Sexually Transmitted Infections and the Associated Risk Factors among Young Women Aged 18-24 Years in Kisumu City, Kenya.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

This work is dedicated to my loving parents and siblings for their unreserved support and motivation throughout this study.

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LIST OF ABBREVIATIONS

AOR	Adjusted Odds Ratio
BV	Bacterial vaginosis
C.I	Confidence Intervals
CDC	Centers for Disease Control and Prevention
CRF	Case report form
DKA	Dual Kinetic Assay
ELISA	Enzyme Linked Immunosorbent Assay
FSWs	Female Sex Workers
HSV-2	Herpes Simplex Virus type 2
IgG	Immunoglobulin G
KAIS	Kenya AIDS Indicator Survey
KDHS	Kenya Demographic Health Survey
NAAT	Nucleic Acid Amplification Tests
NASCOP	National AIDS and STD Control Program
OR	Odds Ratio
PCR	Polymerase Chain reaction
RPR	Rapid Plasma Reagin
RTI	Reproductive Tract Infection
STI	Sexually Transmitted Infections
TMA	Transcription Mediated Amplification
TPPA	*Treponema Pallidum* Particle Agglutination Assay

ABSTRACT

Sexually transmitted infections including HIV/AIDS are common in developing countries. Young women continue to be one of the populations at greatest risk for HIV infection. The STIs and HIV epidemics are inter-dependent. Behaviours such as frequent unprotected intercourse with different partners, place people at high risk of both infections, and there is clear evidence that conventional STIs increase the likelihood of HIV transmission. The main objective of this study was to investigate the prevalence of and risk factors for STIs among young women (aged 18-24 years) in Kisumu city. This was a cross-sectional analysis of 312 women screened for participation in a phase 1 randomized placebo controlled microbicide trial. Prevalent cases of gonorrhea, chlamydia, trichomoniasis were identified through Nucleic acid amplification test (NAAT), HIV and herpes simplex virus (HSV)-2 were diagnosed using enzyme linked immunosorbent assay (ELISA), syphilis was tested using rapid plasma reagin (RPR) test , bacterial vaginosis was identified through Nugent scoring criteria and yeast infection was identified through wet mount. Demographic information and behavioral risk factors were assessed using structured questionnaires. Data analysis was carried out using Epi Info™ version 3.3. Results showed that herpes simplex virus type 2 was the most prevalent STI at 30.4%, HIV was 6.7%, in addition, non-classical STIs such as bacterial vaginosis and yeast infection were diagnosed in 19.9% and 10.6% of the women, respectively. *Neisseria gonorrhoeae* and syphilis had a prevalence of 0.6% while *Chlamydia trachomatis* was 4.5%. In bivariate analysis results indicated that, sexual debut before 18 years of age, HSV-2 sero-positivity, and low levels of education were associated with HIV infection. Whereas in multivariate analysis, only HSV-2 sero-positivity (Adjusted

(A) OR 7.2 95% CI 2.0-26.4) was associated with HIV infection. Being married was the only risk associated with HSV-2 sero-positivity (AOR 8.6, 95% CI 1.8-39.8) in multivariate analysis. In conclusion, from the data above HSV-2 is the most prevalent STI in young women aged 18-24, where as HSV-2 and HIV as the most prevalent co-infections. Factors such as early sexual debut and low levels of education are the high risk factors for HSV-2 and other STIs. Based on these findings, issues such as advocacy for delayed sexual debut, condom use and development of female-controlled methods need to be an integral part in interventional strategies such as educational campaigns which are aimed at minimizing the risk of sexually transmitted infections.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Sexually transmitted infections (STIs) are responsible for an enormous burden of morbidity and mortality in many developing countries such as Kenya, because of their effects on reproductive and child health and their role in facilitating the transmission of HIV infection (WHO, 2001). Poor socio-economic status, inadequate knowledge, lack of sufficient diagnostic facilities, and shortages of effective treatment all contribute positively to the high incidence of STIs (Gerbase *et al.*, 1998).

Majority of STIs are asymptomatic in women (60-70% of gonococcal and chlamydial infections) leading to a greater number of undiagnosed and therefore untreated infections (CDC, 2000); women suffer severe sequelae of STIs including infertility, pelvic inflammatory diseases, ectopic pregnancy and cervical cancer (Holmes & Aral, 1991). Perhaps the most important complication of STI in areas with a rampant HIV epidemic is the increased probability of acquisition and transmission of HIV in the presence of other STIs (Buve´ *et al.*, 2001a).

Women's exposure to STIs is the result of inter-relationships among socio-demographic and behavioural risk factors. Socio-demographic risk factors associated with the acquisition of STIs among women include; young age, female sex and urban residence (CDC, 2000; 2003). Behavioural risk factors include sexual initiation at below 15 years (Ford & Norris, 1996), multiple, sequential, or concurrent sexual relationships (Rosenberg *et al.*, 1999), inconsistent use of condoms (Finer *et al.*, 1999), characteristics of sex partners, such as older age and frequent use of alcohol

and illicit substances, which is often associated with risky sexual behaviours (Ericksen & Trocki, 1994).

Systematic and comprehensive STI surveillance is virtually non-existent in developing countries. Most epidemiological data have been obtained from prevalence studies and from sentinel surveillance sites in a few countries. Prevalence surveys suffer from the disparity of population groups surveyed, which have included university students, antenatal clinic attendees, STI clinic attendees and sex workers (Ferguson & Morris, 2007). Although data from sexually transmitted disease clinics and family planning clinics are useful to estimate prevalence of and risk factors for sexually transmitted infections (STIs), it may overestimate the prevalence of STIs in young women. This is because many patients are seeking treatment for STIs and other reproductive-health concerns. Conversely, data from population-based studies tend to elicit self-reported histories of STIs, which are prone to respondent biases that may inhibit the disclosure of STIs, resulting in an underestimation of STIs in women (Boyer *et al.*, 2006). Women enrolled for a microbicide trial represent a potentially ideal nonclinical cross-section of young women. They reflect a large cohort of young, healthy women who are not seeking treatment for STIs or reproductive health care at the time of screening.

1.2 Literature review

Sexually transmitted infections and other reproductive tract infections (RTIs) are a rapidly growing health problem throughout the world. Although the impact of STIs is serious in both developed and developing countries, it is most profound in the developing world. It is estimated that 75-85% of the 340 million new cases of curable sexually transmitted infections occur in these countries (Kettler *et al.*, 2004). Today, STIs and other RTIs are among the most common problems for which people in the developing world seek health care services (Ahamed *et al.*, 2001).

It is difficult to determine the trends of STIs in developing countries over time because data has not been systematically collected and mostly comes from sporadic studies of specific clinics or high risk population (WHO, 1991). Part of the reason for lack of knowledge in this area is the fact that STIs have not been assigned sufficient priorities in most developing countries to reflect their role as a major cause of acute and chronic ill health. In fact, STIs are ranked among the top five illness categories in patient seeking health services in some African countries (WHO, 1991). More regional information on the prevalence and incidence of individual STI is urgently required to motivate for re-evaluation of national health priorities in order to allocate sufficient resources to this problem (Aral & Gorbach, 2002).

Monitoring trends in STIs incidence and prevalence provides a valuable insight into the likelihood of the importance of sexual transmission of HIV within a country. Previous findings in Mwanza, Tanzania suggest that early diagnosis and treatment of STIs might reduce the risk of HIV transmission (Korenrompa *et al.*, 2005). As a result, World Health Organization (WHO) advocated STI treatment as a cost-effective strategy to minimize HIV transmission (WHO, 2001). These trends also

assist in assessing the impact of behavioural interventions, such as delaying sexual debut, reducing the number of sex partners and promoting condom use (UNAIDS/WHO, 2004).

Therefore identifying people with STIs allows for not only the benefit of treating the STI, but for prevention education, HIV testing, identifying HIV-infected persons in need of care, and partner notification for STIs or HIV infection. Consequently, monitoring different components of STI prevention and control, such as condom use can also provide information on HIV prevention and control activities within a country (UNAIDS/WHO, 2004).

1.2.1 Bacterial Infections

1.2.1.1 Chlamydia

Chlamydia trachomatis infection is the most commonly reported bacterial STD in the United States, and is a significant public health concern worldwide (CDC, 2006b). In 1999, WHO estimated the global prevalence of chlamydial infection to be 92 million (WHO, 2001). Women under age 25 are more likely to develop pelvic inflammatory disease (PID) than those older than 25. This is because the cervix of teenage girls and young women is not fully matured, increasing their susceptibility to Chlamydia and other STIs that are linked to PID like gonorrhea (Westrom & Eschenbach, 1999). Having multiple sexual partners is also a risk factor for developing PID, because of the potential for more exposure to infectious agents (CDC, 2008).

In women, chlamydial infections, are usually asymptomatic and may result in PID, which is a major cause of infertility, ectopic pregnancy, and chronic pelvic pain (CDC, 2008). Data from a randomized controlled trial of *chlamydia* screening in the United States suggests that screening programs can lead to a significant reduction in

the incidence of PID by as much as 60% (Scholes *et al.*, 1996a). As with other inflammatory STIs, chlamydial infection can facilitate the transmission of HIV infection (Brunham *et al.*, 1996). In addition, pregnant women infected with *chlamydia* can pass the infection to their infants during delivery, potentially resulting in neonatal ophthalmia and pneumonia (CDC, 2006a). Due to the large burden of disease and risks associated with infection, CDC recommends annual screening of sexually active women under the age of 26 years of age for *chlamydia* annually (CDC, 2006b), older women with risk factors for chlamydial infections (those who have a new sex partner or multiple sex partners), and all pregnant women (CDC, 2008).

1.2.1.2 Gonorrhoea

Gonorrhoea is the second most common sexually transmitted bacterial infection with a global incidence of 62 million cases per year (WHO, 2001). Untreated gonorrhea can cause PID, which may result in chronic pelvic pain and ectopic pregnancy (Aral *et al.*, 2006). In addition, studies suggest that the presence of gonorrhea infection makes an individual three to five times more likely to acquire HIV, if exposed (Fleming & Wasserheit, 1999)

Multiple studies have focused on female sex workers (FSWs) as a high risk group for acquisition and STIs and serving as a core group in the transmission of HIV and STIs in sub-Saharan Africa. However, there is limited data that indicate risk factors for incident *Neisseria gonorrhoeae* infection and its interaction with other STIs (Harrison *et al.*, 2006). Therefore discovering socio-demographic and behavioural correlates of incident infection are important in developing efficient disease control and prevention strategies.

1.2.1.3 Trichomoniasis

Trichomonas vaginalis, an anaerobic, parasitic flagellated protozoan is the causative agent of trichomoniasis, and is the most common pathogenic protozoan infection of humans in industrialized countries (Soper, 2004). The WHO estimates that 180 million new infections are acquired annually worldwide.

Trichomoniasis is a common STI that is easy to cure**.** The infection often has no symptoms although women are more likely than men to get symptoms. Without treatment, trichomoniasis can increase a person's risk of acquiring HIV (CDC, 2010) One prospective study among female sex workers in Kinshasa, democratic republic of Congo, found trichomoniasis to be a risk factor of HIV infection, although the association was of borderline significance (Laga *et al.*, 1993). Similarly, another study in Kisumu City showed a prevalence of 29.3% and an association with a high HIV prevalence in the region, AOR 1.7, 95% CI 1.1-2.7 (Buve´ *et al.*, 2001b). Given the evidence above, that *T. vaginalis* likely promotes HIV infection, the apparent high level of *Trichomonas* infection is cause for concern. Even if *T. vaginalis* increases the risk of HIV transmission by a small or modest amount, it translates into a sizable population effect since trichomonas is so common. Trichomonas infection may be more readily modifiable than sexual behaviour in some high-risk groups.

1.2.1.4 Syphilis

Syphilis is a genital ulcer disease caused by *Treponema pallidum.* In 1999, WHO estimated the global prevalence of syphilis at 12 million (WHO, 2001), with high prevalence rates in South and Southeast Asia and Sub-Saharan Africa. Since 1999, syphilis outbreaks have reemerged in many developed countries like United States among men who have sex with men (CDC, 2004; Doherty *et al.*, 2002). Among

heterosexuals, sexual contact with sex workers and having multiple sexual partners are important risk factors, a study in Yaounde´, Cameroun showed AOR 2.6, 95%CI 1.3-5.3 and AOR 5.5 95% CI (2.7-11.1) respectively (Buve´ *et al.*, 2001).

Congenital syphilis can cause still-birth, death soon after birth, physical deformity and neurological complications in children who survive (CDC, 2005). Syphilis, like many other STDs, facilitates the spread of HIV, increasing transmission of the HIV at least two- to five-fold (CDC, 1998).

Syphilis is the classic example of an STI that can be successfully controlled by public health measures due to the availability of a highly sensitive diagnostic test and a highly effective and affordable treatment. In Africa, syphilis prevalence rates amongst pregnant women vary from 2.5% in Burkina Faso to 17.4% in Cameroon. A study in Kisumu city has shown a prevalence rate of 3-4% in both men and women aged 15-45 (Buve´ *et al.*, 2001).

1.2.2 Viral infections

1.2.2.1 Herpes Simplex Virus type -2 (HSV-2)

Herpes simplex virus type II (HSV-2) is the most common cause of genital ulcer diseases and one of the most prevalent sexually transmitted diseases worldwide (Schomogy *et al.*, 1998). Through disruption of the epithelial barrier and inflammation of the genitals, it may increase the risk of HIV-1 transmission (Ramjee *et al.*, 2002). HSV-2 infection is strongly associated with HIV infection, and it is thought that HSV-2 infection plays an important role in the spread of HIV in sub-Saharan Africa (Weiss *et al.*, 2001). A study of HIV- discordant couples from Rakai, Uganda, demonstrates that at all levels of HIV viral load in the HIV-positive partner, HSV-2 infection in the susceptible partner increased the per-contact risk of

acquisition of HIV five-fold and genital ulcer disease (GUD) in the HIV-source partner increased the per-contact risk of HIV transmission five-fold (Corey *et al.*, 2004).

1.2.2.2 Human Immunodeficiency Virus (HIV)

HIV/AIDS is a major health problem in Kenya. Over one million adults (15–49 years) are currently infected with HIV (NASCOP, 2004). A higher proportion of women age 15-64 (8.7 percent) than men (5.6 percent) are infected with HIV according to KAIS 2007. This pattern is similar to what was observed in 2003. This means that 3 out of 5 HIV-infected Kenyans are female (KAIS, 2007). Among youth age 15-24, women are 4 times more likely to be infected than men (6.1 percent compared to 1.5%).

Results from Kenya AIDS Indicator Survey (KAIS) indicate that 7.4 percent of Kenyan adults aged between 15-64 are infected with HIV (KAIS, 2007). Previous estimates of adult prevalence, based on surveillance among pregnant women, showed the prevalence at almost 10% (NASCOP, 2004), but these were later revised downwards using new information from the national survey on differentials between female and male prevalence and rural and urban prevalence. This revision has caused some confusion as some people have interpreted the lower estimate from the national survey as evidence of a decline in prevalence when it is actually just a better estimate. At the same time, evidence on prevalence trends from surveillance among pregnant women has gone largely unnoticed (Cheluget *et al.*, 2006).

1.2.3 Other Reproductive Tract Infections

1.2.3.1 Bacterial vaginosis

Bacterial vaginosis (BV) is a polymicrobial syndrome that represents the main cause of abnormal vaginal discharge worldwide, with prevalence in the general population ranging from 20 to 50% (Nagot N. *et al.*, 2007). Bacterial vaginosis (BV) is the most common vaginal infection in women of childbearing age (CDC, 2010). It is not considered to be a sexually transmitted infection by the CDC. BV is not transmitted through sexual intercourse but is more common in women who are sexually active (BASHH, 2006).

