SEROPREVALENCE, PREDICTORS AND ESTIMATED INCIDENCE OF MATERNAL AND NEONATAL HERPES SIMPLEX VIRUS TYPE 2 IN WOMEN AGE 15-34 YEARS IN KILIFI, KENYA

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Seroprevalence, Predictors and Estimated Incidence of maternal and neonatal Herpes Simplex Virus Type 2 in women age 15-34 years in Kilifi, 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 thesis is dedicated to God almighty, coming this far has taken his grace, and in memory of my late grandfather Daniel Dzoro for instilling in me the value of hard work and persistence in life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
CCR5	C-C Chemokine receptor type 5.
CD4+ T	Cluster of Differentiation 4 of T helper cells.
DC-SIGN	Dendritic Cell Specific Intercellular adhesion molecule-3-Grabbing Non-integrin.
ELISA	Enzyme Linked Immunosorbent Assay.
ERC	Ethical Review Committee.
FDA	Food and Drug Administration.
GOK	Government of Kenya.
HIV	Hyper Immune Virus.
HSV-1	Herpes Simplex Virus type 1.
HSV-2	Herpes Simplex Virus type 2.
iDC	Immature Dendritic cells.
IgG	Immunoglobulin G.
JKUAT	Jomo Kenyatta University of Agriculture and Technology.
KEMRI	Kenya Medical Research Institute.
NASCOP	National AIDS and STD/STI Control Program.
STD	Sexual Transmitted Disease.
STI	Sexual Transmitted Infection.

ABSTRACT

Herpes Simplex Virus type 2 (HSV-2) has public health importance as a leading cause of genital ulcers, a co-factor in HIV-1 acquisition and transmission and as a cause of neonatal herpes infections. Little is known of the epidemiology and burden of disease in Coastal Kenya. The objective of this study was to describe the serological prevalence of HSV-2 infection, factors associated with infection and the potential risk for vertical transmission among women aged 15-34 years. Plasma samples of 826 women who participated in an HIV-1 survey in Kilifi in 2004 were screened for HSV-2 IgG antibodies using HerpeSelect ELISA. The sample comprised 563 women selected randomly from a demographic surveillance system (DSS) and 263 women who presented for voluntary counseling and testing (VCT). Predictors for HSV-2 seropositivity and HIV-1/HSV-2 co-infection were determined using multivariate logistic regression. The incidence of maternal HSV-2 infection and risk of neonatal herpes were estimated by a simple catalytic model fitted to age-seroprevalence data. The overall HSV-2 seroprevalence was 36% (296/826), and differed between DSS and VCT recruits (32% vs. 44%, P<0.001). The HIV-1 prevalence was 8% and 12% (P = 0.12) among the DSS and VCT recruits, respectively. Independent risk factors for HSV-2 infection in all women were: older age (30-34 years; odds ratio (OR) 10.5, 95% confidence interval (CI): 5.2 - 21.0), recruitment from VCT (OR 1.5, 95% CI: 1.1 - 2.1), history of genital ulcers (OR 1.7, 95% CI: 1.2 - 2.3) and HIV infection (OR 2.7,

95% CI: 1.6-4.6). Education beyond primary (OR 0.7, 95% CI: 0.5 - 0.9) was inversely associated with HSV-2 infection. Predictors for HSV-2/HIV co-infection were genital ulcers (OR 2.4, 95% CI: 1.4 – 4.9) and presence of other sexual transmitted infections (OR 2.8, 95% CI: 1.3 – 5.9). In the DSS sample, estimated HSV-2 incidence was 6 cases (95% CI: 5.3 – 6.8) per 100 women per year, 21 cases (95% CI: 20-22) per 1,000 pregnancies per year and 41 neonatal cases (95% CI: 39-42) per 100,000 births per year. In conclusion, HSV-2 prevalence in this population is similar to the national observed prevalence among women. HIV-1 is a strong predictor for HSV-2 infection. The rate of HSV-2 transmission is rapid following the onset of sexual activity. Nevertheless, the burden of neonatal HSV-2 can be predicted to be low.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Herpes Simplex Virus type 2 (HSV-2) infection among women of the general population is of considerable public health importance as a leading cause of genital ulcer disease(Corey & Handsfield, 2000; Gupta *et al.*, 2007), neonatal herpes infections (Brown *et al.*, 1991; Brown *et al.*, 1997; Brown *et al.*, 2003; O'Riordan *et al.*, 2006) and due to its role in enhancing HIV-1 acquisition and transmission (Corey & Handsfield, 2000; Gupta *et al.*, 2007; Abu-Raddad *et al.*, 2008; Bollen *et al.*, 2008).

Establishing the burden of HSV-2 ulcer disease can be difficult because incident cases and reactivations are often missed clinically (Brown *et al.*, 1991; Mertz *et al.*, 1992; Brown *et al.*, 2005; Schillinger *et al.*, 2008), and vertical transmission is a rare occurrence in populations with low HSV-2 seroprevalence (Mindel *et al.*, 2000; Poeran *et al.*, 2008; Corey & Wald, 2009). Most (80%) neonatal herpes infections arise from primary genital HSV-2 infection acquired late in pregnancy and can result in significant morbidity to the newborn child (Ades *et al.*, 1989; Brown *et al.*, 1991; Brown *et al.*, 1997; Brown *et al.*, 2003). In the USA, neonatal herpes incidence has been estimated as 1 case per 3200 live births in a study involving over 58000 live births born in the period 1982-1999 (Brown *et al.*, 2003). In Africa, there has been no information on the proportion of pregnant women who acquire HSV during

pregnancy or on the incidence or prevalence of neonatal HSV-2 infection (Mullick *et al.*, 2005).

According to the National AIDS and STI/STD Control Programme (NASCOP), Kenya lacks national health policies that emphasize the need to screen pregnant women for HSV-2 infection(Gok, 2006). While, in other well resource settings, maternal screening for HSV-2 during pregnancy and prevention of neonatal herpes infection by cesarean section and suppressive antiviral therapy is in practice even though not supported by any guidelines (Roberts, 2009). Besides neonatal herpes, maternal HSV-2 seropositivity has been found to increase the risk of perinatal HIV transmission (Bollen *et al.*, 2008).

Since most of HSV-2 transmission occurs in the absence of clinical symptoms,(Mertz *et al.*, 1992) the use of commercially available serological assays to detect the type-specific HSV surface glycoprotein-G (gG-2) would provide a marker to determine if an individual was previously infected (LeGoff *et al.*, 2008; Ngo *et al.*, 2008). In Western Kenya, an HIV survey among women aged 13-34 years conducted in 2003-4 revealed an HSV-2 prevalence of 53% (Amornkul *et al.*, 2009). While, a more recent National AIDS Indicator survey among general population of women aged 15 – 64 years estimated HSV-2 prevalence of 42% (KAIS, 2009). However, data on the possible impact of maternal transmission of HSV-2 were not provided in these surveys. Prevalence data however, allows for an estimation of HSV-2 incidence.

Hence, vertical transmission can be inferred (Ades *et al.*, 1989; Brown *et al.*, 2003; Caviness *et al.*, 2008).

In view of the above, this study sought to explore the prevalence of and risk factors associated with HSV-2 seropositivity. It also estimated rate of maternal infection and the potential risk of vertical transmission from within a well-enumerated population of semi-urban women from coastal Kenya, Kilifi. Information on HSV-2 burden in the general population could provide a reference tool for the hospital management team to educate mothers on the consequences of active HSV-2 infection to newborns and on to the underlying risk of sexual HIV-1 acquisition from an infected partner.

1.2 PROBLEM STATEMENT AND JUSTIFICATION

HSV-2 is a major cause of genital ulcer disease, neonatal herpes and one of the most prevalent sexually transmitted disease known to enhance spread of HIV globally. However, information on its burden among women of reproductive age has been lacking in coastal Kenya which is also one of the potential HIV-1 vaccine trial sites. This study assessed the prevalence and estimated the potential for vertical transmission through serological surveys for specific antibodies.

1.3 HYPOTHESIS

This was a descriptive study and did not test for any hypothesis.

1.4 OBJECTIVES

1.4.1 General Objective:

To determine serological prevalence, factors associated with HSV-2 infection and to estimate the potential underlying risk of vertical HSV-2 transmission among women aged 15-34 years old, participants of a 2004 HIV-1 survey in Kilifi, Kenya.

1.4.2 Specific Objectives:

1. To determine the serological prevalence of HSV-2 in women aged 15-34 years who participated in a 2004 HIV-1 sero-prevalence study in Kilifi.
2. To determine the socio-demographic characteristics of these women with HSV-2 infection.
3. To determine the correlation of HSV-2 and HIV-1 and identify predictors of co-infection.
4. To estimate maternal HSV-2 incidence and the potential risk of vertical transmission.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herpes Simplex Virus Type 2 Occurrence and Epidemiology

Herpes Simplex Virus type 2 is the main cause of genital ulcers globally and is one of the 3 most sexually transmitted diseases (Corey & Handsfield, 2000; Gupta *et al.*, 2007). The prevalence of antibodies to HSV-2 infections in the general population is estimated to range from 10-60% in both developed and developing countries (Gupta *et al.*, 2007). Most HSV-2 infections are acquired through sexual transmission but on rare occasions, vertical transmission occurs, which results to neonatal herpes. HSV-2 infection in adults is not a life threatening disease but has a great health effect to the general public. It enhances sexual and perinatal HIV acquisition and transmission (Bollen *et al.*, 2008), it causes psychosocial distress to immunocompetent people besides being a leading cause in genital ulcer disease and neonatal HSV-2 infections(Corey & Handsfield, 2000; Gupta *et al.*, 2007; Abu-Raddad *et al.*, 2008).

Herpes Simplex Virus type 2 is normally transmitted through close personal contact(Gupta *et al.*, 2007; Abu-Raddad *et al.*, 2008). In susceptible individuals, mucocutaneous infection follows inoculation of the virus into mucosal surfaces (oropharynx, cervix, conjunctiva) or through abraded or cracked skin. Signs of herpes tend to develop within 3-7 days of skin-to-skin contact with an infected person. Most

HSV-2 infections may manifest as blisters or lesions on the genitals (Gupta *et al.*, 2007).

Other signs of HSV-2 infection observed in people particularly with the first episode include fever, muscle aches, headaches, vaginal discharge or painful urination, swollen and tender lymph glands in the groin. Recurrent episodes have much less severe symptoms.

