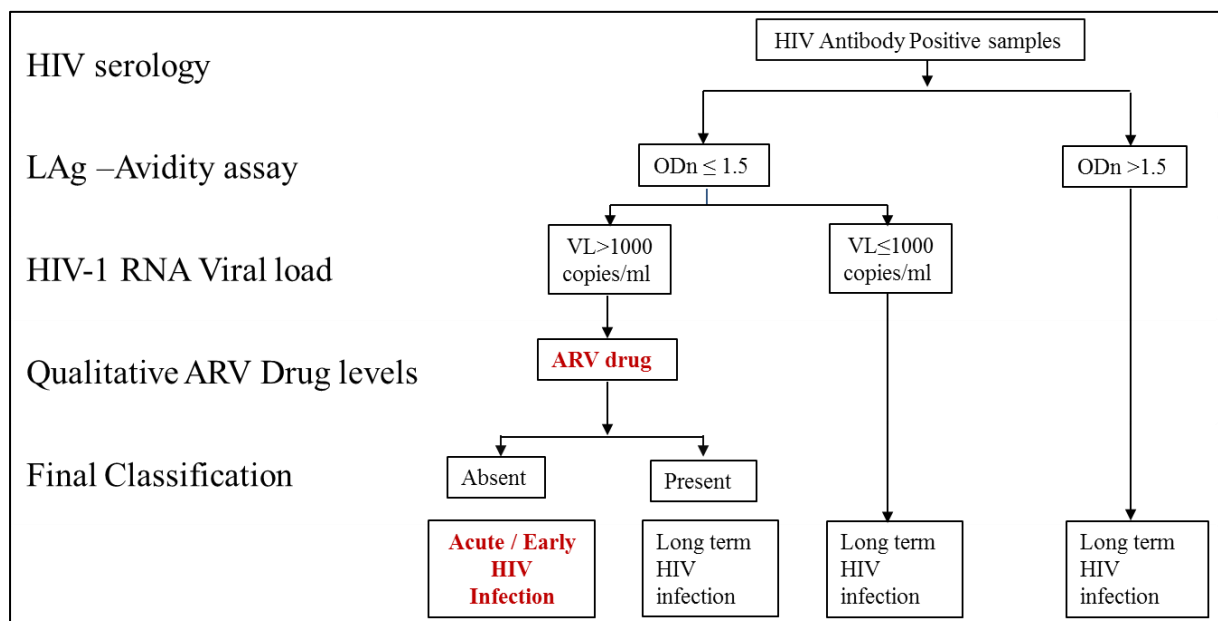
☐ Yes

☐ No :If no, why not: _____

APPENDIX 3: HIV ANTIBODY TESTING ALGORITHM



APPENDIX 4: HIV INCIDENCE TESTING ALGORITHM



APPENDIX 3: QUESTIONNAIRES

CAPRISA 085 Demographic Information

Version 1.0- 1 Dec 2015

Participant identification number

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Demographics Information

I would like to ask you some questions about yourself. Please remember that your name will not be recorded anywhere in this questionnaire and the information you give will be kept confidential.

Your honesty is much appreciated.

1. Gender

☐ Male

☐ Female

2. How old were you at your last birthday?

years

3. What is your date of birth?

dd mm yy

4. What is the participants race (observed)?

☐ Black (Zulu)

☐ Other

5. What is your highest level of education completed?

☐ No schooling/ crèche/ pre-primary

☐ Primary (grade 1 – 7),-

☐ Completed secondary (grade 12/NTC3),

☐ Incomplete secondary (grade 8 – 11/NTC1/NTC2)

☐ Some tertiary (incomplete tertiary education) Tertiary (completed diploma/ degree)

☐ No response

6. Are you currently

☐ still in school

Grade

☐ Employed

Occupation_____

☐ Part time employed

Occupation_____

☐ Not employed

7. What is your source of income?

☐ Self-generated income

☐ Salary/wage Employed

☐ Partners income

☐ Social grants

☐ Family support

☐ No income

☐ Other, specify _____

8. How long have you lived in this community?

☐ Always

- ☐ Some years/months
- ☐ Don't know
- ☐ No response

9. Who do you live with?

With biological

- ☐ mother and father
- ☐ mother only
- ☐ father only

with non biological

- ☐ mother and father
- ☐ mother only
- ☐ father only
- ☐ With other family members
- ☐ With siblings

10. The person you live with, is this person at home

- ☐ Always
- ☐ weekends only
- ☐ end of the month only
- ☐ end of the year only

11. Do you travel away from home for more than one consecutive week

- ☐ Yes
- ☐ No

12. In your home (Household economic situation)

- | | | |
|---|------------------------------|--------------------------|
| Is there enough money for basic things like food and clothes? | ☐ yes | ☐ |
| no | | |
| Money for food and clothes but short in money for other things? | ☐ yes | ☐ |
| no | | |
| Do you have most of the important things but few luxury goods? | ☐ yes | ☐ |
| no | | |
| Do you have money for extra things such as holidays and luxury goods? | ☐ yes | ☐ |
| no | | |