Having BV can increase a woman's susceptibility to HIV infection if she is exposed to the HIV virus. In addition it increases the chances of an HIV-infected woman passing the HIV virus to her sexual partner (CDC, 2006b). Several studies have suggested bilateral interactions between BV and HIV-1. Cross-sectional studies have reported that HIV sero-positive women are more likely to have BV episodes (Sewankambo *et al.*, 1997). Reciprocally, longitudinal studies have suggested that BV could facilitate HIV acquisition (Martin *et al.*, 1999; Myer *et al.*, 2005). Increased rates of infection with HSV-2, *C. trachomatis* and *N. gonorrhoeae* have been reported in women with bacterial vaginosis (Nagot N. *et al.*, 2007).

In Kenya, a study done in Nairobi the prevalence of BV was 44% and was significantly associated with trichomoniasis (OR 13.2), HIV infection (OR 5.7), and an odds of 2.5 in the women who reported more than one sexual partner (Bukusi *et al.*, 2006) .

1.2.3.2 Yeast Infection

Vaginal yeast infection is often referred to as vaginal Candidiasis or Candida vulvovaginitis and in most cases caused by *Candida albicans*. About 25-30% of reproductive age women have some yeast present in the vagina (Mendoza *et al.*, 1999) An estimated 75% of women will have at least one episode of vulvovaginitis, and 40%–45% will have two or more episodes (CDC, 2006b). Infection rates are higher in women treated with broad spectrum antibiotics, pregnant women , diabetic women and women with HIV/AIDS, women with low CD4+ cell counts are associated with higher rates of Candida colonization (Burns *et al.*, 1997). Recurrent vulvovaginitis affects a small percentage of women (<5%). Vulvoginitis can be easily treated with topical fungicides(CDC, 2006b). The incidence of VVC in HIV-infected women is unknown. Vaginal *Candida* colonization rates among HIV-infected women are higher than among those for sero-negative women with similar demographic characteristics and high-risk behaviors, and the colonization rates correlate with increasing severity of immunosuppression (CDC, 2006b)

1.2.4 Special Concerns for Women

Women are biologically more vulnerable to diseases of the genital tract than men. This is because lining of the vagina is a mucosal membrane that is readily permeable to infection than the skin on the outside of the penis. Women's genitals have more surface area through which infection can occur. Younger women are particularly vulnerable because their cervical tissues may be less mature and more readily penetrated by organisms (e.g. gonorrhea and chlamydial infections). Older women are more likely to get small abrasions in the vagina during sexual activity because of the thinning of the tissues (EngenderHealth, 2005).

This vulnerability is compounded by women's unequal social status, which in many communities makes them economically dependent on men. Economic vulnerability amplify women's risk for infection. For example, many women lack sufficient economic resources and are fearful of abandonment or of violence from their male partner. They therefore have little control over how and when they have sex, which in turn hampers their ability to protect themselves from infection (EngenderHealth, 2005).

Such dependence of women on men in some communities often leads to feelings of powerlessness and low self-esteem of women. As a result, women tend to become sexually active at younger ages than men and are less likely to negotiate safer sexual practices (WHO, 2001). Indeed, many women do not have a say in sexual relations, although absolute fidelity is expected of them. This societal demand holds true for both married and unmarried women. Moreover, women may be less likely to seek medical care not only because their infections are often asymptomatic, but also because of poor economic resources and a sense of modesty (UNAIDS/WHO, 2004). Polygamy and the early marriage of many girls also contribute positively towards the vulnerability of women to STIs.

Women who already have an infection (particularly one that causes genital lesions) are more likely to acquire or transmit HIV, and since women are often asymptomatic when infected with an STI, they are often not aware of this increased risk. Other risks for women include; the use of vaginal douches (which increase the risk of PID) and the influence of hormonal contraceptives on acquiring or transmitting STIs, although this relationship is not yet fully understood (EngenderHealth, 2005).

1.2.5 STI epidemic and risk factors

The epidemiology of STIs reflects patterns of sexual behaviour and network of sexual mixing. Multiple sexual partners is a major determinant of STI rates. Sexual behaviour across societies is extraordinarily heterogeneous. Unfortunately, from the perspective of behavioural intervention, little is known regarding sexual behaviour and this is especially in the developing countries (Brunham & Ronald, 1991)

Young age is particularly an important variable in STI epidemiology in developing countries because sexual partner change rate is age dependent (Brunham *et al.*, 1996). The proportions of the population aged between 15-30 years are at a greater risk particularly in developing countries. This large segment of the population contributes greatly to the increased incidence of STIs seen in developing countries (Brunham & Ronald, 1991).

Sexual behaviours vary from individual to individual in the social context and within different societies. One of the critical categories of differentiation is gender. Girls, boys, women and men are socialized into different gender roles that significantly influence their sexual behaviour. Both age and gender influence physical characteristics and social roles that in turn affect the expression of sexual behaviour patterns (Brunham & Ronald, 1991).

1.2.6 Demographic and societal factors influencing risk

Sexual behaviour is linked to the social conditions under which people live and work. These conditions have a bearing on people's thinking and attitudes, and they represent the "causes behind the causes" of poor health (WHO, 2000). Such social conditions influence the many risk determinants for STI infection, including age, gender, partner preference and number, and health-care–seeking and protective

behaviour. The environmental determinants of behaviour include cultural norms and practices, demographics, socio-economic status, availability and quality of health care services, peer opinions, social pressures, societal attitudes and prejudices, and access to services. STI prevalence tends to be higher among urban residents, unmarried individuals, and young adults. STIs tend to occur at a younger age in females than in males, which may be explained by differences in patterns of sexual activity and the relative rates of transmission from one gender to the other. Girls tend to have sex with older males who are likely to be having other sexual partners hence at high risks of STIs, where as boys tend to have sex with girls of the same age group who have fewer sexual partners (WHO, 2000). Other high-risk groups include sex workers, refugees, and highly mobile people such as long-distance truck drivers and migrants.

Individual determinants are influenced by factors such as low self-esteem, lack of information, the inability to negotiate safer sex, and lack of knowledge about the risks of different sexual behaviours (Brugha *et al.*, 1997). These factors are most obvious among youth, whose STI prevalence rates are among the highest. Age is therefore an important risk determinant in terms of both risky behaviour and increased biologic susceptibility to infection, especially among females (Brabin, 2002).

The youth are more likely to have multiple partners either sequentially or concurrently. They are also less likely to buy or to use condoms, and they tend to have a low awareness of the risks of STIs (Olayinka & Osho, 1997).These tendencies are often because of either a lack of money or a feeling of modesty, as most African

communities equate sexual activity in the young with promiscuity (Ojwang & Maggwa, 1991).

1.3 Research Question

What is the prevalence of sexually transmitted infections among young women aged 18-24 years not seeking treatment at the time of screening, and what are the risk factors associated with specific STIs the young women in Kisumu City, Kenya?

1.4 Justification

In Kenya as in many other developing countries there is an inadequate STI surveillance system. Most data on sexually transmitted infection is obtained from either sexually transmitted disease clinics, family planning clinics or from sentinel data. In this respect few studies have focused on prevalence of these infections in a young women population who are not seeking STI treatment. Understanding prevalence rates and risk factors for STIs is essential for designing prevention strategies that target specific populations.

Coverage by clinical services, partner management, and treatment may be different with co-infection than with independent infections. Although co-infections of HIV and other STIs have received a great deal of attention in recent years, researchers have not focused on overlaps among other STIs in a similar systematic manner. Empirical data on co-infection are limited. Most studies conducted in developed countries have focused on co-occurrences of chlamydial and gonococcal infections. The epidemiology of co-infection with more than one sexually transmitted pathogen may have important intervention such as treatment which may be different with co-infection than with independent infections.

This study will estimate the prevalence and rates of co-infection of non-HIV STIs such as HSV-2, *N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, bacterial vaginosis, syphilis and yeast infection, in this group of young women. In addition the study will also provide baseline data for targeted STIs and HIV prevention intervention programmes by evaluating the risk factors associated with these infections.

1.5 Objectives

1.5.1 General objectives

To determine the prevalence and risk factors associated with sexually transmitted infections in young women aged 18-24 years in Kisumu City, Kenya.

1.5.2 Specific Objectives

1. To determine the etiology and prevalence of STI among young women aged 18-24 years.
2. To determine the prevalence and types of co-infection of STIs in this population.
3. To determine risk factors associated with single or multiple STIs in this population.

1.6 Research Hypothesis

Young women aged 18-24 years in Kisumu City, Kenya, engage in risky behavior that predispose them to sexually transmitted infections

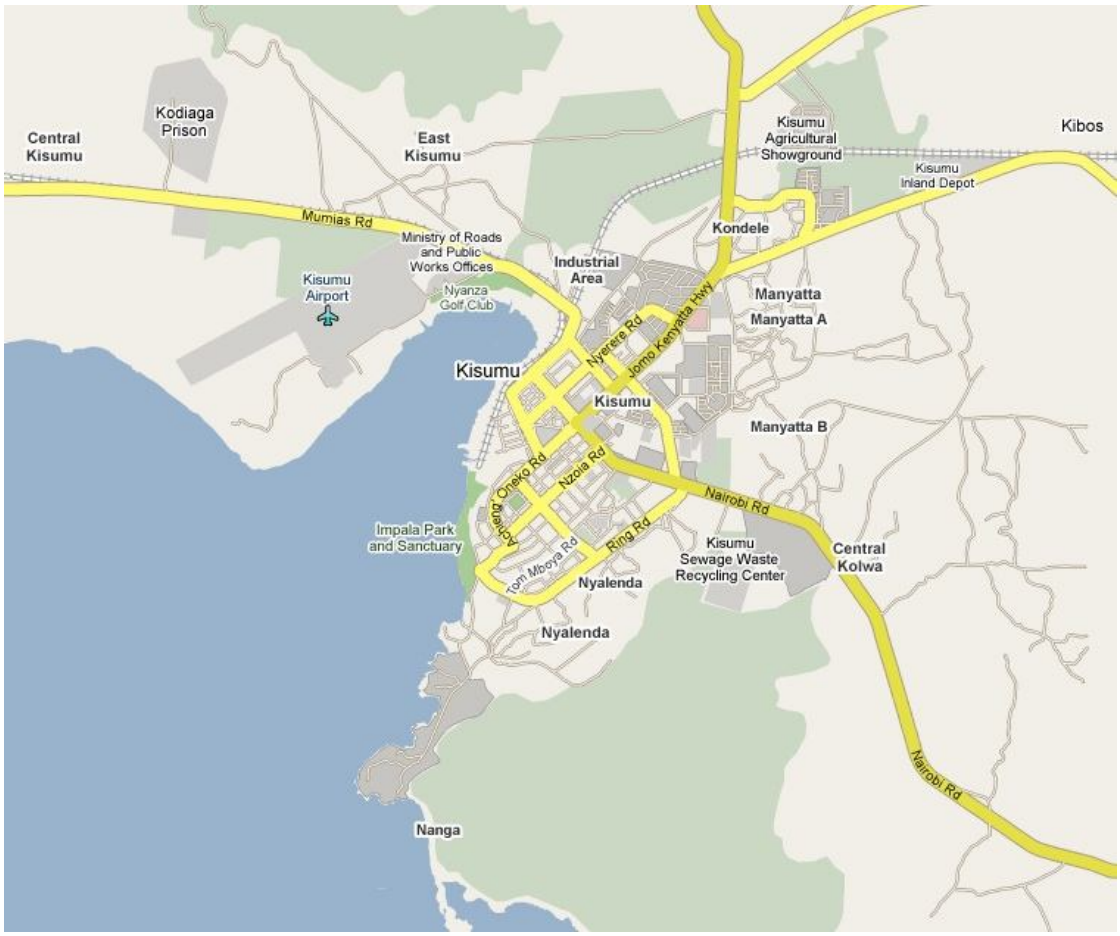
CHAPTER TWO

2.0 Methodology

2.1 Study site: Kisumu City

Kisumu City is the administrative center of Nyanza Province in Western Kenya, situated along Lake Victoria in Nyanza Province. It has a population of 504,359 (as of 1999) and a land area of 919 sq km (KDH 2003) (Figure 1). Kisumu District is bordered by Kericho District to the east, Nandi and Vihiga Districts to the north, Homa Bay District to the south and Lake Victoria to the west. The main economic activities are subsistence agriculture and fishing on Lake Victoria. Kisumu City is the main commercial centre in Western Kenya.

Figure 1: Map of Kisumu City



Google maps

2.2 Study design

This was a descriptive cross-sectional analysis of women screened for participation in a phase 1 randomized placebo controlled microbicide trial; Expanded phase I randomized placebo controlled trial of the safety and tolerability of 3% w/w SPL7013 Gel (VivaGelt^{TM}) in healthy young women when administered twice daily for 14 days. VivaGel^{TM} (3% w/w SPL7013 Gel) is a vaginal microbicide that is thought to prevent transmission of STIs including HIV and HSV-2, under

development by Starpharma Ltd (Melbourne, Australia). 312 women were screened for participation in the randomized trial, and this made the evaluable sample size used in this study.

2.3 Study population

The target population was sexually active young women aged between 18-24 years. The study population was composed of women aged 18-24 years with a history of sexually activity. Study participants were recruited from Kisumu City.

2.4 Inclusion criteria

Participants were eligible for enrolment if they met the following:

1. They had voluntarily given written informed consent to participate in the study.
2. The participant was female and aged between 18 and 24 years, inclusive.
3. She was sexually active.

2.5 Exclusion criteria

1. The participant had received an investigational drug within 30 days or 10 half-lives of the drug, whichever is longer, prior to entering this study, or is planning to receive another investigational drug while participating in this study.
2. She had an active, uncontrolled medical condition (e.g., neurological, gastrointestinal, renal, hepatic, cardiovascular, pulmonary, metabolic, endocrine, hematological, genitourinary or other major disorder), or psychiatric illness (e.g., depression, schizophrenia).
3. Had a clinically significant illness within 30 days of the start of the study.
4. Participant had used a vaginal preparation within 30 days prior to screening.

2.6 Sample size determination

From a previous study (Feldblum *et al.*, 2001), the prevalence of STIs in the general population is about 23.9% (24%). Therefore using this prevalence the minimum sample size was determined using the following formulae (Fishers *et al.*, 1998);

$$N = \frac{Z^2 P (1-P)}{\delta^2}$$

Whereby;

N is the minimum sample size

P is the estimated prevalence (24%)

δ^2 is the degree of precision, which is 0.05

α is the level of significance (95%)

Z is the standard normal deviate that corresponds to 95% confidence interval

Therefore, $n = \frac{(1.96)^2 \times 0.24(1-0.24)}{(0.05)^2} = 280$

This was the minimum sample size expected = 280

Analyzed sample size = 312 women

As this study was nested, the sample size from the Vivagel^{TM} microbicide study was used. The Vivagel^{TM} participants were selected through random sampling. Therefore

the sample size for this study was 312 women. But some of the study participants did not fill all variables in the questionnaires and also some participants were eliminated from the larger study once ineligibility was determined and therefore were not tested for all the STIs analyzed in this study. This resulted in different sample size in the variables that were analyzed.

2.7 Research Variables

2.7.1 Predictor variables

Data on the following variables was collected:

1. Number of sexual partners
2. Frequency of condom use
3. Level of education
4. Marital status
5. Age of coital debut
6. Presence/absence of yeast infection
7. Presence/absence of bacterial vaginosis

2.7.2 Outcome variable:

Presence or absence of the aetiological pathogens; *N. gonorrhoeae* (GC), *C. trachomatis* (CT), *T. vaginalis* (TV), Herpes Simplex Virus type 2 (HSV 2), syphilis and HIV. And this was used to determine the prevalence of each organism in this population.