Reactivation of HSV-2 infection presents as pain or a tingling sensation in the area of the infection without any lesions. The sensation is a result of irritation and inflammation of the nerves leading to the infected area of skin and it is during this period when an infected person is highly contagious (Mertz *et al.*, 1992; Corey & Handsfield, 2000; Gupta *et al.*, 2007; Abu-Raddad *et al.*, 2008; Bollen *et al.*, 2008). Gupta *et. al* 2007, observed that the subclinical reactivation of HSV-2 accounted for a large proportion of total HSV shedding (Gupta *et al.*, 2004). Whereas, Mertz *et. al* 1992, in a study for risk factors for HSV-2 transmission in heterosexual partners, found that, in 70% of patients, transmission appeared to result from sexual contact during periods of asymptomatic viral shedding (Mertz *et al.*, 1992). In Mertz study, the risk for acquisition of HSV-2 was observed to be higher in women than men(Mertz *et al.*, 1992).

Herpes Simplex Virus type 2 is ubiquitous, it affects both urban and rural populations worldwide. Most HSV-2 infection frequently occurs in the absence of overt symptoms

and hence an assessment of infection prevalence is best undertaken through serological surveys of specific antibodies (Corey *et al.*, 2004; Gupta *et al.*, 2007).

The serological prevalence of HSV-2 infection in the United States is estimated to be 22% in the general population while in Europe, it is about 16% (Corey *et al.*, 2004). Japan and Philipines have the least HSV-2 sero-prevalence in women, about 7% and 9% respectively (Corey *et al.*, 2004). However, the prevalence varies between different selected populations. For instance, STD clinic attendees in Peru, have HSV-2 prevalence of 83% while blood donors in London UK have showed a sero-prevalence of 8%(Corey *et al.*, 2004). HSV-2 prevalence is less common in pregnant women in Australia(12.5%) (Mindel *et al.*, 2000) than in the United States (22%) (Xu *et al.*, 2007).

Studies on the African continent show a wide variation in HSV-2 prevalence across diverse populations of women, ranging from 22% among adults in Tanzania, 68% urban adults in Kenya to 90% among Commercial sex workers in Zaire (Weiss *et al.*, 2001; Corey *et al.*, 2004). A recent survey for AIDS Indicators in Kenya revealed HSV-2 sero-prevalence among women in the general population to be about 42.3% (KAIS, 2009) .

The variation in distribution of HSV-2 acquisition and sero-prevalence in different communities have been attributed to the sexual behavior of that community (Mindel *et*

al., 2000). This has frequently raised concern especially on HSV-2 prevention strategies. Some studies have suggested that, prevention strategies need to be different for different communities depending on the HSV-2 serological prevalence (Mindel *et al.,* 2000).

Based on the reviews of prevalent studies, the data is still scanty among pregnant women from the general population in Sub Saharan Africa.

2.1.1 Herpes Simplex Virus type 2 Pathogenesis

Understanding the pathogenesis of HSV-2 infection will be an important step towards effective diagnosis, treatment and formulation of prophylactic vaccines for preventive strategies.

At initial infection, after personal contact with an infected person, HSV-2 enters the body through skin or mucosal surfaces and initiates cytolytic replication in epithelial cells at the site of entry. Virions within epithelial cells are seen histologically as intranuclear inclusions, and HSV-2 induces cells to fuse and form multinucleated giant cells (Gupta *et al.,* 2007).

Cell damage in the skin causes epithelial cells to detach and form fluid-filled blisters containing cellular debris, inflammatory cells, and progeny virions. An inflammatory response takes place as HSV-2 penetrates through the dermis and enters the ends of

peripheral sensory nerves innervating the infected cells (Gupta *et al.*, 2007). The nucleocapsid, containing the double-stranded DNA genome, is taken up by the sensory nerve axons and transported in a retrograde manner to the neural soma in the sensory nerve root ganglia. Some viral replication continues in the neural tissue. The virus also spreads via the lymphatic system to the local and regional lymph nodes. In immunocompetent people, the spread of the virus is restricted by humoral and cell-mediated immunity. However, in rare cases, cutaneous or visceral dissemination occurs, which often has a fatal outcome (Gupta *et al.*, 2007).

Neuronal HSV-2 infection is not thought to lead to cell death. Instead, HSV-2 enters a latent state in the sensory ganglion, where it can persist for the life of the infected person. The HSV-2 genome is maintained in unintegrated latent state, with expression of a few proteins but without active replication or cytotoxicity. The mechanism for the establishment and maintenance of latency is not yet fully understood. The virus's ability to evade recognition by the host's immune system is facilitated by latency in immune-privileged sites and by active down regulation of host immune responses by viral proteins (Jean *et al.*, 2001; Gupta *et al.*, 2007).

Studies suggest that cytotoxic T cells have a role in maintaining latency by active surveillance (van Lint *et al.*, 2004). All people infected with HSV-2 presumably have latent virus in nerve ganglia, whether or not the initial infection was symptomatic. Recurrent episodes take place when HSV-2 reactivates in neurons with latent infection

and is transported in the peripheral nerves back to the mucosal or skin surface where it exits at the nerve ending of any branch of the axon and cause lesions. Specific conditions trigger reactivation and replication of latent HSV-2, including local trauma (eg, surgery or UV light) and systemic stimuli (eg, immunosuppression or fever). In most cases, however, no precipitating factors can be identified, and the triggers are poorly understood (van Lint *et al.*, 2004; Gupta *et al.*, 2007).

Reactivation of HSV-2 can result in clinically evident disruption of mucosa (i.e, recurrences accompanied by viral shedding), or viral shedding might occur in the absence of clinically recognised symptoms. People who are sero-positive for HSV-2 antibodies shed HSV-2 intermittently, and both sexual and vertical transmission of HSV-2, usually occur during periods of subclinical shedding (Mertz *et al.*, 1992; Gupta *et al.*, 2004).The frequency of clinical and subclinical viral reactivation varies widely depending on host factors.

The immunogenetic determinants of disease severity are unknown but even among otherwise healthy people, disease activity varies from asymptomatic to life-threatening, with most people having mild disease. Cell-mediated immunity seems the most important process for control of viral replication (Jean *et al.*, 2001). Previous infection with HSV-1 could offer a small margin of protection against HSV-2 infection; conversely HSV-2 infection seems to protect against HSV-1 acquisition(Gupta *et al.*, 2007).

2.2 Diagnosis and Detection of HSV-2 Infection

2.2.1 Diagnosis of HSV-2 Infection

In the absence of clinical symptoms, screening for antibodies specific to HSV-2 would provide an objective measure of HSV-2 infection. HSV-2 serology can provide a marker of the extent of polypartnerism, early age sexual debut, acquisition rates of genital herpes and as an indicator in HIV-1 transmission studies (Laeyendecker *et al.*, 2004; Ngo *et al.*, 2008).

Herpes Simplex Virus Type-2 belong to the family *Herpesviridae,* which are large double-stranded DNA viruses. HSV-2 and HSV-1 share 83% of their protein coding and their genome structures are alike but can be distinguished serologically using IgG type specific tests (Ades *et al.*, 1989; Brown *et al.*, 2007; Gupta *et al.*, 2007). HSV-2 infection can also be diagnosed by virus culture and DNA amplification methods, but these diagnostic techniques are complex, very expensive, and not suited for settings with limited resources or for use in large-scale epidemiological studies (van Dyck *et al.*, 2004; Gamiel *et al.*, 2008).

Currently, three HSV IgG test kits for the detection of specific antibodies against HSV-2 and approved by the United States Food and Drug Administration (FDA) are commercially available. These kits are the HerpeSelect HSV-2 ELISA (Focus Technologies), Kalon HSV-2 ELISA (Kalon Biologicals Ltd) and Biokit HSV-2 (Biokit) (Gamiel *et al.*, 2008; Ngo *et al.*, 2008; Ngo *et al.*, 2008). HerpeSelect and

Kalon enzyme-linked immunosorbent assays (ELISAs) have shown high sensitivities (93% to 100%) and specificities (95% to 100%) in comparison to various "gold standards" with serum samples from western populations. A rapid membrane assay, the Biokit assay, was developed as a point-of-care test specific for HSV-2 antibodies and showed premarket evaluations of 96% sensitivity and 98% specificity (Ngo *et al.*, 2008).

There has been debate concerning the performance of the IgG type specific tests on serum samples from African populations and populations with high HIV-1 sero-prevalence (Laeyendecker *et al.*, 2004; van Dyck *et al.*, 2004; Gamiel *et al.*, 2008; Ngo *et al.*, 2008; Ngo *et al.*, 2008). Nevertheless, most validation studies have recommended the Kalon kit as appropriate for use in sera from Sub Saharan Africa (Laeyendecker *et al.*, 2004; van Dyck *et al.*, 2004; Gamiel *et al.*, 2008; LeGoff *et al.*, 2008). This study used the HerpeSelect kit even though it has a low specificity. The reason being, the sera used was from the general population, for a seroepidemiological survey and not from STD clinics or people diagnosed with genital herpes. The Kalon kit has shown to be very insensitive for the detection of antibody to early HSV-2 infection. Morrow *et.al* 2003, documented the median times for detecting sero-conversion as,120 days by the kalon assay, 21 days by Focus assay and 68 days by western blotting assay.

As described by Laeyandecker, in his assessment of the Kalon and Focus HSV-2 IgG ELISA test kits, the performance of Focus Kit can be optimized by using higher cut off values than recommended by the manufacturer (Laeyendecker *et al.*, 2004). For instance, a cut off value of >3.4 would yield a sensitivity of 84.9% and a specificity of 84.6% and reduce the probability of false positive to 8%. The index cut off value of >3.4 is also recommended for use if the test is for epidemiologic research in which it is not being used for diagnostic purposes and thus the sensitivity need not be optimized at the cost of specificity (Laeyendecker *et al.*, 2004). The manufacturer's cut off index value of 1.1 can be used to maximize sensitivity (Laeyendecker *et al.*, 2004; van Dyck *et al.*, 2004; Gamiel *et al.*, 2008).

2.2.2 Determinants of HSV-2 Infection

Herpes Simplex Virus type 2 serology represents a biological marker for risky sexual behavior. In a prevalence study of male factory workers in Zimbabwe, HSV-2 infection was associated with marital status, history of STD, older age and higher income (McFarland *et al.*, 1999). A plausible explanation for association of higher income with increased HSV-2 infection was that money may afford increased access to sexual partners, particularly prostitutes, to a greater extent this urban working population in Sub-Saharan Africa (McFarland *et al.*, 1999). In a study done in the United States low income among women was associated with increased HSV-2 prevalence (Breining *et al.*, 1990). Other studies have found HSV-2 to be associated

with age, HIV sero-status, perception about partner's STI status and circumcision, lack of education, number of sexual partners and ethnicity (Hunter *et al.*, 1994; Chawla *et al.*, 2008; Kramer *et al.*, 2008; Ng'ayo *et al.*, 2008).