2.8 Measures

2.8.1 HIV sero-status

HIV serostatus was determined at the screening visit using Vironostika HIV Uni-Form II Ag/Ab (Biomerieux, France) positive results were confirmed by two consecutive rapid tests. The final HIV status from the screening visit was used as the primary outcome in these analyses.

2.8.2 HSV-2 and Syphilis Serostatus

HSV2 Sero-status was determined at the screening visit through an enzyme immunosorbent assay (EIA) using HerpeSelect Kit (Focus Technologies Inc., Cypress, California, USA.).

Syphilis was determined using BD Macro-Vue RPR card test (Diagnostic Systems, Maryland, USA.).

2.8.3 *N. gonorrhoeae, C. trachomatis* and *T. vaginalis*

Infection with these organisms were determined using Nucleic acid amplification tests **(**Multiplex PCR Aptima, Gen Probe, Inc., San Diego, California.)

2.8.4 Other variables

Other variables that were measured in structured questionnaires included; ever having used a male or female condom; lifetime number of sexual partners (one or more than one partner); education (years of formal education); currently living with partner; ever practiced anal sex; marital statu; and if they had ever engaged in transactional sex.

2.9 Data collection

Laboratory data, demographic information, and data on sexual behaviour were collected during the Vivagel^{TM} microbicide trial (Cohen *et al.*, 2006). I authenticated

the data by carrying out laboratory tests using random archived samples from the Vivagel^{TM} study trial.

2.9.1 Collection of biological samples

The Vivagel^{TM} study was introduced to the women and permission sort for those willing to participate in the study. Women who were found to be eligible to participate in the study were taken through the consent process.

Swab specimens were collected by a certified nurse for *N. gonorrhoeae, C. trachomatis, T. vaginalis,* bacterial vaginosis and yeast infection testing. Blood specimen was collected for HIV, HVS-2 and syphilis testing. The specimens were labeled with the study identification and stored in a cold box for transport the laboratory where they were tested for sexually transmitted infections.

2.9.1.1 *N. gonorrhoeae, C. trachomatis* and *T. vaginalis*

Diagnosis of *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* was done by nucleic acid amplification tests (NAAT) (Multiplex PCR Aptima, Gen Probe, Inc., San Diego, California, USA.). The Multiplex PCR Aptima assays utilize target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) technologies for the in vitro qualitative detection and differentiation of ribosomal RNA (rRNA) from *C. trachomatis* and/or *N. gonorrhoeae*.

TMA system used in this test amplifies a specific 23S rRNA target for *C. trachomatis*, 16S rRNA for *N. gonorrhoeae* and 18S rRNA for *T. vaginalis* via DNA intermediates. AMP was performed with endocervical specimens by using a 23S rRNA 16S rRNA and 18S rRNA-based amplification method according to manufacturer's instructions.

Nucleic Acid Amplification Tests Procedure

i. Equipment preparation

Prior to starting the assay the equipments, work surfaces and pipettors, were wiped down with house hold bleach. The bench surface on which the test was performed was covered with clean, plastic-backed absorbent bench covers. Sufficient number of Ten Tip Cassettes were placed into the Target Capture System (TCS). The TCS wash bottle was be filled with APTIMA wash solution and the aspirator connected to the vacuum pump

ii. Reagent Reconstitution/ Preparation

To reconstitute the APTIMA Combo 2 Enzyme, Amplification, and Probe Reagents:

The reconstitution solution was paired with the dried reagent (The labels are color coded so the paired reagents have the same color bands).

Preparation of the Target Capture Reagent plus Target Capture Reagent B (TCR plus TCR-B)

The number of reactions to be performed (specimens plus controls) were determined. The volumes of Target Capture Reagent (TCR) and Target Capture Reagent B (TCR-B) was calculated as per manufacturer's instructions and transferred to an appropriately dry container. The calculated volume of TCR-B was added into the TCR and the solution was thoroughly mixed.

iii. Target Capture

The controls and swab specimens were allowed to reach room temperature prior to processing. A hundred µL of the TCR was added into each reaction tube. 400 µL of the Positive Control, *C. trachomatis*/Negative Control *N. gonorrhoea*, was added to

the first reaction tube. In the same manner, 400 µL of the Positive Control, *N. gonorrhoea* /Negative Control, *C. trachomatis* was added to the second reaction tube. 400 µL of each specimen was added into the remaining tubes. A new pipette tip was used for each specimen and control.

The tubes were incubated at 62 ℃ for 35 minutes followed by a 60 second vortex and a controlled temperature ramp down to 23 ℃ for 30 minutes. The tubes were then placed on the TCS magnetic base for 10 minutes and then washed with the APTIMA wash.

After washing all liquid was aspirated, and 1.0 ml of APTIMA Wash Solution was delivered into each tube. The tubes were covered and vortexed for 10 seconds and placed back on TCS magnetic base for 7 minutes after which all liquid was aspirated.

iv. Amplification

Seventy five µl of the reconstituted Amplification Reagent was added to each reaction tube. Two hundred µl Oil Reagent was then added and the tubes were covered, vortexed for 10 seconds, incubated at 62 ºC for 10 minutes and ramped down to 42 ºC in 5 minutes. 25 µL of the reconstituted Enzyme Reagent was added after temperature ramp down to each of the reaction mixtures and the tubes were then immediately covered and incubated at 42 ºC for 1 hour.

v. Dual Kinetic Assay

a) Hybridization

One hundred micro litter of the reconstituted probe was added to each reaction tube. The tubes were covered with sealing cards, and placed on the heating block, vortexed for 10 seconds followed by incubation at 62 ºC for 20 minutes after which the tubes were incubated in room temperature for 5 minutes.

b) Selection

Two hundred and fifty µL Selection Reagent was added and the tubes were covered vortexed for 10 seconds followed by a 10 minutes incubation at 62 ºC and a final cool down for 15 minutes at 23 ºC.

c) Detection

The leader HC+ Luminometer will be prepared by placing one empty TTU in cassette position number 1 and the wash protocol will be performed. The TTU will then be loaded into the luminometer and read on a computer program.

vi. Test interpretation

In DKA, differences in the kinetic profiles of the *C. trachomatis* and *N. gonorrhoeae* labeled probes allow for the differentiation of signal. Kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for *C. trachomatis* signal has very rapid kinetics and has the "flasher" kinetic type. The chemiluminescent detection reaction for *N. gonorrhoeae* signal is relatively slower and has the "glower" kinetic type. Assay test results are automatically interpreted by the APTIMA Combo 2 Assay Software and presented as individual *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* test results. A test result may be negative, equivocal, positive or invalid as determined by the kinetic type and total RLU in the detection step. A test result may be invalid due to parameter outside the normal expected ranges. Initial equivocal and invalid test results were repeated.

2.9.1.2 Yeast Infection

Diagnosis of yeast infection was done using the wet mount technique by direct microscopy. The wet preparation and potassium hydroxide (KOH) examination are

simple techniques for microscopic examination of samples from the vaginal mucosa and vaginal opening. The alkaline KOH solution used in the procedure, causes separation and, ultimately, destruction and epithelial cells and allows easy identification of hyphae and spores, which are unaffected or less rapidly affected by the KOH solution.

The vaginal swabs were vigorously mixed in and out of the saline to collect all the material adhering to the side of the tube. The swabs were then removed from the saline and depressed onto clean, dry microscope slides expressing a small amount of fluid. The samples were covered using covers slip. The prepared slides were examined for yeast cells under a light microscope.

2.9.1.3 Bacterial Vaginosis

Diagnosis of BV was done using the Nugent Criteria which is a gram stain scoring technique (Nugent *et al.,* 1991). The scoring is calculated by assessing for the presence of large gram positive rods whose decrease in numbers is scored as 0-2, small gram variable rods scored as 0-4 and curved gram negative rods scored as 0-2.

2.9.1.4 HIV

HIV sero-status was determined using two consecutive rapid tests; Uni- Gold (Trinity Biotech PLC. Bray, Ireland) and Determine^{TM} (ABBOTT Laboratories, Abbot Park, Illinois, USA.). Positive results were confirmed by Enzyme Linked Immunosorbent Assay (ELISA) using Vironostika HIV Uni-Form II Ag/Ab kit (Biomerieux, France)

i. Rapid Tests

a) Uni- Gold

The Trinity Biotech Uni-Gold™ HIV test kit is a single reagent assay for the detection of antibodies to HIV1/2 in serum, plasma or whole blood. The antigen proteins are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. During the test, antibodies react with the colloidal gold linked antigens. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control regions of the device. In the presence of antibodies to HIV a pink/red band in the test region of the device is visualized. In the absence of antibodies to HIV no band is formed on the test region. Excess conjugate forms a second pink/red band in the control region of the device. Fifty μL of serum sample were pipette on to the sample port. The test was allowed to react for 20 minutes before the results were read

b) Determine™ HIV-1/2

Detremine HIV1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the colloid antigen. This phase continues to migrate through the solid phase to the immobilized recombinant antigens at the patient window site. If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and mixes with the selenium colloid and to the antigen at the patient window site. If antibodies are absent, the antigen-

selenium colloid flows past the patient window, and so no red line is formed at the patient window site.

Fifty µL of serum sample was dispensed to the sample pad (marked with the arrow symbol). The test was allowed to react for 20 minutes and then the results were read.

ii. Interpretation of rapid tests

Positive (Two bars): Red bars appeared in both the control window (labeled "Control") and the patient window (labeled "Patient"). Any visible red color in the patient window was interpreted as positive.

Negative (One bar): One red bar appeared in the control window and no red appeared in the patient window of the strip.

Invalid (No bar): If there was no red bar in the control window of the strip, and even if a red bar appears in the patient window of the strip, the results was considered invalid and repeated.

iii. ELISA Assay procedure

Reagents and samples were equilibrated to room temperature (25 ºC) before beginning the assay and they remained at room temperature during testing. The strip holder was fitted with the required number of microelisa strips and the strip sealers were removed. 100 µl of the specimen diluent was pipetted into all wells including the control wells to dissolve the conjugate. 50 µl sample or control was pipetted into the assigned wells, three negative controls and one anti- HIV-1 positive control were included in each strip holder. The strips were incubated at 37 ºC for 60 minutes. Each well was washed and soaked six times with phosphate buffer. The well contents were completely aspirated and the wells were filled with phosphate buffer and allowed to soak for one minute. The wash and soak procedure was repeated five times for a total

of six washes. A hundred µl of TMB substrate was pipetted into each well and the strips were incubated at 25 ºC for 30 minutes. The reaction was stopped by adding 100 µl sulfuric acid to each well and the resultant colour change was quantified by a microplate photometer (Multiskan EX, Thermo Fisher Scientific Inc. MA, USA) reading of optical density (OD) at 450nm.

iv. Interpretation of test results

Sample optical density readings were compared with the calculated cut-off OD reading to determine results. The cut-off was the mean OD of the negative controls plus 0.1.

2.9.1.5 HSV-2

HSV2 sero-status was determined through an enzyme immunosorbent assay using HerpeSelect kit (Focus Technologies Inc., Cypress, California, USA.).

i. Assay Procedure

In this assay, the microtitre wells are already coated with a recombinant antigen. The wells were filled with 1X wash buffer solution and allowed to soak for five minutes after which the buffer was aspirated. The emptied antigen wells were blotted to remove residual wash buffer. 100µL of the sample diluent were dispensed into the "blank" wells and 100µL of each diluted specimen, control or calibrator into the appropriate wells. The plates were covered with sealing tape and incubated for 60 minutes at room temperature (25 ºC) to allow antibodies in the sample to react with the antigen coated on the microtitre wells. The wells were then washed three times using the wash buffer to remove unbound antibodies, and then 100µL of peroxidase-conjugate anti-human immunoglobulin G (IgG) was dispensed to all wells and incubated for 30 minutes at room temperature (25 ºC) to allow reaction with specific

IgG. Excess conjugate was removed by washing three times with wash. A hundred µl of the enzyme substrate reagent was pipetted into all wells, and the plates were incubated for 10 minutes at room temperature. The reaction was stopped by adding 100µL of Stop Reagent (sulfuric acid) to all wells.

After adding the Stop Reagent, the resultant colour change was quantified by a microwell spectrophotometer (Multiskan EX, Thermo Fisher Scientific Inc. MA, USA) reading of optical density (OD) at a wavelength of 450 nm. Sample optical density readings were compared with reference cut-off OD reading to determine results.

ii. Interpretation of test results

All results were reported as index values relative to the Cut-off Calibrator. To calculate index values, the specimen OD values were divided by the mean of the Cut-off Calibrator absorbance values.

Positive: An index value of >1.10 was presumptive for the presence of IgG antibodies to HSV-2.

Equivocal: An index value of ≥ 0.90 but ≤ 1.10 was considered an equivocal result. These samples were re-tested.

Negative: An index value of < 0.90 indicated no IgG antibodies to HSV-2 were detected.

2.9.1.6 Syphilis

Syphilis infection was diagnosed using rapid plasma regain (RPR) 18mm circle card test (Quorum Diagnostics, Vancouver, Canada.

i. Preparation of reagent

All the reagents were removed from the refrigerator and allowed equilibrated to room temperature before use. The reagents were mixed gently before use, and the carbon antigen was vigorously agitated for 30 minutes to ensure homogeneity.

ii. Assay Procedure

This test detects non-specific treponemal antibodies in serum. One free-falling drop of serum was dispensed onto the card test area. A fresh stirrer pipette was used for each sample. The process was repeated by adding one free-falling drop of reactive, Weak Reactive or Nonreactive controls from the dropping vials supplied and the location of each sample was noted. Using the flat end of the stirrer, the sample was spread over the entire area of the test circle. One free-falling drop of the carbon antigen suspension was dispensed onto each sample while holding the bottle in a vertical position. The card was mixed on a shaker at 100 rpm, for 8 minutes under humidifying cover. Following rotation, a brief rotating and tilting of the card by hand (3 or 4 to-and-fro motions) was made to help differentiate noncreative from minimally reactive results. The results were immediately read visually in the "wet" state under high intensity light source.

iii. Interpretation of results:

Reactive: Presence of aggregates in the center or periphery of test circles, ranging from slight to marked and intense

Nonreactive: A smooth gray appearance within the test circle or button of non-aggregated carbon particles in the center of the circle, showing none of the clumping characteristic of a reactive result.

2.9.2 Collection of demographic data and sexual behaviour data

The participants' demographic information and data on sexual behaviour included history of a male or female condom use; age at first vaginal sex; lifetime number of sexual partners; number of sexual partners in the last three months; currently living with partner; education (years of formal education); history of anal sex; marital status (married women were defined as women who were living with their regular sexual partners); and history of transactional sex work. Transactional sex was defined as sex in exchange for money, food, drugs or shelter.

2.10 Data Management and statistical analysis

All questionnaire data and laboratory data was entered into a computer using Microsoft access program. Data collected for the study was thoroughly checked and cleaned for analysis.

Descriptive, bi-variate and multivariate analysis was done using Epi-info software (Version 3.3). Variables with p-values less than 0.2 were put into a logistic regression model and significant variables identified through backward elimination process. In the analysis an odds ratio (OR) ≥ 1 was considered a risk factor. P- Value of < 0.05 was considered significant.

2.10 Ethical consideration

The larger study was cleared ethically by both University of California San Francisco Institutional Review Board and National Ethical Review Committee of the Kenya Medical Research Institute (ERC, KEMRI). Research clearance for this study was also sought from National ERC, KEMRI.

CHAPTER THREE

3.0 RESULTS

3.1 Socio-demographic and sexual risk behaviours

Socio-demographic and sexual risk behaviours characteristics were collected using structured questionnaires. The mean age of the participants was 21 years with a range of 18-24 years. Of the 312 women screened, 286 (91.67%) of the women were single, 22 (7.05%) were married 2 (0.64%) and 2 (0.64%) were separated and widowed respectively. About 40.38% (126) had tertiary education, while, over seventy percent (225, 72.12%) of the women had a regular sexual partner. The women reported a median of two (range 1 – 7) lifetime number of sexual partners, and a median of one (range 0 – 3) sexual partner within the last three months.

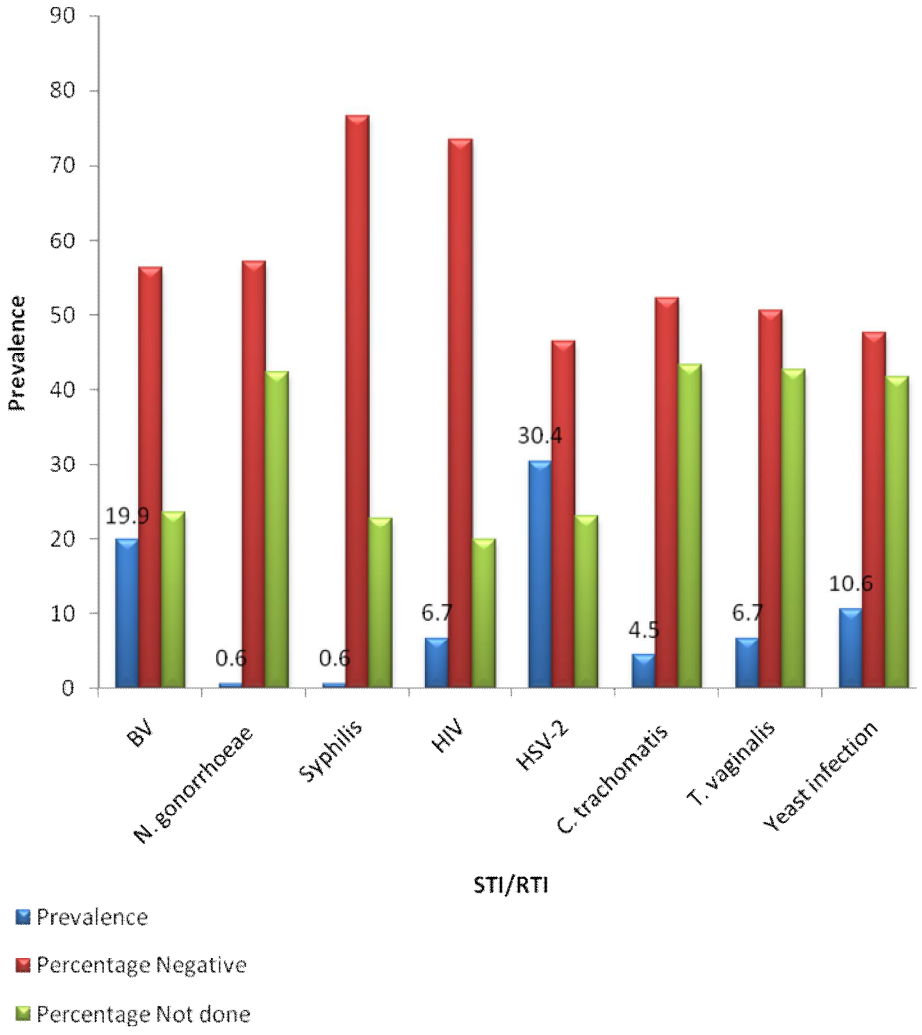
History of ever use of male condom was reported by 212 (67.95%) of the women, while 128 (41.03%) reported using a male condom in the three months preceding the study. Only two (0.64%) women had ever used a female condom, and only two (0.64%) had ever used a spermicide. History of ever use of a male condom was reported by 76/119 (63.86%) of the women who had one sexual partner and 136/186 (73.11%) of the women who had two or more sexual partners.

Over a third, (115, 36.86%) had their sexual debut before 18 years of age, 119 (38.14%) had one male sexual partner in their life and 186 (59.62%) had had two or more male sexual partners, while 237 (75.96%) had one male sexual partner in the three months preceding the study. Few had engaged in transactional sex 8 (2.56%) three (37.50%) of whom had a history of male condom use, only four (1.28%) had had anal sex half (2/4) of whom reported use of a male condom. Almost fourteen percent (43/312) reported ever having had an STI/RTI in their life (Appendix 1).

3.2 Prevalence of Sexually transmitted infections including bacterial vaginosis and yeast infection

HSV-2 infection was the most prevalent STI in this population at 30.4%. HIV was 6.7%. Bacterial vaginosis and yeast infection though not classical STI were also prevalent at 19.9% and 10.6% respectively, of the study population (Figure 2).

Figure 2: Prevalence of STIs including HIV, BV and yeast infection



3.3 Bi-variate analysis

3.3.1 Factors associated with having at least one STI

Married women were at a significantly higher risk of having an STI (odds ratio [OR] 3**,** 95% confidence interval [C.I] 1.2-7.5). Women who had bacterial vaginosis were also significantly more likely to be infected with an STI (OR 2.5, 95% C.I 1.4-4.6). Women reporting two or more sexual partners had a reduced odds of STI infection (OR 0.5, 95% C.I 0.3-0.8). Age at sexual debut, history of transactional sex, history of male condom use, yeast infection, condom use three months before the study, and history of anal sex were not associated with diagnosis of an STI (Table 1).

Table 1: Factors associated with having any sexually transmitted infection

Variable (Exposure)	*Frequency of STI -/ve (%)*	*Frequency of STI +/ve (%)*	*Odds ratio(OR)*	*Confidence interval (C.I 95%)*
Married	8 (36.4)	14 (63.6)	3.0	1.2-7.5
Having BV	22 (35.5)	40 (64.5)	2.5	1.4-4.6
Sexual debut before 18years of age	65 (56.5)	50 (43.5)	1.3	0.8-2.2
History of transactional sex	6 (75)	2 (25)	0.5	0.1-2.5
History of anal sex	2 (50)	2 (50)	1.6	0.2-11.7
Have a yeast infection	20 (60.6)	13 (39.4)	0.8	0.3-1.6
≥2 sexual partner	85 (71.4)	34 (28.6)	0.5	0.3-0.8
History of male condom use	132 (62.3)	80 (37.7)	0.9	0.5-1.5
Condom use in the 3 months before the study	79 (61.7)	49 (38.3)	1.0	0.6-1.6

*Any STI is any of the following alone or in combination: HIV, HSV-2, *N. gonorrhoeae*, *T. vaginalis*, syphilis and *C. trachomatis*

3.3.2 Factors associated with having HSV-2 infection

Being married was associated with HSV-2 sero-positivity (OR 10.3, 95% C.I 2.3-47.9). Women reporting two or more sexual partners (OR 0.5 95% CI 0.3-0.8) and a history of male condom use (OR 0.5 95% CI 0.3-0.9) were less likely to be infected with HSV-2. Other factors including age at sexual debut, condom use in the last three months before the study, history of transactional sex and history of anal sex were not associated with HSV-2 infection (Table 2).

Table 2: Factors associated with HSV-2 infection

Variable	*Frequency of HSV-2 -/ve (%)*	*Frequency of HSV-2 +/ve (%)*	*Odds ratio*	*C.I (95%)*
Married	2 (14.3)	12 (85.7)	10.3	2.3-47.9
Sexual debut before 18years of age	47 (56.0)	37 (44.0)	1.3	0.8-2.4
History of transactional sex	1 (33.3)	2 (66.7)	3.1	0.3-34.9
History of anal sex	1 (50)	1 (50)	1.6	0.1-26.0
≥2 sexual partner	69 (71.1)	28 (28.9)	0.5	0.3-0.8
History of male condom use	114 (65.5)	60 (34.5)	0.5	0.3-0.9
Condom use in the 3 months before the study	68 (66.0)	35 (34.0)	0.6	0.4-1.2

3.3.3 HSV-2 co-infection with other reproductive tract infections

Women with HSV-2 infection were not at a significant risk of being infected with other reproductive tracts infection except for a trend towards infection with *T. vaginalis* (OR 2.2, 95% CI 0.9-5.6) (Table 3)

Table 3: HSV-2 co-infection with other reproductive infections

Infection	*Frequency of HSV-2 –/ve (%)*	*Frequency of HSV-2 +/ve (%)*	*Odds Ratio*	*C.I (95%)*
T. vaginalis	10 (47.6)	11 (52.4)	2.2	0.9-5.6
Bacterial Vaginosis	35 (56.5)	27 (43.5)	1.3	0.7-2.3
C. trachomatis	9 (64.3)	5 (35.7)	1.0	0.3-3.1
Yeast infection	22 (66.7)	11 (33.3)	0.9	0.4-2.0
N. gonorrhoeae	1 (50%)	1 (50%)	1.8	0.1-29.7

3.3.4 Factors associated with HIV sero-positivity

Factors found to have a statistically significant association with HIV infection include; women who had their sexual debut before 18 years of age (OR 2.5, C.I 1.0-6.3) and HSV-2 sero-prevalence (OR 7.5, C.I 2.1-27.1). Condom use in the 3 months before the study was not protective against HIV infection (OR 0.3, 95% CI 0.1-1.1). Being married, having two or more sexual partner and having ever used a male condom were not significantly associated with HIV infection (Table 4.

Table 4: Factors associated with HIV sero-positivity

Variable	*Frequency of HIV-/ve (%)*	*Frequency of HIV+/ve (%)*	*Odds ratio*	*C.I (95%)*
Married	13 (81.3)	3 (18.8)	2.7	0.7-10.6
Sexual debut before 18years of age	78 (86.7)	12 (13.3)	2.5	1.0-6.3
2 or more sexual partner	91 (92.9)	7 (7.1)	0.7	0.3-1.9
History of male condom use	169 (92.9)	13 (7.1)	0.5	0.2-1.4
Condom use in the 3 months before the study	101 (95.3)	5 (4.7)	0.3	0.1-1.1
HSV-2 infection	82 (86.3)	13 (13.7)	7.5	2.1-27.1

3.3.5 Factors associated with having *T. vaginalis* infection

No factors were associated with *T. vaginalis* infection Variables including history of transactional sex, being married and history of anal sex had no significant association with *T. vaginalis* infection (Table 5).

Table 5: Factors associated with having *T. vaginalis* infection

Variable	*Frequency of TV -/ve (%)*	*Frequency of TV +/ve (%)*	*Odds Ratio*	*C.I (95%)*
Sexual debut before 18years of age	55 (90.2)	6 (9.8)	0.7	0.3-2.0
2 or more sexual partner	69 (93.2)	5 (6.8)	0.3	0.1-1.1
History of condom use	122 (89.7)	14 (10.3)	0.6	0.2-1.6
Condom use in the 3 months before the study	74 (88.1)	10 (11.9)	1.0	0.4-2.5

3.3.6 *T. vaginalis* co- infection with other reproductive infections

T. vaginalis infection was associated with bacterial vaginosis(OR 7.7, 95% C.I 2.7-22.3). Two (6.3%) of the women who were infected with *T. vaginalis* also had a yeast infection but this association was not statistically significant (Table 6).

Table 6: *T. vaginalis* co-infection with other reproductive infections

Variable	*Frequency of TV -/ve (%)*	*Frequency of TV +/ve (%)*	*Odds Ratio*	*C.I (95%)*
Having BV	44 (73.3)	16 (26.7))	7.7	2.7-22.3
Having a yeast infection	30 (93.8)	2 (6.3)	0.5	0.1-2.1

3.3.7 Factors associated with having *C. trachomatis* infection

History of condom use, having two or more sexual partner, and age at sexual debut were not found to have a statistically significant association with *C. trachomatis* infection Other factors that were not significant included being married, history of transactional sex and a history of anal sex (Table 7).

Table 7: Factors associated with *C. trachomatis* infection

Variable	*Frequency of CT -/ve (%)*	*Frequency CT +/ve (%)*	*Odds Ratio*	*C.I (95%)*
Sexual debut before 18years of age	52 (89.7)	6 (10.3)	1.6	0.5-4.8
2 or more sexual partner	68 (94.4)	4 (5.6)	0.6	0.2-1.8
History of condom use	123 (91.8)	11 (8.2)	1.2	0.3-4.5
Condom use in the 3 months before the study	73 (88.0)	10 (12.0)	3.0	1.0-10.1

3.3.8 *C. trachomatis* co-infection with other reproductive tract infections

Infection with *C. trachomatis* was associated with *T. vaginalis* infection (OR 3.4, 95% C.I 1.0-12.0) and bacterial vaginosis (OR 4.0, 95% C.I 1.3-12.6) (Table 8).

Table 8: *C. trachomatis* co-infection with other reproductive infections

Variable	*Frequency CT -/ve (%)*	*Frequency CT +/ve (%)*	*Odds Ratio*	*C.I (95%)*
T. vaginalis	17 (81.0)	4 (19.0)	3.4	1.0-12.0
Having BV	48 (84.2)	9 (15.8)	4.0	1.3-12.6

3.3.9 Prevalence of STIs and RTIs across levels of education

The prevalence of HIV, HSV-2, *C. trachomatis* and *T. vaginalis* decreased with increase in the level of education, whereas prevalence for both bacterial vaginosis and yeast infection increased with increase in level of education. The prevalence of syphilis and *N. gonorrhoeae,* was too small to yield any meaningful analysis (Table 9).

Table 9: Prevalence of STIs/RTIs across levels of education

STI(N)	Primary education (%)		Secondary education (%)		Tertiary education (%)	
	+ve	-ve	+ve	-ve	+ve	-ve
HIV (249)	6 (15.8)	32 (84.2)	10 (9.8)	92 (90.2)	5 (4.6)	104 (95.4)
HSV-2 (239)	21 (56.8)	16 (43.2)	41 (42.7)	55 (57.3)	32 (30.2)	74 (69.8)
Syphilis (240)	2 (5.4)	35 (94.6)	0 (0)	97 (100)	0 (0)	106 (100)
N. gonorrhoeae (180)	1 (4.0)	24 (96.0)	1 (1.5)	65 (98.5)	0 (0)	106 (100)
C. trachomatis (177)	3 (12.5)	21 (87.5)	5 (7.8)	59 (92.2)	6 (6.7)	83 (93.3)
T. vaginalis (179)	4(16.0)	21 (84.0)	10 (14.9)	57 (85.1)	7 (8.0)	80 (92.0)
BV (237)	12 (34.3)	23 (65.7)	22 (22.4)	76 (77.6)	27 (26.0)	77 (74.0)
Yeast (182)	4 (15.4)	22 (84.6)	11 (16.9)	54 (83.1)	18 (19.8)	73 (80.2)

*For each infection, all percentages in the table are calculated using the number of participants in each level of education as the denominator. E.g. of the 24 women with primary education, only 3 had *C. trachomatis* which is 3/24(12.5%).

3.3.10 STI/RTI across levels of education

Primary level of education was used as the reference group to reduce the error rate. Women with secondary level of education had a 42% (1-0.58) reduced odds of HIV infection compared to those with primary school education though the risk was not statistically significant. Those with tertiary level of education had a 74% reduced odds of HIV infection compared to women with primary level of education who were at a higher risk of infection (O.R 0.26 95% C.I 0.1-0.8). Similarly women with tertiary level of education had a 67% reduced odds of infection with HSV-2

compared to those with primary level of education (OR 0.33, 95% C.I 0.1-0.7). Although there was a significant risk of infection with syphilis among women with primary education compared to both women with secondary and tertiary education, the prevalence of syphilis was very low (among the 240 women tested for syphilis, only 2 were infected) to give any meaningful analysis. Levels of education were not significantly associated with *N. gonorrhoeae, C trachomatis, T. vaginalis,* bacterial vaginosis and yeast infection (Table 10).