2.3 Public Health Effect of HSV-2 Infection

2.3.1 Neonatal Herpes

Herpes Simplex Virus type 2 accounts for about 80% of neonatal infections (O'Riordan *et al.*, 2006). Almost all neonate HSV-2 infections are acquired by passage through an infected birth canal. Most mothers who transmit HSV-2 to their children are asymptomatic at delivery and transmission rates are much higher when the mother is experiencing a primary or initial genital infection (> 50%) versus a recurrent infection (< 5%), and where the mother is not yet producing IgG antibodies to HSV-2 (Ades *et al.*, 1989; Gupta *et al.*, 2004; Mullick *et al.*, 2005; O'Riordan *et al.*, 2006). It is estimated that about 1 child in every 3000 live births in the United States is diagnosed with neonatal herpes (Mindel *et al.*, 2000; Rudnick & Hoekzema, 2002).

Neonatal herpes is rare but a condition with high mortality and morbidity (Mindel *et al.*, 2000; O'Riordan *et al.*, 2006). Without therapy; mortality for untreated infants who develop disseminated infection exceeds 70% with half of the survivors developing neurological impairment (O'Riordan *et al.*, 2006). Ades *et al.*, 1989, in his

study to determine the prevalence of herpes simplex antibodies in pregnant women from several antenatal clinics in the United Kingdom observed that sero-prevalence of HSV-1 in black pregnant women born in Africa or Caribbean was nearly 100%, while Brown *et al.,* 2007, established that the predictors of neonatal herpes outcome were maternal HSV-2 sero-positivity and HSV-2 shedding at genital tract during delivery (Ades *et al.*, 1989; Brown *et al.,* 2007). However, most of the HSV-2 neonatal data available, is from studies conducted within the western populations, and therefore few data exists on the proportion of women in developing countries who acquire HSV-2 during pregnancy or on the incidence or prevalence of neonatal HSV-2 (Mullick *et al.*, 2005).

Many studies have put an emphasis on reducing the risk of neonatal herpes especially in areas where HSV-2 sero-prevalence is high (Mindel *et al.,* 2000; Duran *et al.*, 2004; Brown *et al.*, 2007; Xu *et al.,* 2007). Screening for antibodies specific to HSV-2 will be an important tool to identify women of reproductive age with asymptomatic or sub clinical genital HSV-2 infection and those susceptible to primary genital HSV-2 infection(Ades *et al.,* 1989; Mindel *et al.,* 2000; Rudnick & Hoekzema, 2002).

2.3.2 Role of HSV-2 infection in HIV-1 Transmission and Acquisition

Herpes Simplex Virus type 2 is an important co-factor in the global HIV pandemic (Weiss *et al.*, 2001; Serwadda *et al.*, 2003; Barbour *et al.*, 2007; Rebbapragada *et al.*, 2007; Watson-Jones *et al.*, 2007; Abu-Raddad *et al.*, 2008; Bollen *et al.*, 2008). Nearly

80-95% of HIV infected individuals in Africa are sero-positive for HSV-2 (Weiss *et al.,* 2001; Rebbapragada *et al.,* 2007). HSV-2 infection increases the risk of HIV acquisition or transmission of HIV, when co-infected with HSV-2 by two or 3 fold (Rebbapragada *et al.,* 2007; Watson-Jones *et al.,* 2007). It is also believed that, HSV-2 sero-prevalence is on the increase in women in Sub Saharan Africa as a result of its synergistic relationship with HIV-1. However, scanty data due to lack of screening for HSV-2 infection in asymptomatic individuals, limits the evidence to support this assertion.

Reppabragada and group in 2007, in a study among commercial sex workers in Pumwani Kenya, were able to demonstrate the mucosal immune interactions between HIV-1 and HSV-2 in the female genital tract (Rebbapragada *et al.,* 2007). They observed that, there exists a negative mucosal synergy between HIV-1 and HSV-2. In HIV infected females HSV-2 infection was associated with a ten fold increase in cervical immature dendritic cells (iDC) expressing DC-SIGN and 3-fold increase in cervical CD4+ T cells expressing CCR5. The findings suggest a mucosal vicious circle in which HSV-2 infection increases HIV target cells in the genital mucosa, subsequent HIV infection impairs HSV-2 mucosal immune control and local HSV-2 reactivation enhances both HSV-2 and HIV transmission (Rebbapragada *et al.*, 2007). Similar findings were observed in another study at a family planning clinic at Coast General Hospital, Mombasa, Kenya (Scott *et al.*, 2002).

Other observational studies also found a strong association between HSV-2 infection and HIV incidence. HSV-2 accounts for about 63% of new HIV infections (Watson-Jones *et al.*, 2007). A study of HIV sero-prevalence survey by Weiss, in four major cities in Africa (Kisumu, Yaunde, Benin and Ndola) found that HSV-2 was highly prevalent in women (over 50%) even at young ages and was strongly associated with HIV at an individual level (Weiss *et al.*, 2001). From these findings Abu-Raddad *et al* 2008, designed a mathematical model to look at the dynamics of HSV-2 and HIV-1 interaction. He observed that in cities with low HSV-2 sero-prevalence, HIV is concentrated in higher risk groups whereas cities with high HSV-2 sero-prevalence, HIV transmission is observed in large fraction of the general population. His results suggest a more substantial role for HSV-2 in fueling HIV spread in Sub Saharan Africa than other sexually transmitted infections (Abu-Raddad *et al.*, 2008).

A nested case control study in Rakai, Uganda observed the odds ratio of HSV-2 sero-positivity associated with HIV acquisition was 1.7. HIV load was increased in HSV-2 sero-positive case subjects compared with that in HSV-2 sero-negative subjects at 5 months after sero-conversion. Results explain that, HSV-2 sero-positivity is associated with HIV acquisition and HIV infection exacerbate HSV-2 infection increasing the frequency and persistence of herpetic ulceration (Serwadda *et al.*, 2003).

In a zidovudine / placebo randomized controlled trial, among 307 HIV-positive women, 228 (74.3%) were HSV-2 sero-positive and 24 (7.8%) were shedding HSV-2.

Herpes simplex virus type 2 sero-positivity was associated with overall perinatal HIV transmission [adjusted odds ratio, 2.6; 95% confidence interval, 1.0–6.7)], and HSV-2 shedding was associated with intrapartum transmission independent of plasma and cervicovaginal HIV viral load, and zidovudine treatment. These results show evidence of an increased risk of perinatal HIV transmission among HSV-2 sero-positive women and an increased risk of intrapartum HIV transmission among women shedding HSV-2 (Bollen *et al.*, 2008).

Despite the epidemiological evidence of the strong association between HIV-1 and HSV-2, clinical trials to assess if HSV-2 suppression would reduce HIV acquisition have not been very successful (Baeten *et al.*, 2008; Celum *et al.*, 2008; Gray & Wawer, 2008; Watson-Jones, 2008).

2.3.3 Psychosocial Distress and Stigma

Regardless of severity of symptoms, genital herpes frequently causes psychological distress in people who know they are infected (Melville *et al.*, 2003; Rosenthal *et al.*, 2006; Gupta *et al.*, 2007). Numerous qualitative studies to assess emotional and psychosocial impact of serological diagnosis of HSV-2 in asymptomatic individuals revealed discomforts such as fear of telling future partner, feeling socially stigmatized, feeling sexual undesirable, sex avoidance to social responsibility, fear of

transmitting to a new born and relationship concerns relating to the diagnosis (Melville *et al.*, 2003; Miyai *et al.*, 2004; Meyer *et al.*, 2005; Rosenthal *et al.*, 2006).

Despite the concern for psychological burden, this should not deter serological testing for HSV-2 given the epidemiological evidence of HSV-2 and HIV-1 and the fact that 80% of HSV-2 infections are unrecognized or asymptomatic (Meyer *et al.*, 2005). Many of the negative responses may be time limited if pretest and post test counseling is included as part of the diagnosis (Melville *et al.*, 2003; Miyai *et al.*, 2004; Rosenthal *et al.*, 2006).

2.4 HSV-2 Prevention and Control Strategies

Current management of genital herpes is by use of antiviral therapy such as acyclovir, valaciclovir and famciclovir mainly aimed to abating signs and symptoms of HSV-2 such as lesion healing, decreased viral shedding and prevention of new lesions (Gupta *et al.*, 2007). Prevention strategies for HSV-2 sexual transmission in symptomatic patients include condom use and antiviral therapy (Gupta *et al.*, 2007).

Prevention of neonatal herpes has focused on prevention of HSV-2 acquisition among women during pregnancy and suppression of genital herpes viral shedding during delivery (Gupta *et al.*, 2004). Acyclovir and Valaciclovir have both been found to be very effective in prevention of perinatal HSV-2 transmission (Gupta *et al.*, 2004). Pregnant women at risk of HSV-2 acquisition can be counseled to avoid unprotected

oral-genital contact during the last trimester of pregnancy (Gupta *et al.*, 2007). Antiviral therapy is also recommended for infants who show signs of HSV-2 infection (O'Riordan *et al.*, 2006).

Prevention of HIV-1 transmission or acquisition due to HSV-2 infection is based on the hypothesis that HSV-2 suppressive treatment may reduce HIV-1 replication. Clinical trials to suppress HSV-2 and reduce HIV-1 transmission have been conducted (Baeten *et al.*, 2008; Celum *et al.*, 2008; Gray & Wawer, 2008; Watson-Jones, 2008). Suppressive therapy using acyclovir 400mg twice daily on HSV-2 sero-positive women and men to prevent HIV-1 acquisition did not show any significant decrease in HIV-1 incidence between the placebo and acyclovir group (Celum *et al.*, 2008; Watson-Jones, 2008). On another randomized cross-over trial of HSV-2 suppressive therapy with valacyclovir, 500mg twice daily on HSV-2/HIV-1 co-infected women (Baeten *et al.*, 2008), showed a significant reduction of Plasma HIV-1 level was observed in the valacyclovir arm compared to placebo. The results giving hope that suppressive HSV-2 therapy has the potential to reduce HIV-1 infectiousness and slow HIV-1 disease progression. Although, results of these trials are still unclear, a phase III randomized placebo-controlled trial of HSV-2 suppression to prevent HIV-1 transmission among HIV-discordant couples was recently concluded. This trial will directly answer the extent to which HSV-2 infection increases infectiousness of HIV-1/HSV-2 co-infected persons and the relative reduction in HIV-1 transmission among HSV-2 sero-positive persons treated with daily suppressive antiviral therapy.

The biggest challenge in HSV-2 prevention in women especially pregnant women in Kenya is lack of health policies that emphasize need for screening of asymptomatic pregnant women for HSV-2 infection(Gok, 2006). Similarly, most of the policies in place have not been well integrated into the health care system for use by healthcare providers due to poor reporting system of disease prevalence. Ethical issues about screening of asymptomatic individuals and cost effectiveness of the HSV-2 test contribute highly the lack of data on HSV-2 prevalence (Gupta *et al.*, 2007). Some studies on HSV-2 prevalence recommend that the same preventive measures used in HIV infection be applied for prevention of HSV-2 since the risk factors are similar for both infections (Ng'ayo *et al.*, 2008).