Table 10: Logistic regression model of STI/RTI across levels of education

STI/RTI	**Education***	**OR**	**95% CI**
HIV	2 vs.1	0.58	0.2-1.7
	3 vs. 1	0.26	0.1-0.8
HSV-2	2 vs.1	0.57	0.3-1.2
	3 vs.1	0.33	0.1-0.7
Syphilis	2 vs.1	0.001	0.0-0.1
	3 vs.1	0.001	0.0-0.1
N. gonorrohoeae	2 vs.1	0.10	0.0-8.4
	3 vs.1	0.03	0.0-1.8
C. trachomatis	2 vs.1	0.59	0.1-2.7
	3 vs.1	0.59	0.1-2.2
T. vaginalis	2 vs.1	0.92	0.3-3.3
	3 vs.1	0.46	0.1-1.7
BV	2 vs.1	0.55	0.2-1.3
	3 vs.1	0.67	0.3-1.5
Yeast	2 vs.1	1.12	0.3-3.9
	3 vs.1	1.36	0.4-4.4

Education* 1: Primary; **2**: Secondary; **3**: Tertiary education
Primary level of education is the reference group

3.4 Multivariate Logistic Regression

Variables with p-values less than 0.2 from bi-variate analysis were entered in a logistic regression model for multivariate analysis. $P \leq 0.05$ was considered significant. Condom use in the last three months before the study was included regardless of the p-Value.

3.4.1 Independent factors associated with having any STI

Unadjusted multivariable regression analysis showed that being married (p=0.01) having two or more sexual partners (p=0.001) and having bacterial vaginosis (p=0.002) were associated with having an STI. When the model was adjusted for condom use in the three months before the study, infection with bacterial vaginosis, age at sexual debut, and multiple sexual partners; being married (p=0.02), having two or more sexual partners (p=0.01) and having bacterial vaginosis (p=0.001) were the only risks associated with STI (Table 11).

Table 11: Logistic regression models of factors associated with having an STI

Variable	Unadjusted Odds Ratio (95% CI)	P- value	Adjusted Odds ratio (95% CI)	P-value
Married	3.0 (1.2-7.5)	0.01	12.7 (1.5-103.8)	0.02
Sexual debut before 18years of age	1.3 (0.8-2.2)	0.21	1.3 (0.7-2.4)	0.34
2 or more sexual partners	0.5 (0.3-0.8)	0.001	0.5 (0.2-0.9)	0.01
Condom use in the 3 months before the study	1.0 (0.6-1.6)	0.97	0.8 (0.5-1.5)	0.50
Having BV	2.5 (1.4-4.6)	0.002	2.9 (1.5-5.5)	0.001

3.4.2 Independent factors associated with HSV-2 infection

Unadjusted multivariable regression analysis showed being married (p=0.0003 and having 2 or more sexual partners (p=0.01) as risk factors associated with HSV-2 infection. When the model was adjusted for condom use, and multiple sexual partners only being married (p=0.01) and having two or more sexual partners (p=0.01) remained significantly associated with HSV-2 infection (Table 12).

Table 12: Independent factors associated with HSV-2 infection

Variable	Unadjusted Odds Ratio (95% CI)	P-value	Odds Ratio Adjusted Odds ratio (95% CI)	P-value
Married	10.3 (2.3-47.9)	0.0003	7.7 (1.6-36.7)	0.01
2 or more sexual partners	0.5 (0.3-0.8)	0.01	0.5 (0.3-0.9)	0.01
Condom use in the 3 months before the study	0.6 (0.4-1.2)	0.16	0. 7 (0.4-1.2)	0.15

3.4.3 Independent factors associated with HIV infection

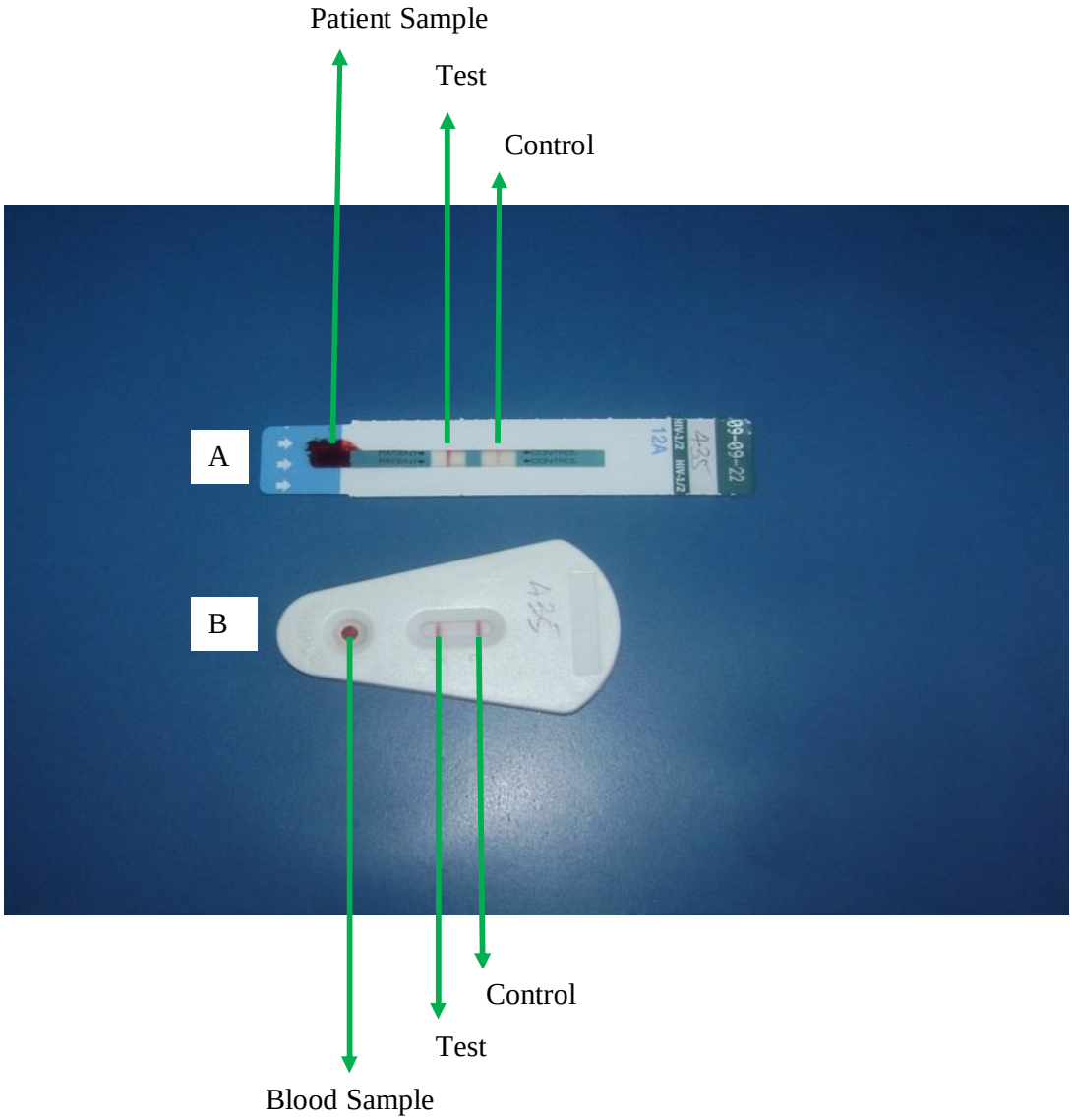
Factors that were associated with HIV infection from the unadjusted model included sexual debut below 18 years of age (p=0.03) and infection with HSV-2 (p=0.0004). When adjusted for multiple sexual partners, condom use in the three months before the study and being married, only HSV infection (p=0.01) was associated with HIV infection (Table 13).

Table 13: Independent factors associated with HIV infection

Variable	Unadjusted Odds Ratio (95% CI)	P-value	Adjusted Odds ratio (95% CI)	P-Value
Married	2.7 (0.7-10.6)	0.12	1.2 (0.27.2)	0.78
Sexual debut before 18years of age	2.5 (1.0-6.3)	0.03	1.7 (0.6-5.5)	0.35
2 or more sexual partners	0.7 (0.3-1.9)	0.54	1.3 (0.4-4.4)	0.64
Condom use in the 3 months before the study	0.3 (0.1-1.1)	0.07	0.3 (0.1-1.3)	0.12
HSV-2 infection	7.5 (2.1-27.1)	0.0004	6.9 (1.8-26.5)	0.01

3.5 Rapid test kits for detection of antibodies to HIV types 1 and 2

Figure 3: Positive HIV test



KEY;

A: Determine™

B: Uni-gold™ device

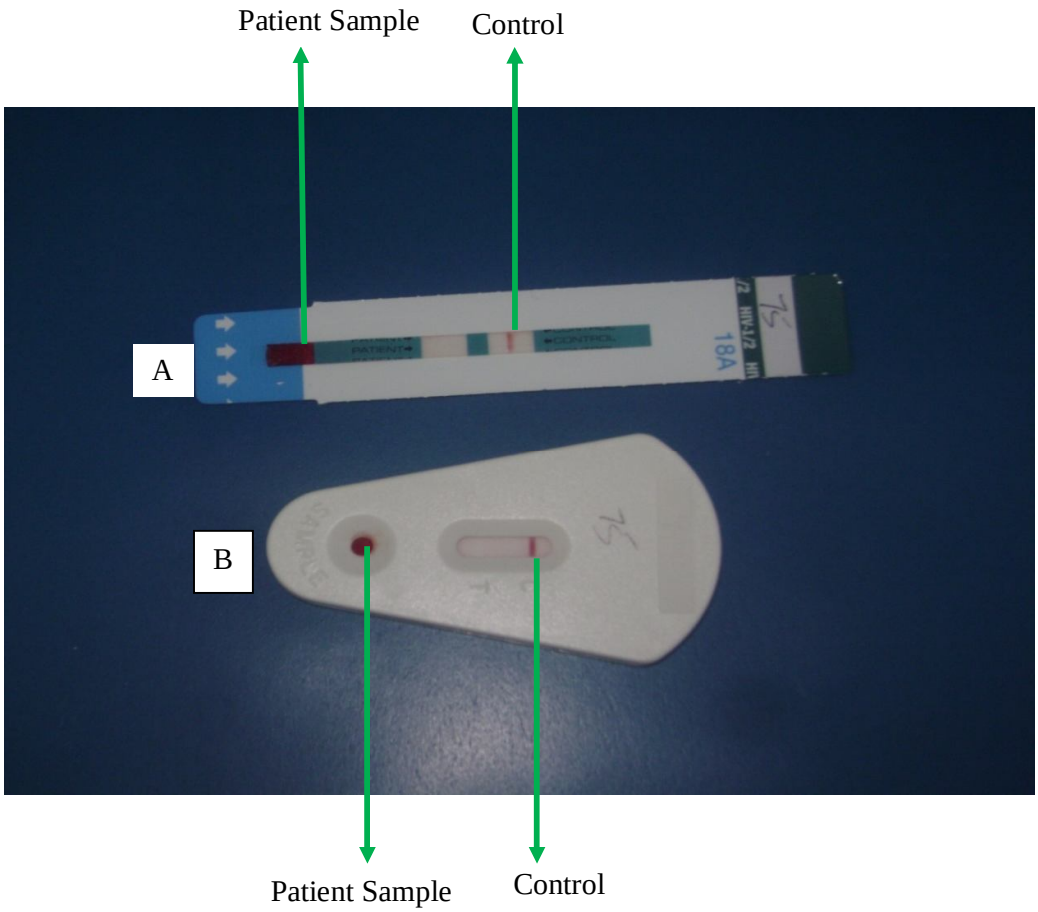
3.5.1 The Abbott Determine™ strip

For a positive reaction red bars appeared in both the control window (labeled "Control") and the patient window (labeled "Patient") of the strip. Any visible color in the patient window was intepreted as positive (Figure 3A).

3.5.2 The Trinity Biotech Uni-Gold™ device

A positive reaction was visualised by a pink/red band in the test region of the deivice. A line of any intensity forming in both the test (labeled "T") and control (labeled "C) regions indicated a positive result (Figure 3B).

Figure 4: Negative HIV test



3.5.3 The Abbott Determine™ strip

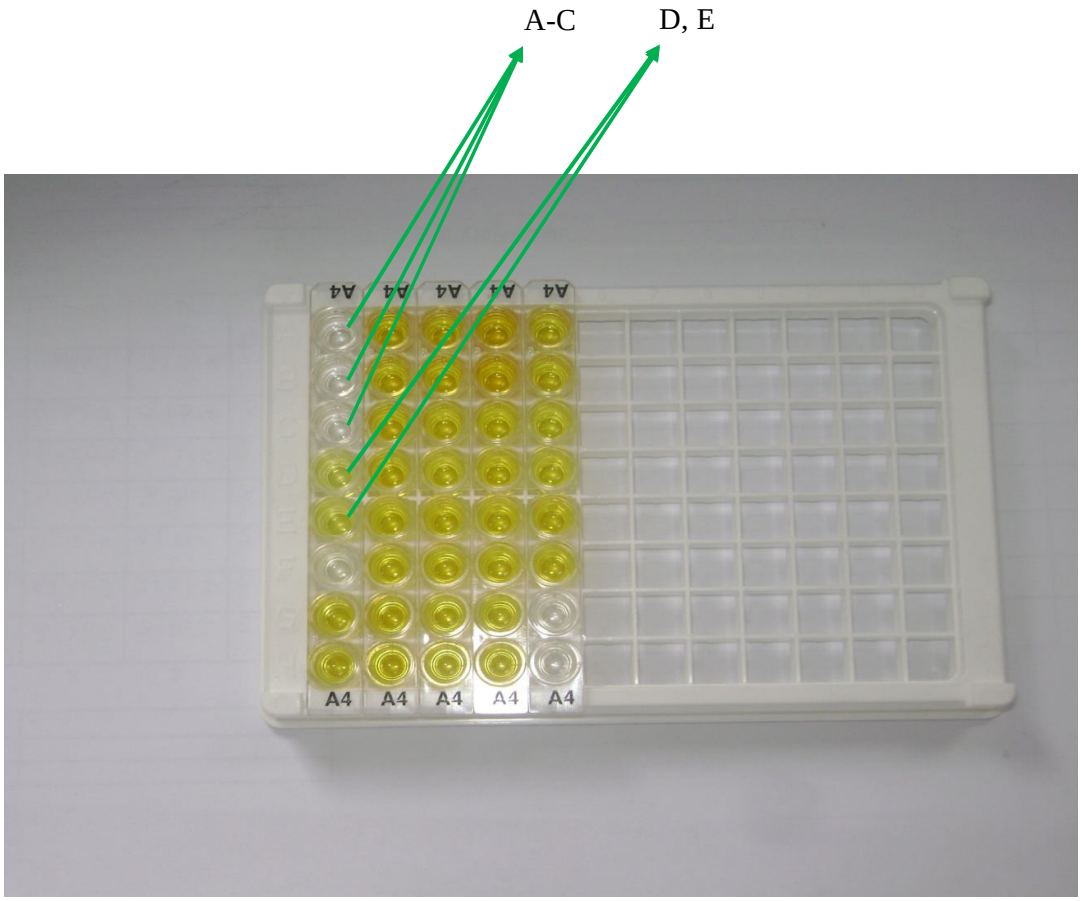
One red apperaing in the control window of the strip (labeled “Control”), and no red bar appearring in the patient window of the strip (labeled “Patient”) was intrepreted as negative (Figure 4A).

3.5.4The Trinity Biotech Uni-Gold™ device

A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the specimen. Concequently no visual detectable band develops in the test (labeled “T”) region of the device. A line in the control region only indicated a negative test result (Figue 4B).

3.6 Enzyme linked immunosorbent assays

Figure 5: HIV ELISA qualitative results



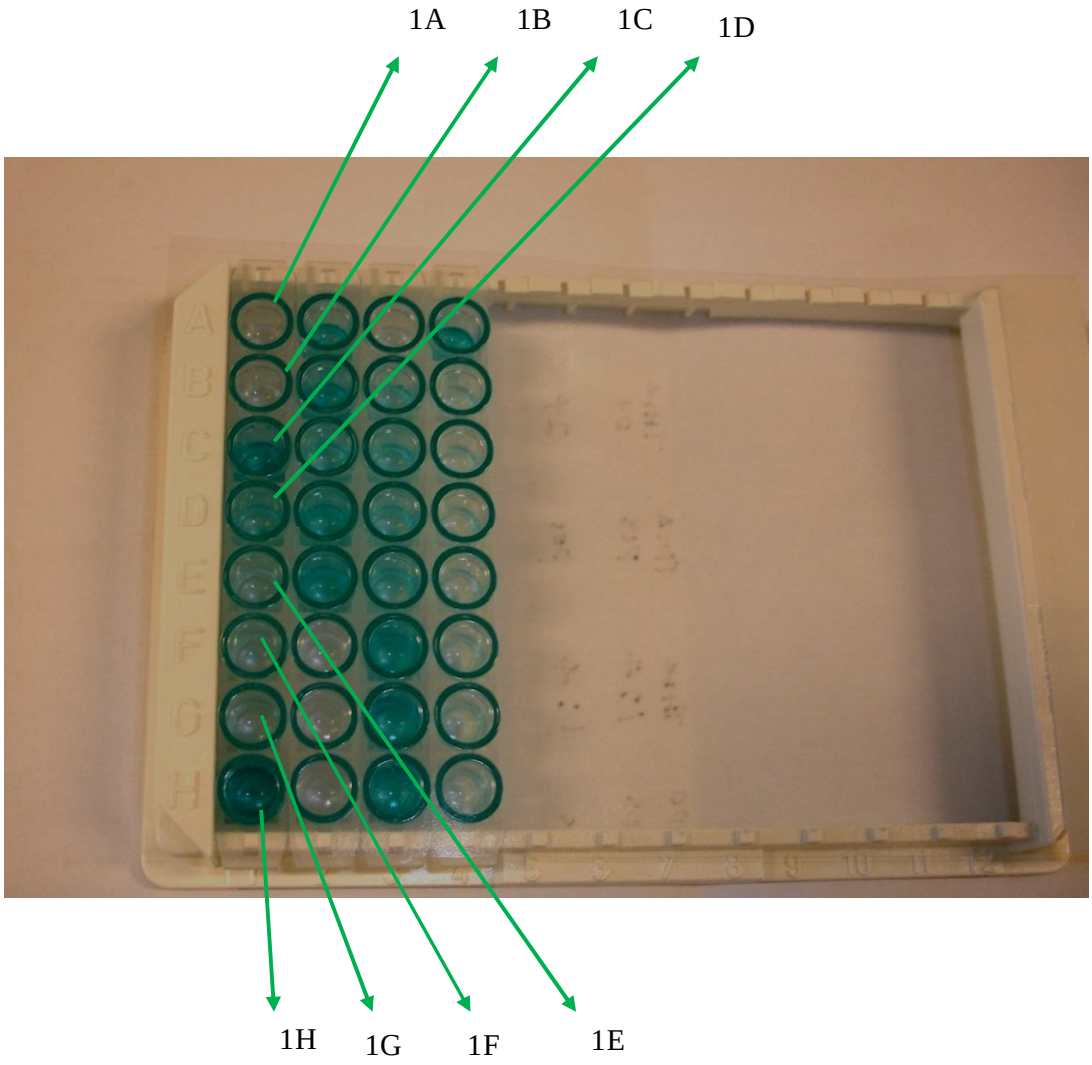
KEY;

A-C: Negative control

D, E: Positive Control

The results were interpreted based on a cut-off value (mean optical density of the negative controls + 0.1). Values above the cut-off were considered positive results while those below the cut off were considered negative. Values equal to the cut off value were re-tested.

Figure 6: HSV-2 ELISA test



KEY:

1A: Blank

1B: Negative control

1C: High positive control

1D: Low positive control

1E: Cut off calibrtator (CAL)1

1F: CAL 2

1G: CAL 3

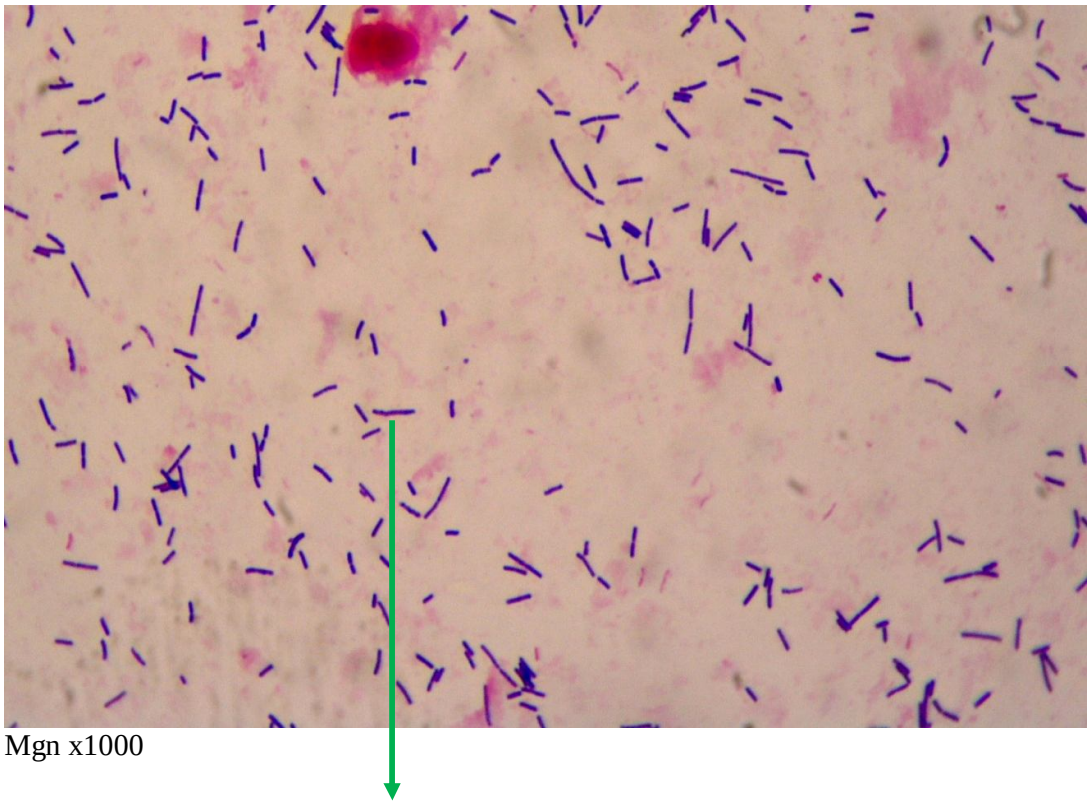
1H- 4H: Patient sample

Results were interpreted as Index values relative to the Cut-off Calibrator. The index value was calculated by dividing specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values. An index value of >1.10 was presumptive for the presence of IgG antibodies to HSV -2, an index value of < 0.90 indicated no IgG antibodies to HSV-2 were detected. An index value of ≥0.90 but ≤1.10 was considered an equivocal result, the sample was restested.

3.7 Microscopic examination of vaginal smears

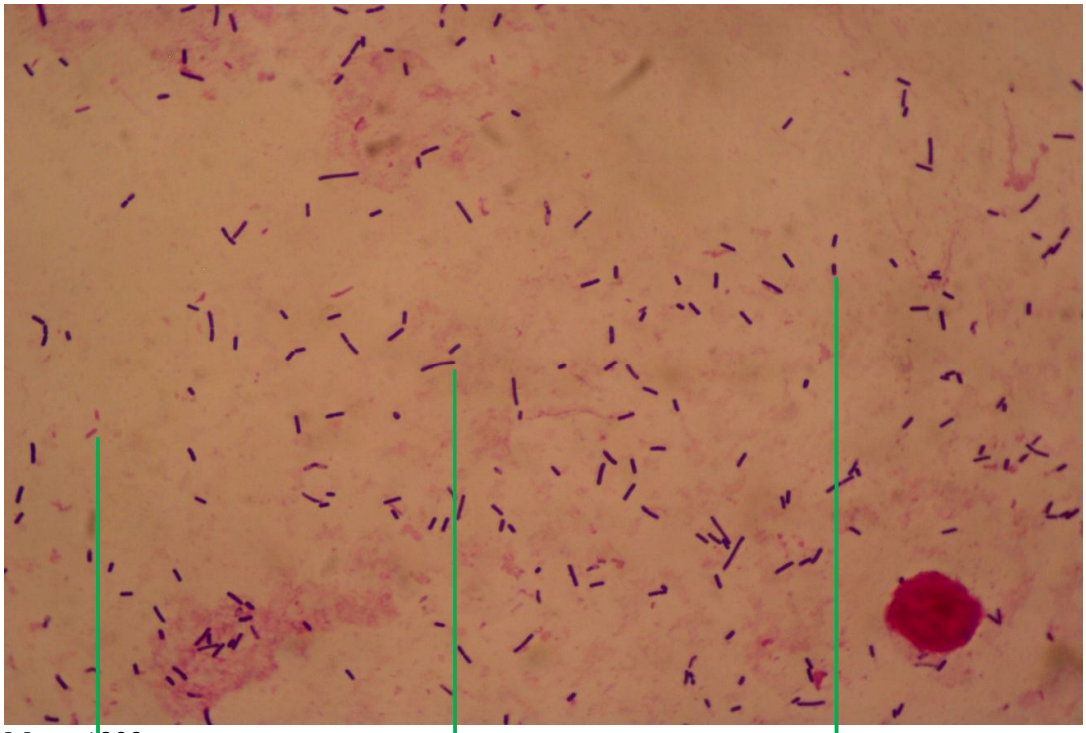
Gram stained vaginal smears were assessed qualitatively as normal, intermediate or consistent with BV.

Figure 7: Normal Vaginal Smear



A normal vaginal smear with lactobacillus species.

Figure 8: Intermediate Score Vaginal Smear



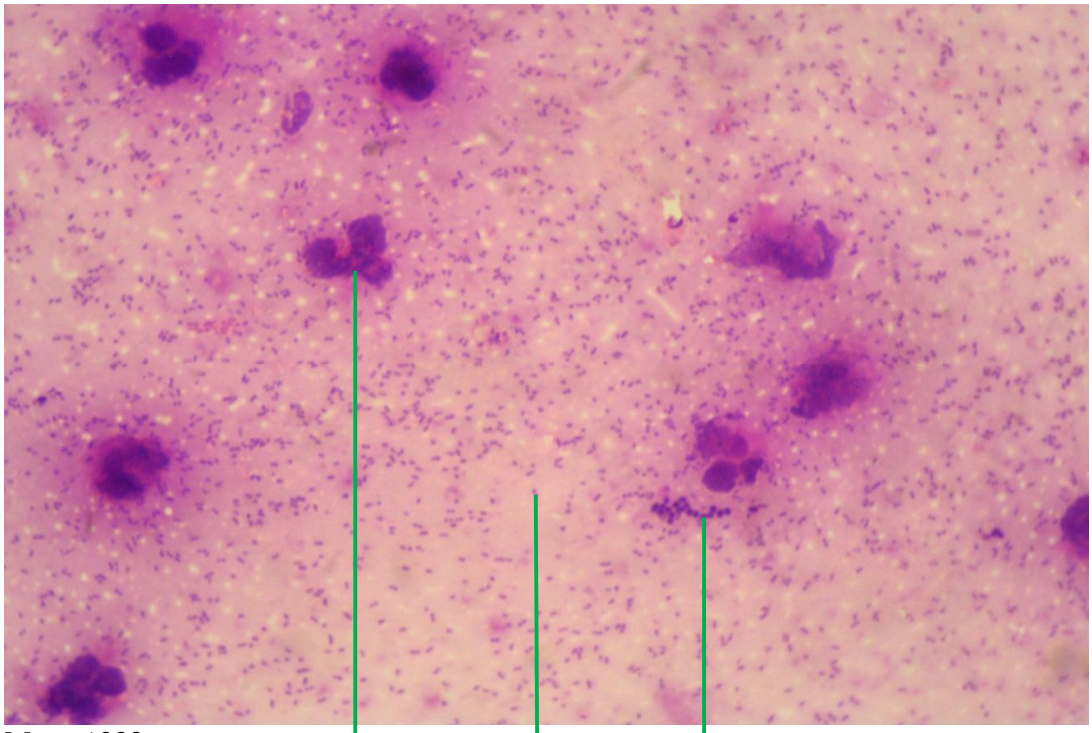
Mgn x1000

Short gram -ve rods

Long gram +ve rods

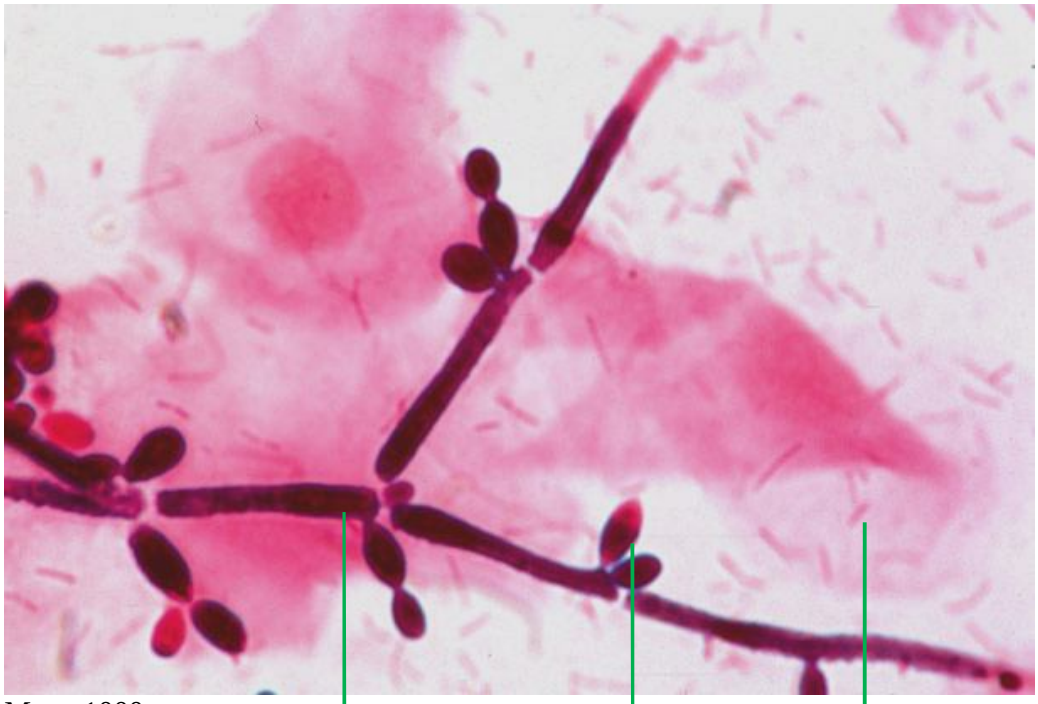
Short gram -ve rods

Figure 9: Bacterial vaginosis vaginal smear



The Nugent scoring criteria involves three morphotypes. The first type is lactobacillus species or large gram positive rods. As the amount of lactobacillus on the smear decreases, the score increases. The other two morphotypes are small gram negative to gram variable rods resembling *Gardnerella vaginalis* or Bacteroides species and curved rods resembling Mobiluncus sp. For these two morphotypes the score increases as the amount on the slide increases. The score ranges from 7-10. A score of 0-3 was considered normal (Figure7), 4-6 intermediate or altered vaginal flora (Figure 8), bacterial vaginosis ranged from 7-10 (Figure 9).