2.5 Development of Vaccines to HSV-2

Prophylactic vaccines for HSV-2 would give a broad and durable effective immunity across all mucosal surfaces (Stanberry, 2004). Several protein subunit vaccines based on HSV-2 envelope glycoproteins have reached advanced-phase clinical trials. The glycoprotein antigens are the targets of neutralizing-antibody responses because they elicit cellular immunity (Koelle & Corey, 2003). A candidate ICP10DeltaPK vaccine has shown to prevent recurrence of disease in 44% of treated subjects and reduces the frequency and severity of recurrences in the subjects that are not fully protected (Aurelian, 2004).

Most HSV-2 vaccine trial studies have shown induced HSV- specific antibody responses alone but failed to protect recipients from recurrences (Straus *et al.*, 1997; Stanberry, 2004). For instance, a study to test HSV-2 vaccine consisting of recombinant glycoprotein-D with a new adjuvant showed efficacy of 39–46% for prevention of HSV-2 infection in the subgroup of women who were sero-negative for both HSV-1 and HSV-2 but could not provide protection from HSV-2 in men or in women sero-positive for HSV-1 (Straus *et al.*, 1997).

Vaccines would still be useful if they could increase the threshold of infection, or prevent clinical disease. However, this has become a significant challenge, as the major determinants of effective immunity have not yet been identified (Stanberry, 2004). As a result, the development of effective prophylactic and therapeutic vaccines against genital herpes has not been very successful (Aurelian, 2004). The difficulties are associated with the complexity of the virus life cycle (latency) and our relatively poor understanding of the mechanism of immune control of primary and recurrent disease (Aurelian, 2004).

It will be important to conduct more research to evaluate the impact of vaccination on HSV-2 infection, clinically apparent genital herpes, and HSV shedding among vaccine recipients who acquire infection (Koelle & Corey, 2003) and to define determinants of immunity to HSV-2, including identifying HSV-2 antigens, in order to design more effective vaccines (Stanberry, 2004).

Through the Center for Disease Control and Prevention's Kenya AIDS Indicator Survey 2007 (KAIS, 2009), and this study, for the first time, HSV-2 data among women of the general population is provided at an aggregate level.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 STUDY SITE

3.1.1 Study area

The enrollment of study participants and data collection for the parent study (HIV-1 survey) took place at Kilifi District, within Kilifi Demographic Surveillance System area specifically Kilifi township and Mtondia on the Kenyan Coast. All HSV-2 assays and data analysis were carried out at KEMRI Center for Geographical Medicine Research – Coast.

Kilifi District is a largely rural district in Coastal Kenya, with an estimated population of 544,305 people (52% female) (Gok, 2002). In the year 2000, the Kenya Medical Research Institute (KEMRI) - Wellcome Trust Research Programme defined and mapped a geographical area in Kilifi District for demographic surveillance and clinical and epidemiological research. The resultant Kilifi Demographic Surveillance System (DSS) monitors a population of around 240,000 residents (Bauni *et al.*, 2007). Kilifi District has a birth rate of approximately 8,000 live births per year, perinatal mortality of 44 per 1000 live births (Bauni *et al.*, 2007) and about 4,000 women attend antenatal care at the district hospital per year (Bauni *et al.*, 2007). The reproductive age of women at Kilifi is between 15-44years, with a crude birth rate of 49.2 per 1000. The

total fertility rate is 6, with literacy level of the female about 35% with majority having less than secondary education(Gok, 2002). Until 2003, in Kilifi District, pregnant women were not routinely tested for HIV-1, and VCT services were not widely available. In 2004, a large HIV-1 survey was conducted at several semi-urban communities in and around Kilifi town and VCT services were strengthened.

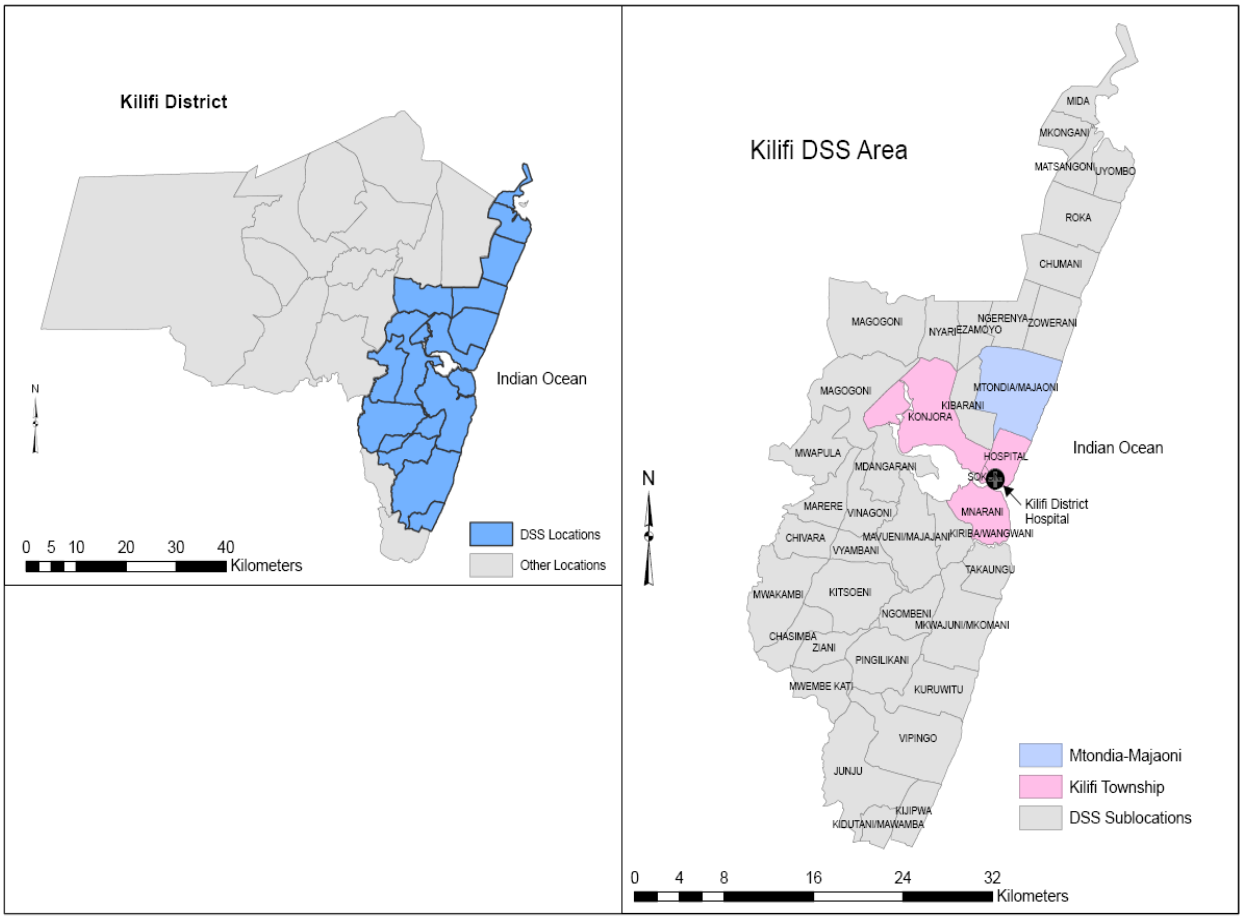

Figure 3.1: Map of Kilifi District Demographic Surveillance System Area.

3.1.2 Study population

The study used serum samples of women aged 15-34 years, from a cohort of volunteers of the HIV-1 survey conducted in 2004. These participants were recruited

on the basis of their willingness to know their HIV status or by self presentation to access VCT services.

3.1.3 Inclusion criteria

Data and serum samples of all women volunteers previously consented during HIV-1 survey, aged 15-34 years from Mtondia and Kilifi township.

3.1.4 Exclusion criteria

Subjects with either missing serum samples or missing HIV-1 survey data.

3.1.5 Study design

This was a descriptive study which used stored serum samples and data of women from a 2004 HIV-1 cross-sectional survey.

3.2 SAMPLING PROCEDURE

This study used archived serum samples and socio-demographic data retrieved from the 2004 HIV-1 survey, which was conducted in Kilifi by the Kenya Medical Research Institute, a study that was financially supported by IAVI (KEMRI SCC #820) (Appendix IV).

During recruitment into the HIV-1 survey, all volunteers were interviewed, counseled and educated about the study prior to enrolment. Acceptance of HIV-1 testing was an enrolment criterion. All volunteers gave informed consent for storage of their blood

samples and for more testing of those samples on other infections related to HIV. A survey questionnaire (Appendix III) was administered through one on one interview for collection of data on socio-demographic characteristics, sexual exposure, medical history and knowledge of sexually transmitted disease (STD) / HIV-1 related infections. A medical history that focused on the past or present STDs was obtained. Medical care for sexually transmitted diseases was provided. Volunteers received pre- and post-HIV test counselling, and were tested for HIV-1 at the enrolment visit using two rapid test kits (Determine, Abbott Laboratories, Abbott Park, Illinois, USA; Unigold, Trinity Biotech plc, Bray, Ireland) in parallel. Discrepant rapid HIV-1 test results were resolved using an ELISA test (Genetic System HIV-1/2 plus O EIA, Bio-Rad Laboratories, Redmond, Washington, USA). Women who tested HIV-1 positive were referred for Comprehensive HIV care including Prevention of Mother to Child Transmission (PMCTC) services, as appropriate, at the District Hospital.

After testing for HIV-1, serum samples were stored for other tests related to HIV-1 infection.

3.2.1 Study sample size calculation

The study group of the HIV-1 survey comprised of women aged 15-34 years, 580 who were randomly selected from the Demographic Surveillance Study area of Kilifi

township and Mtondia, and 270 who self presented to access VCT services. A total of 850 women were available from the HIV-1 survey for the HSV-2 study.

HSV-2 sero-prevalence was estimated based on a prevalence obtained during the 2007 Kenya Aids Indicator Survey of 42.3% (KAIS, 2009)among all women in the general population (Fisher's formula for a single proportion). With the available sample-size of 850 subjects and a sero-prevalence of 42.3%, using the binomial formula for the 95% confidence interval on a simple proportion [Mean +/- 1.96 * SQRT(p*(1-p)/N)] the 95% confidence interval of prevalence of HSV-2 in this population was estimated with a width of 6.6 percentage points(Fisher's formula).