Figure 10: Vaginal smear showing yeast



Gram's stain image of Candida organisms in vaginal secretion. Pseudohyphae and buds (shown in arrows) as well as normal bacterial flora are seen.

CHAPTER FOUR

4.0 DISCUSSION AND LIMITATIONS

4.1 Discussion

Patterns of sexual behaviour among the young women were explored using data from a structured questionnaire. Variables such as years of formal education completed, marital status, age of coital debut, number of sexual partners, history of transactional sex, anal sex and previous STI/RTI infection, were analyzed. These behaviours have been proven to be associated with an increased HIV/STI infection (Pettifor *et al.*, 2005).

High-risk sexual behaviours, such as multiple sex partnerships are underreported, these has been a recurring problem in sex surveys (Catania *et al.*, 1995). In addition, respondents may not practice high-risk behaviour but may be put at risk by the sexual behaviour of their male partners. As a proxy for practicing, or being exposed to, a high-risk behaviour, I looked at the frequency of self-reported sexually transmitted infections. This measure has its own weaknesses that cannot be overlooked, for example, women may have an infection that is asymptomatic and therefore goes unnoticed. Similarly, respondents may experience STI-like symptoms but not recognize them as such, and therefore not report having had an STD. Conversely (though less likely), respondents may experience STI like symptoms that are not actually caused by an STI (Garg *et al.*, 2001). Finally, as with any other socially undesirable behaviour, there is also the likelihood of under reporting.

Low levels of education and being married were risk factors for HSV-2 positivity in bi-variate and multivariate analysis; OR 0.33, 95% C.I 0.1-0.7 and AOR 7.7, 95% C.I 1.6-36.7 respectively. The high sero-prevalence of HSV-2 in Kisumu is

consistent with results from other African studies in urban populations which reported HSV-2 prevalence in adults ranging from 39% in 15-19 year old women to 66% in 20-24 year old women in Kisumu Kenya (Weiss *et al.*, 2001). In the same study in Kisumu, married women were found to be at a significantly higher risk of HSV-2 infection compared to women who were not married women.

The high prevalence of HSV-2 among married individuals is probably due to increased exposure to infection among individuals married to HSV-2 positive partner, and more frequent sexual contact in marriage. Another reason could be married women may not feel the need to use condoms during sexual intercourse as they are in stable relationships. And even if they wanted to use a condom they may not be able to request their partners to use it as it may be construed as mistrust.

Women with low levels of education may be at an increased risk of HSV-2 because of their ignorance about HSV-2 and other STIs including HIV. They probably lack sufficient information on how to practice less risky sexual behaviour. This suggests that improving the level of knowledge of STIs/HIV and their prevention will motivate an individual to alter the behaviour that put them at risk of infection. Inadequate sexual health education and services tailored to adolescents and youth at risk, many of whom are out of school and unemployed, may contributes to high rates of STIs, HIV and unintended pregnancy. Previous research has shown that school-based sexuality education programmes for youth discourages early sexual activity and multiple sexual partners (Manlove *et al.*, 2004); and can increase knowledge and reduce risk behaviour (Magnani *et al.*, 2005).

HIV prevalence in the general population (among adults aged 15-64) in Kenya is 7.4 (KAIS, 2007). Nyanza Province is one of the two provinces with HIV prevalence levels (15.3%) more than double the national estimate. Kisumu itself is among the hardest hit districts in the country by the HIV pandemic, with a prevalence of over 20% in the adult population. The KAIS 2007 found that HIV prevalence ranged from 4% among women age 15–19 years to a high of 11% among those age 25-29 years. The population in this study had a prevalence of 6.7%. More than half (59.62%) of the women reported having had more than one sexual partner in life.

Although the spread of HIV and other STIs depends on having unprotected sex with multiple partners or having unprotected sex in a discordant relationship, in this study women with more than one lifetime sexual partner were not significantly at a higher risk of being infected with HIV compared to women who reported having one sexual partner. Although there is not a universal pattern of risk for the acquisition of STI, it seems clear that many of these young women cannot be said to be at risk simply because of risky patterns of sexual behaviour such as multiple partners. It is possible that the pattern of partnerships among those reporting more than one partner was predominantly serial, and not the overlapping mixing pattern that has been demonstrated to be extremely high risk for the transmission of STIs

This study showed a consistently strong association between HSV-2 and HIV infections; AOR 7.5, 95% CI 2.1-27.1. Several factors could have contributed positively to this strong association. For instance HSV-2 infection likely increases susceptibility to HIV infection because of the lesions it causes that are not always recognized by the infected individual (Holmberg *et al.,* 1988). Secondly, it is also possible that HIV infection increases susceptibility to HSV-2 infection, and this

biological synergy may explain the very strong associations seen. Epidemiological evidence for the role of HSV-2 infection increasing susceptibility to HIV has been demonstrated in several cohort studies of heterosexual acquisition of HIV from Zimbabwe and Kenya. A study done in Kenya found that HSV-2 infection at baseline was associated with increased risk of HIV acquisition: unadjusted rate ratio (RR) 2.9, 95%CI 1.2-7.0 (Rakwar *et al.*, 1999); where as in the Zimbabwe study: unadjusted RR 1.4, p=0.06 (McFarland *et al.*, 1999).

History of condom use was not a protective factor against STI/HIV infection in this population. The history of condom use was likely a proxy for more sexual experience (58.4% of those who reported having had sex within the three months preceding the study used a male condom), and higher risk sex, although in this study women who had one sexual partner were likely to report history of condom use compared to women with two or more partners. Additionally, history of condom use is not a sensitive measure of use as it does not capture the frequency or consistency of use, which are both essential to condom efficacy in HIV prevention.

Condom use in the last three months before the study was a risk factor against *C. trachomatis* infection in bivariate analysis; OR 3.0 95% CI 1.0-10.1. Possibly the women over-reported this practice or lied about it. It could also be possible that even though they used the condoms, they were not using them correctly or consistently. From previous studies women are less likely to use condoms compared to men. Studies conducted in Mwanza, Tanzania in 1992 measured the proportion of individuals who reported ever having used a condom and found 20% men and 3% women had used this form of protection (Munguti *et al.*, 1997). In a similar study in Arusha, Tanzania, 34% men and 14% women reported ever having used a condom

(Mnyika *et al.*, 1997). In the past 15 years, the awareness of African populations towards condom use as a protective device for HIV and other STI has increased dramatically. The 2003 KDHS reported that men were three times (17 %) more likely than women (5 %) to use condoms during sexual encounter with any partner.

Co-infection with more than one sexually transmitted pathogen was common in this study. Those who were infected with HSV-2 were also co-infected with bacteria vaginosis, *T. vaginalis, C trachomatis* and yeast infection. In a study done by Kaul and co-wokers (2007) in Kenya, HSV-2 infection was shown to increases susceptibility to bacterial STIs and/or alterations of vaginal flora, therefore HSV-2 infection enhances the sexual transmission of HIV both directly (through mucosal ulcerations) and indirectly (by increasing the incidence of other genital infections that act as HIV cofactors). This emphasizes the need to screen for HSV-2 as a control strategy to reduce the transmission not only of HIV, but also of other STIs. A previous study carried out in Burkina Faso demonstrated a strong association between bacterial vaginosis (BV) and HSV-2 co-infection (Nagot *et al.*, 2007). This association was not demonstrated in this study though 43.5 % of those infected with BV also were HSV-2 sero-positive.

There was also a strong association between the presence of bacterial vaginosis with both *T. vaginalis* (OR 7.7 95% CI 2.7- 22.3) and *C. trachomatis* infections (OR 4.0, 95% CI 1.3-12.6) in this study. Earlier studies have also revealed a significant association between a past history of trichomoniasis and bacterial vaginosis. In a previous study, 80% of women infected with *T. vaginalis* were diagnosed with bacterial vaginosis. This was evident in *T. vaginalis* symptomatic women and those attending STD clinics (James *et al.*, 1992). However, since mixed vaginal infections

due to *T. vaginalis* and bacterial vaginosis associated microorganisms occur frequently, no etiological relationship can be derived from any statistically significant association between presence of *T. vaginalis* and bacterial vaginosis.

In several studies, bacterial vaginosis has been associated with an increased susceptibility to STIs. In a cross-sectional study in Uganda, bacterial vaginosis was associated with HIV-1 infection (Sewankambo *et al.,* 1997). Abnormal vaginal flora was also associated with HIV-1 acquisition in a prospective trial of Kenyan commercial sex workers (Martin *et al.,* 1999). Data that link abnormal vaginal flora to non-HIV STD acquisition are limited. Studies of pregnant women demonstrated that women with *Lactobacillus* predominant vaginal flora were less likely to be infected with *C. trachomatis, N. gonorrhoeae,* and *T. vaginalis* than women with altered flora (Hillier *et al.,* 1992a)

Young men and women do not wait until they are married to become sexually active. The majority of young people begin sexual activity while still in their teens. Nearly 75% of young people have sexual intercourse before their twentieth birthday, with a median age of first sexual encounter of 17 years for women and 16 years for men (Waithaka & Bessinger, 2001). Early age of coital debut has been shown to be associated with a number of negative subsequent outcomes such as STIs, decreased condom and contraceptive use, and increased numbers of sexual partners (Pettifora *et al.,* 2004). A study of sexual behaviour and HIV infection in four African cities reported that in the two cities with high HIV prevalence (Kisumu and Ndola), young women had their sexual debut earlier than in the two cities with a low HIV prevalence (Yaounde` and Cotonou) (Ferry *et al.,* 2001). The mean age at fist vaginal intercourse was 17 years in this study**.** More than half of the women reported coital

debut above 17 years of age. This profile is almost similar to that seen in other female populations in Kisumu city and rural Siaya District, where the mean age of sexual debut among women aged 15-29 years was 16.5 and 15.7 years respectively (Voeten *et al.*, 2004).

Though many studies have shown that delaying the age of first sex is an important practice in reducing HIV incidences many women are put at risk of HIV infection by the behaviours of their male partners, not their own. In this study early coital debut was only a risk factor for HIV infection in bi-variate analysis.

Transactional sex defined as receiving or giving gifts or money for sex, is considered as a sexual practice that increases the risk of STIs including HIV infection (Waithaka & Bessinger, 2001) Women who have little negotiating power with their partners to insist on use of condoms experience a higher risk of contracting STIs, including HIV/AIDS (Gregson *et al.*, 2002). In this study, only 2.56% of women reported having engaged in transactional sex. An important concern is the validity of this self reported behaviour that may be subject to reporting bias. Since it is considered an immoral practice it is possible that the respondents could have under-reported engaging in transactional sex. This could also suggest that transactional sex is not an important factor in consental sex.

Anal intercourse has been shown to be associated with high risk of HIV acquisition among women and the probability of HIV transmission, per contact, has been estimated to be 10 times higher for penile-anal than penile-vaginal sex (Schwandt *et al.*, 2006). The practice of anal intercourse was reported infrequently in this population (1.28%), and was less common than found in a previous study among female sex workers (FSWs) in Nairobi, Kenya where 14% reported practicing anal

intercourse (Fonck *et al.*, 2000). This may be accounted for by variations in cultural practices and sexual preferences. The high prevalence of anal sex in FSWs maybe related to the financial incentives involved, with anal sex particularly commanding a higher price than vaginal intercourse.

Studies of self-reported behaviours perceived as pejorative or as socially undesirable often are under-reported (Brody, 1995). There is ample evidence of such under-reporting, particularly of number of sexual partners by women (Glynn *et al.*, 2001). Given our understanding of social mores in sub-Saharan Africa blanket denials of anal intercourse and other sexual behaviours in surveys from Africa probably reflect cultural reluctance and perhaps, personal reluctance (Brody & Potterat, 2003).

4.2 Limitations of the study

This study is subject to a number of limitations. Data on sexual behaviour were based on self-reported survey data and these data are subject to both selection and reporting biases. Reporting bias may have been in the form of recall bias (e.g. forgetting to mention past partners) or 'social desirability' bias (reporting the norm rather than true behaviour.)

Another limitation is the possibility of ecological correlation among the factors that have a particular social desirability bias, particularly the reporting of STIs and anal sex. In other words, a woman who is sufficiently confident or bold to report having had an STI may also be more likely to reveal the practice of anal sex, thus leading to a correlation between the two variables. More detailed investigative work is needed to quantify the influence of this possible bias.

And finally this being a cross-sectional study, it was only possible to explore in depth the factors associated with STIs including HIV but could not prove causality.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Herpes simplex type 2 was the most prevalent STI in this population; being married and having low levels of education were strongly associated with infection with HSV-2. HSV-2 was also significantly associated with HIV infection. Although not classical STIs, BV and yeast infections were also highly prevalent, both of which have been associated with HIV infection.

There were prevalent cases of STI co-infection among the women screened. Bacterial vaginosis was the most common reproductive tract infection among the women infected with STIs.

In summary, monitoring trends in STIs prevalence provides valuable insight into the likelihood of the importance of sexual transmission of HIV. These trends also assist in assessing the impact of behavioural interventions, such as delaying sexual debut, reducing the number of sex partners and promoting condom use.

6.2 Recommendations

Programmatic Recommendations

1. Women with bacterial vaginosis, married women, and those with lower levels of education should be identified and targeted for interventions by health authorities, to reduce the transmission of STI and HIV. Besides targeting preventive measures towards them, they should also be encouraged to go for regular medical check-up to capture asymptomatic case and therefore treatment is initiated on time.

2. The four most common infections in this study —bacterial vaginosis, trichomoniasis and chlamydia — are easily curable with antibiotics and yeast infection curable by antifungals. Ensuring accurate and timely diagnosis and the availability of appropriate medications could substantially reduce infection rates. Therefore, treatment of prevailing STIs could potentially lessen risk for future HIV acquisition and reduce subsequent transmission to sex partners.
3. Education offers a measure of protection against HIV/AIDS and STIs, reducing the levels of risk and vulnerability to the disease by providing information and skills, and also increasing literacy. Action to provide sex education to those young people is urgently needed.
4. Current evidence of STI co-infection implies a need to strengthen clinical practice among providers caring for persons at risk for HIV or other STIs. These findings strongly suggest the need for bacterial vaginosis screening for STI infected persons. Appropriate diagnosis of co-infection by comprehensive STI screening is also important for averting potential development of pathogen drug resistance

Areas for further research

5. The availability of data is crucial. Surveillance programmes must be introduced, expanded and sustained to include the collection of data by age, sex and year. Such data are critical to achieving an accurate understanding of the extent of the STIs/HIV among young people, identifying those most affected, and ascertaining patterns of transmission. Monitoring the impact of

HIV/AIDS and STI will better allow health organizations to take appropriate, targeted action.