Given a sample size of 850, the logistic regression of $\beta=0$ ($\alpha = 0.050$ two sided) would have 80% power to detect a β of 0.203 (an odds ratio of 1.225); this assumes only one normally distributed covariate x in the model, and the proportion at the covariate mean is 0.423.

3.3 MEASUREMENTS

3.3.1 Data collection on determinants for HSV-2 infection

Available data collected during the HIV-1 sero-prevalence survey was used to assess risk factors for HSV-2 infection, determine associations and identify predictors of HIV/HSV-2 co-infection. This included all data in questionnaires and study forms on

medical history, risk assessment and other examinations done during the HIV-1 study. (Appendix III)

3.3.2 Specimen collection

This study used archived serum samples of HIV-1 survey participants. Consent to use this blood was obtained from participants during the 2004 HIV-1 sero-prevalence survey at enrollment. The serum samples were stored frozen at -70ºC from 2004 until HSV-2 assays were carried out in 2009. During the HIV-1 survey (in 2004), all serum samples were obtained from the study volunteers through the standard procedures of obtaining venepuncture blood. Approximately 5-6 mls of blood were collected at the enrolment visit according to Schedule of Procedures. The blood collected was allowed to settle and then centrifuged at 3000 revolutions per minute for 5 minutes. The suspension was aspirated out and aliquoted into 1.5mls cryovials for storage and for HIV testing. After HIV testing, about 3ml of serum sample was stored frozen at KEMRI/Wellcome Trust laboratory for identification and sequencing of circulating viruses and other tests related to HIV.

3.3.3 Laboratory HSV-2 Serological Assays

Testing for HSV-2 was done by an ELISA method using HerpeSelect kit (Focus Technologies, Cypress, California, USA). This test uses Glycoprotein G protein, which is an envelope protein from the virion and contains predominantly type-specific

epitopes unique to HSV-2. Antibodies to gG2 are used as markers of infection for the virus (Diagnostics, 2007).

The principle of the HSV-2 HERPESELECT ELISA assay was based on a sandwich principle where the antigen (Glycoprotein G protein) was precoated on the microtittre plates. HSV-2 IgG antibodies in serum reacted with HSV-2 antigens immobilized on the ELISA plate to form Ag/Ab complexes. These complexes were acted upon by anti - human IgG (secondary antibodies) conjugated to HRP enzyme which upon the addition of TMB substrate formed color, intensity of which was proportional to the amount of antibodies present. OD of the colour formed was measured spectrophotometrically using an ELISA plate reader at 450nm.

Validation of this test has shown it to have a sensitivity of 88-96% and specificity of 63-81% with sera from Sub-Saharan Africa (Morrow *et al.*, 2003; van Dyck *et al.*, 2004; Gamiel *et al.*, 2008).

3.3.4 Procedure of the Assay

The procedure for testing of HSV-2 specific antibody status was conducted according to the manufacturer's protocol (Appendix I) with the following exception. The protocol recommends a cut off index value of 1.1 to determine HSV-2 infection (Appendix I). Due to a lower specificity of the HerpeSelect test on serum samples

from African populations (Morrow *et al.*, 2003; Ashley-Morrow *et al.*, 2004; LeGoff *et al.*, 2008), a cut off value of > 3.5 was used to determine HSV-2 prevalence. A lower cut off value of 1.5 was used to estimate HSV-2 incidence (Appendix 2).

3.3.5 Laboratory Quality control

An independent laboratory technologist conducted a retest of 2.5% of all serum samples for quality control and assurance check. This retest included all samples with intermediate HSV-2 sero-status (index values between 0.9-1.1). The results were compared with those of the first run and a final result status considered as mean of the indexes.

3.4. DATA MANAGEMENT

3.4.1 Data storage

All study participants' information collected in the HIV-1 sero-prevalence study was retrieved from the 2004 HIV-1 survey database by the principal investigator and verified. The HSV-2 Laboratory data was entered into excel spreadsheets, verified, uploaded to STATA and merged with the HIV-1 survey data. Statistical analysis was conducted using STATA version 10 (StataCorp, College Station, Texas, USA). All study records were stored at KEMRI after the study and only investigators in this study are allowed to have access to this information.

3.4.2 Data analysis

3.4.2.1 Risk factor analysis

All risk factor analyses was conducted using STATA version 10 (StataCorp, College Station, Texas, USA). Chi-squared and Fisher's exact tests were used to determine univariate associations with prevalent HSV-2 infection. Multivariate logistic regression was used to identify independent predictors of HSV-2 seropositive status and HIV-1/HSV-2 co-infection. Variables were introduced sequentially into a multivariate model, beginning with those with the strongest univariate association (lowest P values) and including only those which provided a significantly improved fit to the data (a likelihood ratio test, LRT, $P<0.05$). The correlation coefficient between two variables was used to determine whether to exclude a variable from analysis due to excessive colinearity (i.e., $r^2 > 0.2$).

The risk factors assessed included socio-demographic characteristics (age, marital status, occupation, education level, residence, religion and ethnicity) sexual exposure (number of sexual partners, sexual debut, condom use), medical history (presence of genital ulcer disease or discharge and HIV) and knowledge of STD/HIV related infections. The expected outcome for this study was HSV-2 sero-prevalence and HSV-2/HIV-1 co-infection, maternal and neonatal HSV-2 incidence.

3.4.2.2 Incidence of maternal and neonatal HSV-2 infection

The incidence of maternal HSV-2 infection and risk of neonatal herpes were estimated using a simple catalytic model as described by (Cutts & Vynncky, 1999) and (Ades *et al.*, 1989). In this model, the force of infection (i.e. per person incidence) was calculated by fitting age-stratified prevalence of specific antibodies to an exponential decay model by maximum likelihood. An assumption was made that seronegative status equates to the absence of HSV-2 infection (past or present), and that following infection there was no reversion to seronegativity. In other words it was assumed that, the seronegative status is equated with being susceptible to infection. It was further assumed that all women were seronegative at age 14 years and thereafter exposed to a force of infection (per year), λ, constant across all ages and unchanging over time (where $\lambda>0$). Hence the proportion susceptible, $s(a)$, at age a was defined as

$$s(a) = \exp\left[-\lambda(a-14)\right] \qquad (1),$$

where $14<a<35$. The proportion seropositive at age a, $p(a)$, was therefore $1-s(a)$. This model was fitted by maximum likelihood to the observed proportions remaining seronegative, $S(i)$, representing $r(i)$ seronegative individuals out of $n(i)$ sampled for each single year of volunteer age, i=15-34, to identify the value of the force of

infection best supported by the data and estimate the 95% confidence intervals (Cutts & Vynncky, 1999).

Another assumption was made that the force of infection acting on pregnant women was equivalent to that estimated from the seroprevalence survey, and the childbearing age range at Kilifi was 15-44 years. With average proportion susceptible $\bar{s}$ derived from (1), and given a gestation period of 40 weeks, it follows that the average annual risk of primary maternal HSV-2 infections per pregnancy, I_m, is

$$I_m = \bar{s}\left[1-\exp(-\lambda 40/52)\right] \qquad (2),$$

which for small λ approximates to

$$I_m = \bar{s}\lambda 40/52 \qquad (3).$$

Assuming that vertical HSV-2 infection only arises if a pregnant woman sheds virus at the time of birth (Brown *et al.*, 1991), that is, only for a primary infection during the final 11 days of pregnancy (Corey *et al.*, 1983), and that this transmission risk, v, is 50% (Ades *et al.*, 1989), then from equation (3) the risk of neonatal HSV per pregnancy, I_n, is

$$I_n = \bar{s}\lambda 11/365v \qquad (4).$$

The number of maternal infections per 1,000 pregnancies and neonatal infections per 100,000 live births can thus be defined from equations (3) and (4), respectively; the latter assuming there is no excess mortality attributable to HSV-2 infection.

3.4.3 Enhancing Causal Inference

This was a cross-sectional study therefore the results only established associations but cannot be used to make causal inferences. Stratification and multi-variant model of analysis was used at analysis stage to eliminate any source and evidence of confounding.

3.5 ETHICAL CONSIDERATIONS

3.5.1 Ethical Approval

Approval to conduct this study i.e testing specimen for HSV-2 was obtained from KEMRI Ethical Review committee. The HIV-1 sero-prevalence study had already gained ethical approval to test serum samples for other sexual transmitted infections other than HIV. (Appendix II).

3.5.2 Informed Consent

Consent to store and use serum samples to screen for other sexual transmitted infections, was previously obtained in writing from all participants in HIV-1 survey. Consent for women aged 15-17 years in the parent study followed the World Health Organisation and country guidelines of consent for minors as previously applied in the Kenya Health Demographic Survey conducted in 2003.

3.5.3 Risks

There was no risk involved to the participants in this study. De-indentified data and samples were used and there were no participant contact.

3.5.4 Benefits to the participating subjects and to the community as a whole

There were no direct benefits to the participating individuals in this study. However, given the strong association between HSV-2 and HIV-1, the data will be of use to the HIV clinical and research program within KEMRI Centre for Geographical Medicine Research Coast and IAVI. It is hoped that information in this study will improve knowledge on HSV-2 disease among women of the general population at the Kenyan Coast.

3.5.5 Confidentiality

Participants' confidentiality was adhered to. There were no names used but coded data and samples that could not be linked to the study participant. All records for results of this study are securely stored in KEMRI, Kilifi.

CHAPTER FOUR

4.0. RESULTS

4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

The 2004 HIV-1 survey, recruited a total of 850 women who were available for the HSV-2 study, 270 were recruited from Kilifi KEMRI research VCT and 580 randomly recruited from the DSS area. During testing for HSV-2 seropositivity, 24 of the participants selected from the archived database were missing stored serum samples and were excluded from the study. The final analysis included data for 826 participants (263 VCT and 563 DSS area) who had both serum samples and HIV-1 survey data.

The median age of these women was 24 years (Interquartile range (IQR): 15-34 years), of whom 62% were married, 24% had no education, 62% were Christian, and 66% were of the Giriama ethnicity. Of these women, 33% had a history of genital ulcers and 9% (77/826) were HIV-1 positive and 36% (296/826) were HSV-2 antibody positive. The two sampling groups differed in their marital status and religion. Women attending VCT, when compared to the random sample from the DSS, were more likely to have used condoms, to have had casual sexual relationships, had more sexual partners in the previous year, and also to have genital ulcers (either presently or ever). The two groups did not differ significantly in HIV-1 prevalence

(12% in VCT vs. 8% in DSS, P = 0.10), ethnic composition, educational level attained, or age distribution.

Table 4.1: Characteristics of Participating women in Kilifi, Kenya

Characteristic	DSS[µ]	%	VCT[α]	%	Total	%	P*
Sample size	563		263		826		
Age, years							
Median (range)	24 (15-34)		24(18-34)		24(15-34)		0.536^
15-19	105	19	30	11	135	16	0.001
20-24	179	32	118	45	297	36	
25-29	142	25	67	25	209	25	
30-34	137	24	48	18	185	22	
Marital status							
Single	172	31	70	27	242	29	0.002
Married monogamous	314	56	129	49	443	54	
Married polygamous	36	6	26	10	62	8	
Widow/separated	41	7	38	14	79	10	
Education							
Completed primary	247	44	119	45	366	44	0.764
Religion							
Christian	325	58	183	70	508	62	0.005
Muslim	135	24	46	17	181	22	
Other	103	18	34	13	137	17	
Ethnicity							
Giriama	375	67	171	65	546	66	0.128
Chonyi	60	11	19	7	79	10	
Other	128	23	73	28	201	24	
Condom use sometimes							
Yes	74/419	18	74/228	32	148/647	23	<0.001
Casual partners in last year							
Yes	66/428	15	49/158	31	115/586	20	<0.001
Partners in last year							
None	237/429	55	93/158	59	331/587	56	<0.001
One	162/429	38	39/158	25	201/587	34	
More than one	29/429	7	26/158	16	55/587	9	
History of Genital ulcer							
Yes	161	29	114	43	275	33	<0.001
HIV status							
Pos	46	8	31	12	77	9	0.122
HSV-2 status (>3.5 cut off)							
Pos	181	32	115	44	296	36	<0.001

*(µDSS - Demographic Surveillance System, αVCT - Voluntary Counselling and Testing, and *P - Chi squared P value.)*

There was a higher seroprevalence of HSV-2 in VCT-women compared with DSS-women (44% (115/263) vs. 32% (181/563), P<0.001). Of 77 HIV-1 positive women, 50 (65%) were HSV-2 positive, compared to 33% (246/749) of HIV-1 negative women (p=0.001). Results of characteristics of the study participants are presented in Table 4.1 above.

4.2: HSV-2 PREVALENCE AMONG WOMEN AT KILIFI

The serological prevalence of HSV-2 for all women was 36%. Prevalence increased with increasing age. Below 20 years very few people tested sero-positive, while more than 50% tested HSV-2 seropositive at age above 25 years. This trend demonstrates that HSV-2 prevalence is cumulative and once infected there is no reversion to seronegavity. As a result, high prevalence is observed in a cohort of older women (30-34 years). The HSV-2 prevalence can also be used as a measure of sexual activity.

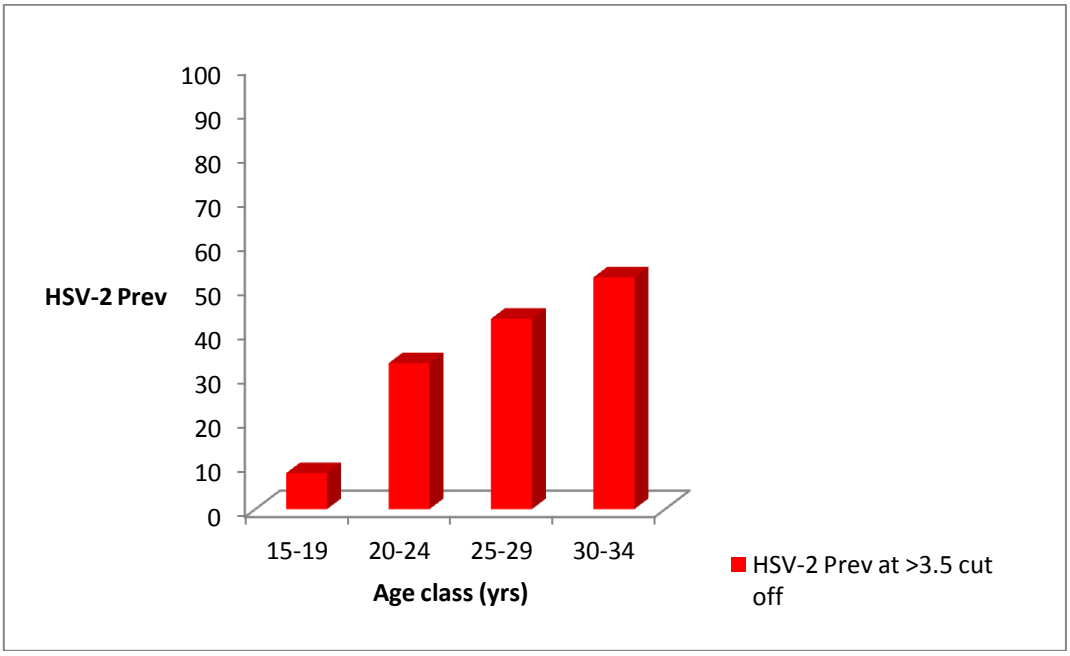

Figure 4.1: Variation of HSV-2 prevalence with age

In comparing the two categories of HSV-2 study participants, HSV-2 prevalence among VCT volunteers was persistently higher than that of the DSS volunteers at all ages. Prevalence of HSV-2 was 32% and 44% among DSS and Research VCT volunteers respectively.However at age class 30-34, HSV-2 prevalence for the two groups was similar. The age adjusted odds ratio of testing HSV-2 seropositive when recruited from VCT was 1.6 (95% Confidence interval 1.2-2.2).

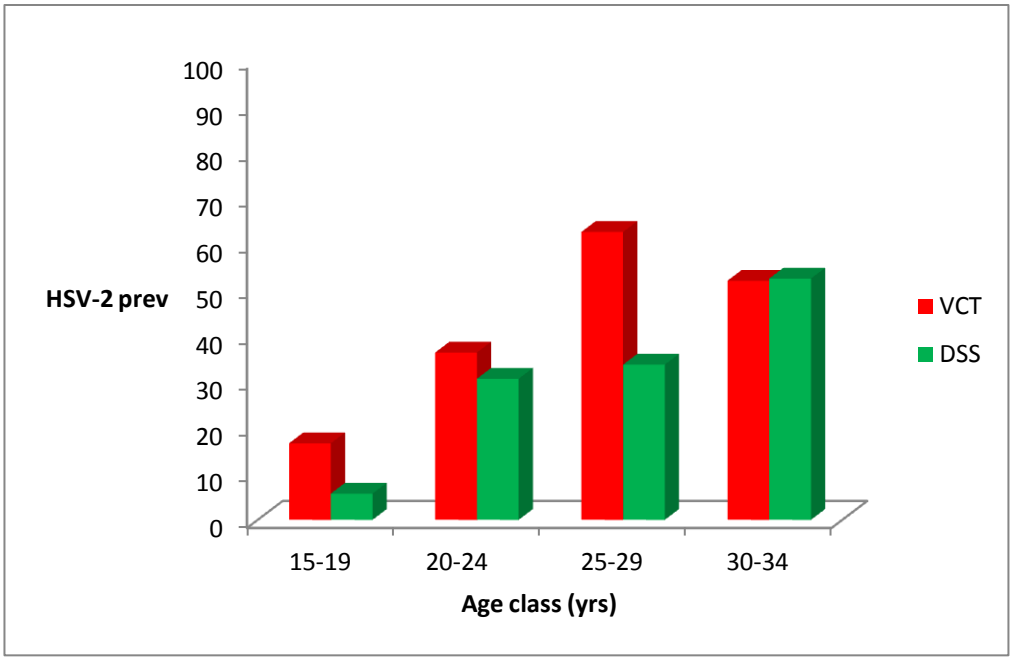

Figure 4.2: Variation of HSV-2 prevalence between DSS and VCT participants

4.2.1 *Effect of Age on HSV-2 Prevalence in Relation to Other Risk Factors*

The effect of other risk factors on prevalence was assessed in relation to age. Seroprevalence for HSV-2 and HIV-1 had a similar trend at ages 15 to 29 years.

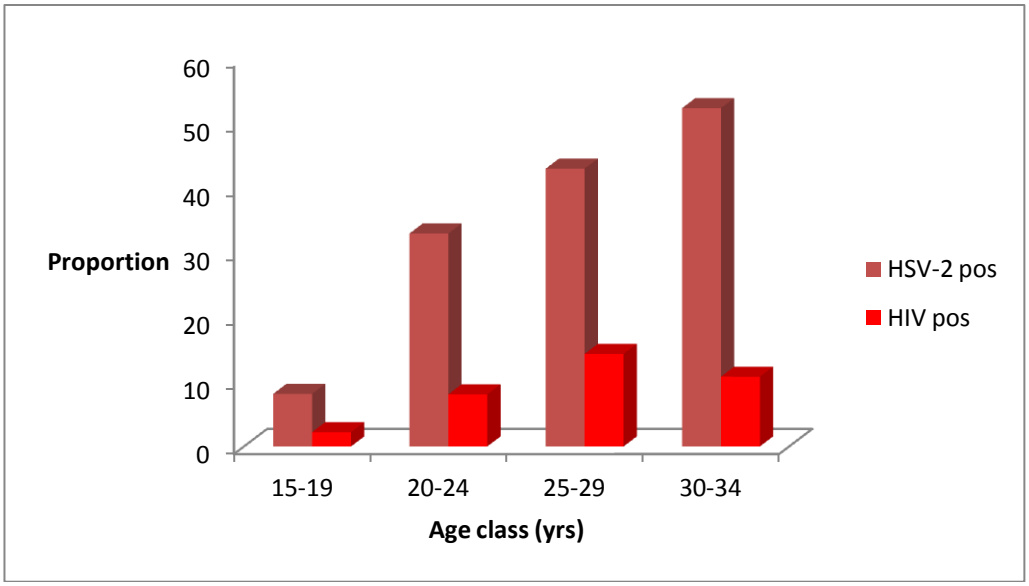

Figure 2.3: Variation of HSV-2 and HIV-1 prevalence with age in Kilifi women

However at ageclass 30-34, the prevalence for HIV-1 decreased to 11% from 14% at age 25-29.