6. Interventions must also be designed to increase testing of sexually active men and treatment of those men who are infected or who are contacts to persons with STIs. In addition, observational studies to determine the behaviours of both members of sexual dyads and not just women would be important in the design of future behavioural and educational interventions.

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APPENDICES

Appendix 1: Socio-demographic and sexual risk behaviours

Variable	Frequency	Percentage
Years of formal education		
Tertiary education	126	40.38
Secondary education	132	42.31
Primary education	54	17.31
Marital Status		
Married	22	7.05
Single	286	91.67
Separated	2	0.64
Widowed	2	0.64
Divorced	0	0.00
Regular sexual partner		
Yes	225	72.12
No	86	27.56
No response	1	0.32
Live with regular sexual partner		
Yes	21	6.73
No	204	65.38
No response	87	27.88
History of male condom use (ever)		
Yes	212	67.95
No	100	32.05
Male condom use in the last 3 months		
Yes	128	41.03
No	182	58.33
No response	2	0.64
History of female condom use (ever)		
Yes	2	0.64
No	309	99.04
No response	1	0.32
Female condom use in the last 3 months		
Yes	2	0.64
No	310	99.38

***Table continued*: Socio-demographic and sexual risk behaviours**

Variable	Frequency	Percentage
History of spermicide use		
Yes	2	0.64
No	308	98.72
No response	2	0.64
Coital debut		
Below18 years	115	36.86
Above 18 years	190	60.90
No response	7	2.24
Male partners in lifetime		
1 Male partner		
2 or more sexual partners	119 186	38.14 59.62
No response	7	2.24
Male partner in last 3 months		
0	54	17.31
1	237	75.96
2	11	3.53
3	2	0.64
No response	8	2.56
History of transactional sex		
Yes	8	2.56
No	296	94.87
No response	8	2.56
History of anal sex		
Yes	4	1.28
No	299	95.83
No response	9	2.88
Used condom last had anal sex		
Yes	2	50
No	2	50
Ever had STI/RTI*		
Yes	43	13.78
No	265	84.94
No response	4	1.28

Appendix 2: Demographic Sexual Reproductive History form

1. How old are you……☐☐ Years
2. How many years of formal education have you completed?.....☐☐ Years
3. Do you have a regular sexual partner …….. .☐ Yes ☐ No
4. Do you currently live with your regular sexual partner?.....☐ Yes ☐ No
5. What is your current marital status?

   ☐ Married ☐ Divorced ☐ Separated ☐ Widowed ☐ Single
6. Have you ever used the following to prevent sexually transmitted infections

   6a. Male condom.....☐ Yes ☐ No

   6b. Female condom…….....☐ Yes ☐ No

   6c. Spermicide……..☐ Yes ☐ No
7. How old were you when you first had vaginal sex?.....☐☐years
8. How many male sexual partners have you had in your life?

   ☐☐☐ Partners
9. How many male sexual partners have you had in the last 3 months?

   ☐☐☐ Partners
10. Have you ever had sex in exchange for money, food, drugs or shelter?

    ☐ Yes ☐ No
11. How many times have you had vaginal sex in the past month?

    ☐☐☐ Times per: week/month
12. Have you ever had anal sex?..... ☐ Yes ☐ No
13. Did you use a condom the last time you had anal sex?.....☐☐years

STI History

14. Have you ever had a reproductive tract infection or sexually transmitted infection (yeast infection, Chlamydia, gonorrhea, etc)? ……☐ Yes ☐ No
15. Have you ever been diagnosed or treated for a reproductive tract or sexually transmitted infection by a clinician? …….....☐ Yes ☐ No
16. Have you ever had a urinary tract infection? ……...……..☐ Yes ☐ No
17. Have you ever been diagnosed or treated for a UTI by a clinician?.... ☐ Yes ☐ No

Appendix 3: Subject Test Results

1. Study Number ☐☐ ☐☐☐

2. Lab Name _____

3. Registration Card Number/Record Number ☐☐☐☐☐

4. Date Specimens Collected Day ☐ Month ☐ Year ☐

5. Date Report Sent Out Day ☐ Month ☐ Year ☐

6. *T. vaginalis* (wet mount) Positive ☐ Negative ☐

7. *T. vaginalis* NAAT (cervical swab) Positive ☐ Negative ☐

8. *N. gonorrhoea* NAAT (cervical swab) Positive ☐ Negative ☐

9. *C. trachomatis* NAAT (cervical swab) Positive ☐ Negative ☐

10. Syphilis RPR Positive ☐ Negative ☐

11. Syphilis RPR/TPHA Positive ☐ Negative ☐

12. HSV-2 EIA Positive ☐ Negative ☐

13. HIV- EIA Positive ☐ Negative ☐

13. Bacterial vaginosis Positive ☐ Negative ☐

14. Yeast infection Positive ☐ Negative ☐

Appendix 4: Informed Consent

Title of study

An expanded phase 1 randomized placebo controlled clinical trial of the safety and tolerability of 3% w/w SPL7013 Gel (VivaGel□) in healthy young women when administered twice daily for 14 days. (SPL7013-004) DMID Protocol #: 05-0121

Institutions and Investigators

Researcher	**Institution**	**Contact**
Dr. Craig Cohen	University of California, San Francisco	000-1-415-597-9192 (USA)
Dr. Elizabeth Bukusi	Kenya Medical Research Institute	254 733-617-503 (KENYA)
Dr. Barbara Moscicki	University of California, San Francisco	000-1-415-476-5139 (USA)

Introduction

You have decided to take part in the VivaGel™ study. While you are in this research study there may be some blood taken from you that might be useful for future research. You are being asked if you would agree to the storage of these samples. This consent form gives you information about the collection, storage and use of your samples. The study staff will talk with 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 consent form. You will get a copy of this form to keep.

How will you get the samples from me?

There will be no additional samples taken from you for storage. After all the tests are done for this research study, there may be some left over blood samples. If you agree, left over blood samples will be kept and used for future research.

What will happen if I agree to let my samples be stored?

After you have completed your participation in the study, instead of discarding your leftover samples we will save it in what is called a "tissue bank" for possible future research. We also will collect and save information from your study forms, including things like results of physical examinations, tests, questionnaires, and treatments. We do not know for sure if your specimen or study forms will be used, but they might be used to look for additional evidence of infection with HIV or other agents, damage caused by infection, your body's response to the study gel or other infections (such as examining cells, proteins, and other chemicals in your body), or genetic factors that may make individuals susceptible to sexually transmitted infections.

The researchers do not plan to contact you or your health care providers with any results from tests done on your stored samples. This is because research tests are often done with experimental procedures that are not approved and may not give reliable results, so the results are generally not useful or appropriate for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify your study clinician and your study clinician will try to contact you. If you wish to be contacted with this type of test result, you must notify the study clinician of any change to your address and/or phone number.

Also, if you want your health care provider to be told about this type of test result, you must provide the study clinician with their name, address and phone number.

Your samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by the National Institutes of Health and a special committee at the researcher's institution (an Institutional Review Board).

How long will you keep my samples?

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

How will my samples be stored?

Your samples will be stored at special facilities at KEMRI or UCSF that are designed to store samples safely and securely. The storage facilities are designed so that only approved researchers will have access to the samples. Some employees of the storage facilities will need to have some access to your samples in order to store them and to keep track of where they are, but these people will not have information that directly identifies you. An Institutional Review Board will oversee the storage facilities to protect you and other research volunteers from harm.

Does storage of my samples benefit me?

There are no direct benefits to you. The benefit of doing research on stored samples includes the researchers learning more about your body's reaction to the study gel, as well as information about other sexually transmitted diseases.

What are the risks?

There are few risks related to storing your samples. When tests are done on the stored samples there is a small but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (such as

information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems getting a job or insurance.

Will my records be kept private?

In order to keep your information private, your samples will be labeled with a code that can only be traced back to your research clinic. Your personal information (name, address, phone number) will be protected by the research clinic. When researchers are given your stored samples to study they will not be given your personal information. The results of future tests will not be included in your health records.

We will do everything we can to protect your privacy. The tissue bank staff will continue to protect your personally identifiable health information as described in this consent form. Also, any publication of the research will not use your name or identify you personally.

What financial issues should I consider before allowing my samples to be stored?

You will not be charged for the storage of your samples. You will not be paid for allowing us to store your samples. If any new products, tests or discoveries that result from this research have potential commercial value, you will not share in any financial benefits. However, the study investigators will act in good faith to ensure that such products or discoveries resulting from this research benefit study participants and their communities.

What alternatives do I have?

If you choose not to allow us to store your samples for future research, your left over samples will be thrown away after you have completed your participation in the study.

What are my rights as a study participant?

Allowing your samples to be stored is completely voluntary. You may decide to not have any samples stored for future research and still be in this study or any future study.

If you decide now that your samples can be stored for future research, you may change your mind at any time. You must contact your study clinician and let them know that you do not want your samples used for future research. Your samples will then not be used and they will be discarded after you have completed your participation in the study.

What do I do if I have more questions?

For questions about the storage of your samples, contact the VivaGel™ Study Coordinator, Maria J. Oziemkowska (Mobile: 0733 436 418 or by e-mail: vivagel@kemri-ucsf.org).

For questions about your rights related to the storage of your samples for research, call the office of Dr C. Wasunna, the chairperson of the KEMRI Scientific Steering Committee, and the secretary of the KEMRI ERC (a group of people who review the research to protect your rights) at 020-272-2541, or 020-272-6781.

Your statement of consent and signature

Please carefully read the statements below and think about your choice. No matter what you decide, it will not affect your care, or your ability to participate in the VivaGel™ study:

I agree to have my left over blood samples stored and tested for future research:

_____	_____
Participant's name (print)	Participant's signature and date
_____	_____
Study staff conducting consent discussion (print)	Study staff signature and date
_____	_____
Witness' name	Witness's signature and date

Appendix 5: Reconstitution of reagents for HSV-2 detection

IgG Conjugate: One vial of affinity-purified and peroxidase-conjugated goat anti-human IgG (heavy chain specific). Contains protein, buffer and non-azide preservatives.

IgG High Positive Control and IgG Low Positive Control: 1 vial of human serum each. Contains non-azide preservatives.

Negative Control and IgG Cut-Off Calibrator: 1 vial of human serum each. Preserved with 0.1% sodium azide.

HerpeSelect® ELISA Sample Diluent: 1 vial of protein, surfactant, and non-azide preservatives in PBS.

10X Wash Buffer: 1 vial of surfactant in Phosphate buffered saline (PBS) with non-azide preservatives

Substrate Reagent: 1 vial of tetramethylbenzidine (TMB) and organic peroxide in buffer.

Stop Reagent: 1 vial 1 M sulfuric acid

Preparation of 1X Wash Buffer solution

To prepare a 1X Wash Buffer solution, 100 mL 10X Wash Buffer was mixed with 900 mL distilled water and the solution was swirled to mix.

Preparation of Specimen, Controls and Calibrator Preparation

Each specimen, control and calibrator was diluted to 1:100. 10 µL of specimen, control and calibrator was mixed with 1000 µL of sample diluents

Appendix 6: Reconstitution of ELISA reagents for HIV1/2 detection

Phosphate buffer: The phosphate buffer solution was prepared by diluting the concentrate with distilled water to a concentration of 1:25

TMB substrate: Contains tetramethylbenzidine in citric acid preserved in 1 g/l 2-chloroacetamide.

To prepare the TMB substrate, the required amount of TMB solution was combined with urea peroxide solution according to the number of wells being run (40 wells: 3m TMB solution + 3ml urea peroxide solution), the solution was then mixed well.

Sulfuric acid: To make a 1mol/l of diluted sulfuric acid 50ml concentrated sulfuric (18mol/l) acid was added slowly to 850 ml of distilled water while stirring

Negative control: Human serum non-reactive for anti-HIV and HIV antigen. This was ready to use as supplied

Anti-HIV-1 positive control: Human serum containing human monoclonal anti-HIV-2. This was ready to use as supplied.

Anti-HIV-2 positive control: Human serum containing murine monoclonal anti-HIV-2. Ready to use as supplied

Specimen diluent: Contains stabilizing protein and detergent. It is read to use as supplied

Appendix 7: Reconstitution of NAAT Reagents

Reconstituting the APTIMA Combo 2 Enzyme, Amplification, and Probe Reagents:

i) **Enzyme reagent:** Dried reverse transcriptase and RNA polymerase dried

Enzyme reconstitution solution: Buffered solution containing a surfactant and glycerol.

ii) **Amplification reagent:** Nucleic acids dried in buffered solution containing < 5% bulking agent

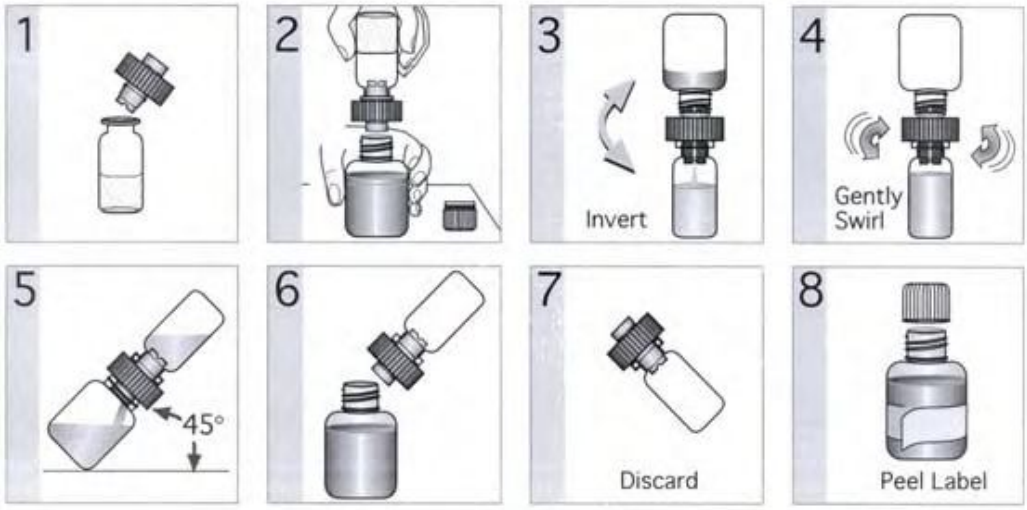
Amplification reconstitution solution: Aqueous solution containing preservatives.

iii) **Probe reagent:** Non-infectious chemiluminescent DNA probes

Probe reconstitution solution: Succinate buffered solution containing < 5% detergent.

The reconstitution solution was appropriately paired with the dried reagent. The labels have been color coded so the paired reagents have the same color bands. The dried reagent was opened and the notched end of the reconstitution collar was firmly inserted into the glass vial (figure 1). The reconstitution solution was opened and, while holding the solution bottle on the bench, the other end of the reconstitution collar was firmly inserted into the bottle (figure 2). The assembly was inverted, and the solution was to drain into the glass container (figure 3), and then swirled gently (figure 4). The assembly was again inverted and tilted at a 45° angle (figure 5), all of the liquid was allowed to drain back into the plastic bottle. The reconstitution collar

and the glass vial were removed as shown (figure 6) and discarded. The plastic bottle was recapped and labeled appropriately.



(Gen-Probe™ Aptima™ Combo 2 kit insert)

Preparation of TCR plus TCR-B:

The number of reactions to be performed (specimens plus controls) was determined.

The volumes of TCR and TCR-B reagents were calculated as follows

Volume of TCR (mL) = (number of reactions + 5 extra reactions) x 0.1 mL

Volume of TCR-B (mL) = Volume of TCR (mL)/100

TCR plus TCR-B Preparation (Example)

Number of Reactions	TCR	TCR-B
96 + 5	10.1 ml	0.1 ml