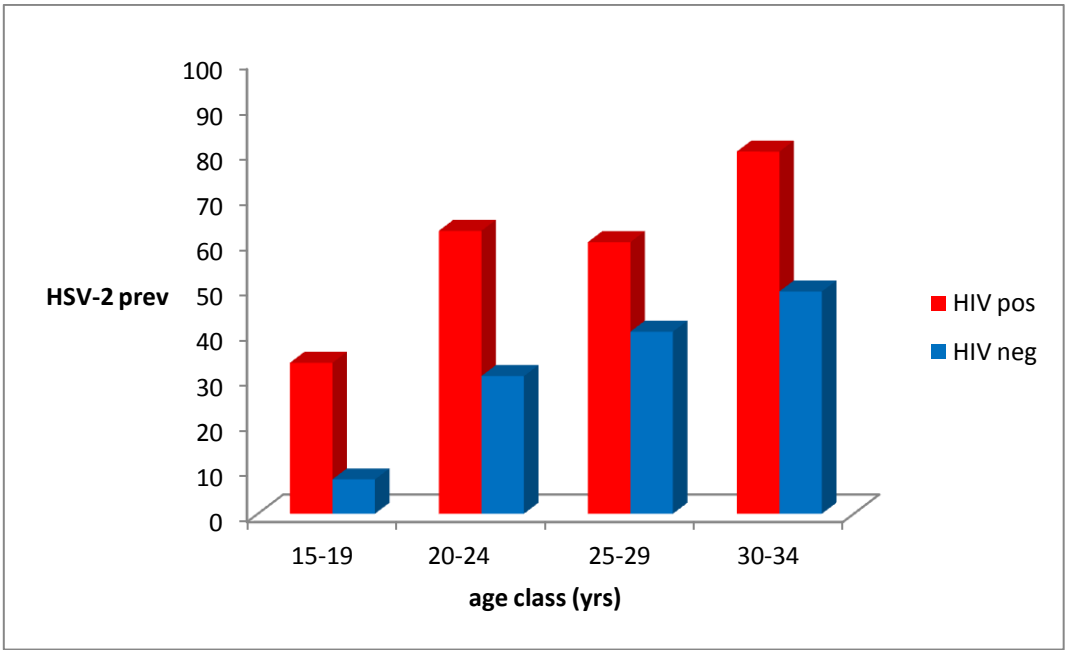

Figure 4.4: Variation of HSV-2 prevalence with HIV status in Kilifi women
[Age adjusted Odds Ratio was 3.2 (95% confidence interval 1.9 -5.3)]

On assessing HSV-2 seropositivity between the HIV negative and HIV positive women, those that were HIV seropositive were 3 times more likely to test HSV-2 positive than the HIV negative women.

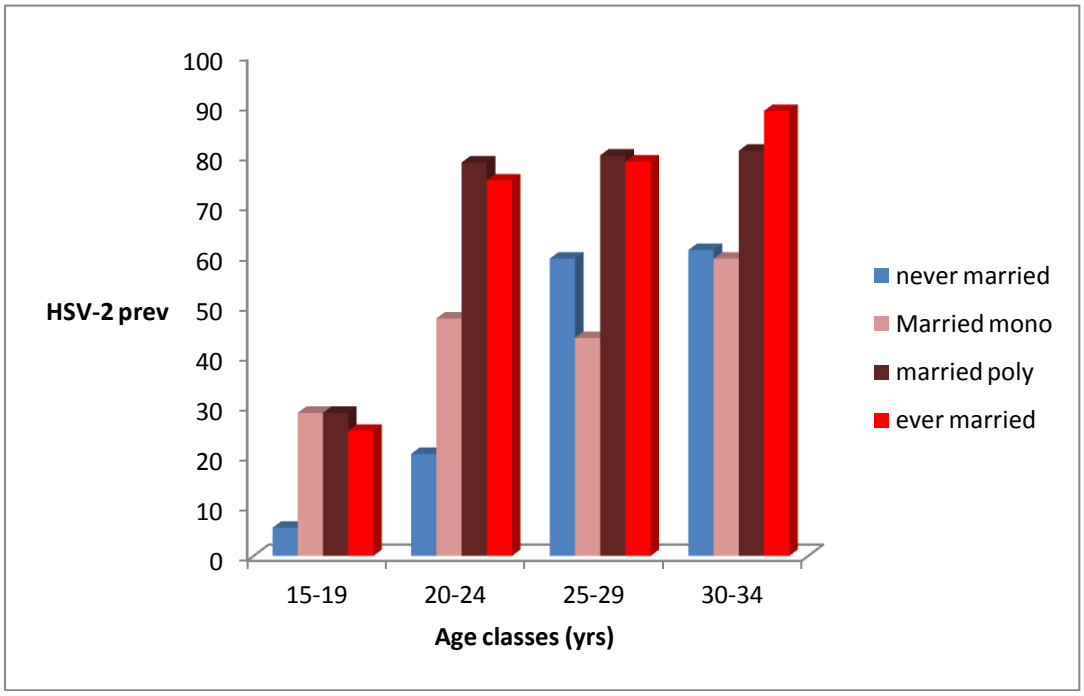

Figure 4.5: Variation of HSV-2 prevalence with marital status in Kilifi women

An evaluation of marital status as a factor associated with HSV-2 seropositivity among the different ageclasses showed a consistent increase of HSV-2 prevalence with age among single women. Women who were ever married (separated, divorced or widowed) and over 20 years had the highest HSV-2 sero-prevalence (79%-89%).Ever married women were more likely to test HSV-2 seropositive regardless of age with an Odds Ratio of 7.4 (95% Confidence Interval 4.2-12.9). The prevalence of

HSV-2 among women who reported to have never married was over 50% from age 25 to 34.

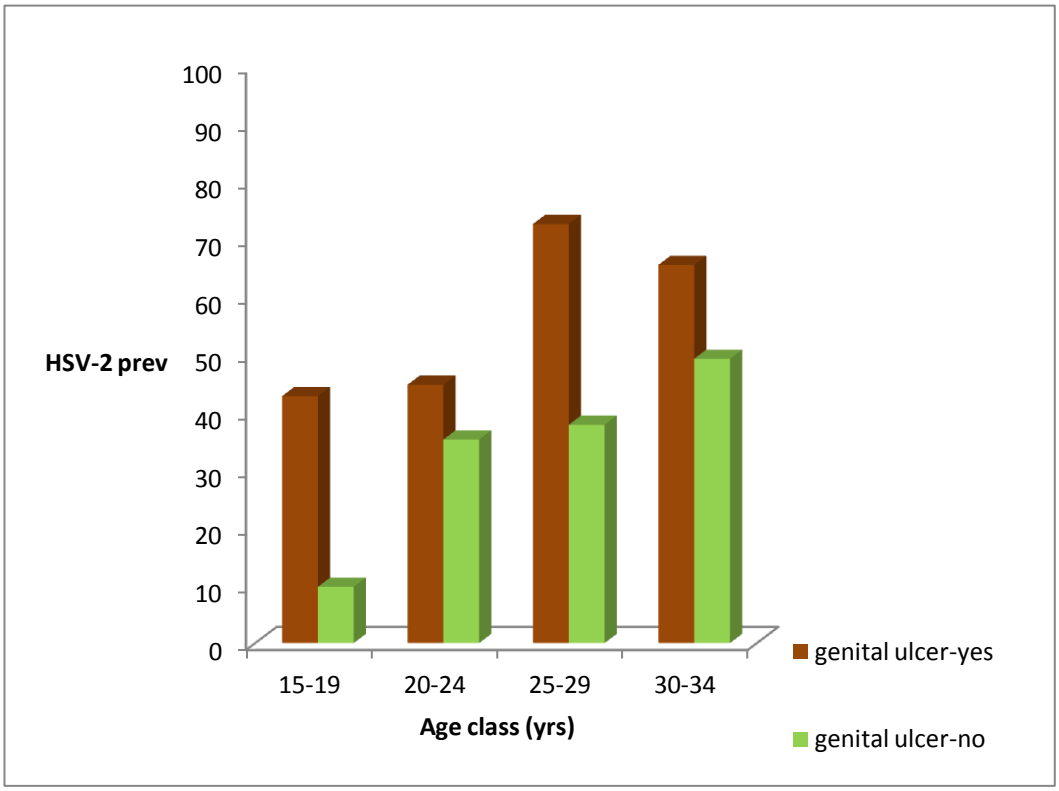

Figure 4.6: Variation of HSV-2 prevalence with genital ulcers in Kilifi women

[Age adjusted Odds Ratio 2.5 (95% Confidence Interval 1.6-3.9)]

Women who reported to have genital ulcers had a high HSV-2 prevalence of 73% at ageclass 25-29 compared to those who reported not to have genital ulcers. Genital ulcers were strongly associated with HSV-2 infection (*P <0.001* at 95% Confidence interval).

The Odds of testing HSV-2 seropositive among those with genital ulcers was 3 times more than those without genital ulcers.

4.3: FACTORS ASSOCIATED WITH HSV-2 SEROPOSITIVITY IN KILIFI: UNIVARIANT ANALYSIS

HSV-2 as a sexually transmitted infection was more associated with sexual risk behavior factors than other socio-demographic characteristics. Significant associations from the univariate analysis showed that the probability of testing HSV-2 seropositive was significantly higher in women who were older, those sampled from the VCT clinic, those ever married, those less educated, had used condoms at least sometimes, had causal partners in the previous year, had ever had genital ulcers, or those who were HIV-1 antibody positive. Marital status was correlated ($r^2>0.2$) with multiple factors (including educational status, HIV-1 status, religion, use of condoms and history of genital ulcers) and was dropped from further analysis in the multivariant model.

Table 4.2: Risk Factors for HSV-2 seropositivity (univariant analysis)

	HSV2			95%CI			
Characteristic	**pos**	**n**	**%**	**OR**	**LCL**	**UCL**	**P**
Age, years							
15-19 (ref)	11	135	8.1				
20-24	98	297	33.0	5.6	2.9	10.8	<0.001
25-29	90	209	43.1	8.5	4.3	16.7	<0.001
30-34	97	185	52.4	12.4	6.3	24.5	<0.001
Study group							
DSS (ref)	181	563	32.1				
VCT	115	263	43.7	1.6	1.2	2.2	<0.001
Marital status							
Never married (ref)	42	242	17.4				
Married monogamous	173	443	39.1	3.1	2.1	4.5	<0.001
Married polygamous	33	62	53.2	5.4	3.0	9.9	<0.001
Ever married	48	79	60.8	7.4	4.2	12.9	<0.001
Education							
None or some primary (ref)	183	460	39.8				
Completed primary	113	366	30.9	0.7	0.5	0.9	<0.008
Religion							
Christian (ref)	160	508	31.5				
Muslim	75	181	41.4	1.5	1.1	2.2	0.016
Other	61	137	44.5	1.7	1.2	2.6	<0.005
Ethnicity							
Giriama (ref)	190	546	34.8				
Chonyi	26	79	32.9	0.9	0.6	1.5	0.742
Other	80	201	39.8	1.2	0.9	1.7	0.207
Condom use							
Never (ref)	209	499	41.9				
At least sometimes	50	148	33.8	0.7	0.5	1.0	0.078
Casual partners in last year							
No (ref)	179	471	38.0				
Yes	57	115	49.6	1.6	1.1	2.4	0.024
Number of Partners in last year							
None (ref)	143	331	43.2				
One	68	201	33.8	0.7	0.5	1.0	0.033
More than one	26	55	47.3	1.2	0.7	2.1	0.573
History genital ulcer							
No (ref)	158	551	28.7				
Yes	138	275	50.2	2.5	1.9	3.4	<0.001
Genital ulcer now							
No (ref)	229	636	36.0				
Yes	61	101	60.4	2.8	1.8	4.5	<0.001
HIV status Negative (ref)	246	749	32.8				
HIV status Positive	50	77	64.9	3.8	2.3	6.2	<0.001

Note on table 4.2

(OR, Odds Ratio, LCL, UCL, Lower and upper 95% confidence interval, respectively)

4.4: FACTORS ASSOCIATED WITH HSV-2 SEROPOSITIVITY: MULTIVARIATE ANALYSIS

Factors shown to be independently associated with HSV-2 sero-positivity using multivariate logistic modelling are as shown in Table 4.2 above. In this study HSV-2 positive status was found to be positively associated with increasing age, recruitment from the VCT centre, history of genital ulcers and positive HIV-1 sero-status. Higher educational level was negatively associated with HSV-2 seropositivity. Separate multivariate models for DSS and VCT women were developed, which gave similar results to those of the full model, except that for DSS women the association with present genital ulcers was replaced with ever having had genital ulcers, and for VCT women the association with casual partners in the last year was lost. In the full model no factors significantly interacted with the association between study group and HSV-2 status (Table 4. 3).

Table 4.3: Predictors for HSV-2 seropositivity

Characteristic	OR[α]	95%CI LCL*	UCL*	P
Age, years				
15-19 (ref)				
20-24	4.7	2.4	9.2	<0.001
25-29	6.9	3.5	13.7	<0.001
30-34	10.5	5.2	21.0	<0.001
Study group				
DSS (ref)				
VCT	1.5	1.1	2.1	0.010
Education				
None or some primary (ref)				
Completed primary	0.68	0.50	0.94	0.018
History of genital ulcer				
No (ref)				
Yes	1.7	1.2	2.3	0.002
HIV status				
Negative (ref)				
Positive	2.7	1.6	4.6	<0.001

[α]OR, Odds Ratio,*LCL, UCL, Lower and upper 95% confidence interval, respectively

4.5: FACTORS ASSOCIATED WITH HSV-2 AND HIV CO-INFECTION

About 83% (64/77) women who were sero-positive for HIV-1 infection were also co-infected with HSV-2. Co-infection was 16.98% (64/826).

Analysis of the factors associated with HSV-2 HIV Co-infection was conducted using chi squared tests, and logistic regression for multi-variant analysis was used to determine predictors of co-infection.

Risk factors for co-infection were determined and presented in table 4.4 below.

Table 4.4: Factors associated with HSV-2/HIV co-infection

Risk Factor	Category	HSV-2/HIV Co-Infection						Odds Ratio
		Yes	%	No	%	P*	OR[α]	(95% CI)
Ethnicity								
	Giriama (ref)	38	14.4	226	85.6			
	Chonyi	7	19.4	29	80.6	0.428	1.4	0.6-3.5
	Luo	6	50.0	6	50.0	0.003	5.9	1.8-19.4
	Other	13	16.7	65	13.7	0.621	1.2	0.6-2.4
Partners in last year	None (ref)	32	18.0	146	82.0			
	One	13	14.8	75	85.2	0.51	0.8	0.4-1.6
	Multiple	11	32.3	23	67.3	0.06	2.2	0.9-4.6
Genital Ulcer now	No ulcer (ref)	38	12.5	266	87.5			
	Ulcer	24	32.4	50	67.6	<0.001	3.4	1.8-6.1
History genital ulcer	No (ref)	25	11.7	188	88.3			
	Yes	39	22.0	132	78.0	0.007	2.1	1.2-3.7
STD now	No STD (ref)	48	13.8	299	86.2			
	STD now	16	37.2	23	62.8	<0.001	3.4	1.8-7.3

[α]OR, Odds Ratio,*P ,chi squared P value

An association with HIV HSV-2 co-infection was found with ethnicity, current genital ulcer, having multiple partners in the last 12 months and presence of sexual transmitted diseases. There was no association observed with co-infection with factors such as education, religion, recruitment category, age, condom use and age at sex debut for these women.

The predictors for HIV/HSV-2 co-infection for all women in a multivariant analysis were having genital ulcers at the time of recruitment to the study and presence of other sexual transmitted infections. The number of sexual multiple partners in the last 12

months was dropped from the model since it was interacting with ethnicity, genital ulcers and history of genital ulcers. Participants from the Luo ethnic group were also few and were therefore, excluded from the final logistic model.

4.6: INCIDENCE ESTIMATES OF MATERNAL HSV-2 AND NEONATAL HERPES INFECTION

The proportions of HSV-2 seronegative women recruited from the DSS reduced from 94% in those aged 15-19 years to 47% in those aged >30 years (with cut off >3.5). The corresponding levels for VCT women were 83% and 48%. In 30 DSS women aged 15 –16 years only 3% were HSV-2 seropositive. The fit of the maximum likelihood catalytic models to proportions seronegative by age and by source of recruitment (DSS or VCT) are shown in Figure 4.7 below.

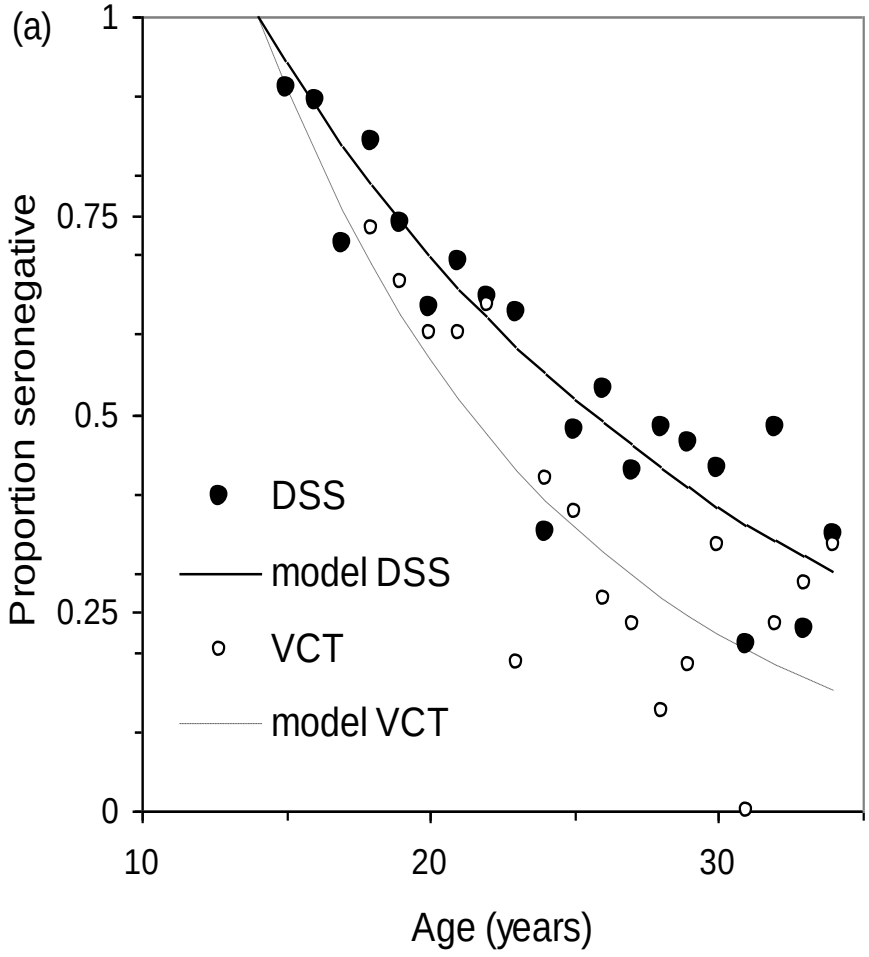

Figure 4.7: Age-stratified proportions HSV-2 seronegative by age (years) and by study group for Kilifi women

From the catalytic model, the estimated incidence of HSV-2 infection was 6.0 (95% CI, 5.3-6.8) per 100 seronegative women per year.

By using the estimates of the force of infection acting on women in the DSS, the estimated average proportion of seronegative women was 45.0 (95%CI 41.3-48.9) over the child-bearing age. Hence the estimated incidence of maternal infections derived from Equation 3 ($I_m = \bar{s}\lambda 40/52$) is 21 (95%CI 15.7-18.3) cases per 1,000 pregnancies per year, and the estimated incidence of neonatal transmission derived from Equation 4 ($I_n = \bar{s}\lambda 11/365v$) is 41cases (95%CI 31-36) per 100,000 pregnancies per year as shown in (Table 4.5).

Table 4.5: Estimated incidence of maternal and neonatal HSV-2 for Kilifi DSS women

	Force of infection (/100 women per year)	Estimated proportion susceptible (%)	Incidence of maternal infection(/1000 pregnancies per year)	Incidence of neonatal transmission(/100,000 births per year)
Estimate	6.0	45.0	21.0	41
LCL*	5.3	41.3	20.0	39
UCL*	6.8	48.9	22.0	42

*LCL, UCL, Lower and upper 95% confidence interval, respectively

CHAPTER FIVE

5.0. DISCUSSION

This study sought to estimate the serological prevalence of HSV-2 among women of semi-urban population of Kilifi, who were presumed to be either a low risk group randomly selected from within a population under demographic surveillance or a self-selected higher risk group recruited from the KEMRI research VCT centre. The study also sought to identify the risk factors for HSV-2 infection and predictors for HIV-1 HSV-2 co-infection. Based on the data on specific HSV-2 antibody status this study explored an estimate for potential risk of vertical transmission among these women.

Women from VCT and DSS did not differ in demographic characteristics such as age, marital status, religion or ethnicity since they all were from the same geographical area mostly dominated by the mijikenda community thus sharing similar demographic factors. Stratification by study group was based on their differences in sexual exposure characteristics. In this study, prevalence for HSV-2 seropositivity was assessed and results presented using a cut off index value recommended for seroepidemiological surveys.

5.1: PREVALENCE OF HSV-2 INFECTION

The overall HSV-2 prevalence of 36% in all women aged 15-34 years (32% DSS and 44% VCT), was in the same range of the 42% national prevalence reported among Kenyan women from 15-64 years of age (KAIS, 2009). Similar to other studies, a

rapid rise in HSV-2 seroprevalence was found from mid-late teenage years upwards coincident with onset of sexual activity (Watson-Jones *et al.*, 2007). Seropositivity was more common in women of older age than young women less than 18 yrs. This trend confirms that HSV-2 infection among these women was mostly through sexual contact, with few women experiencing sexual activity by age of 15 years. The findings in this study also agree with other studies on HSV-2 prevalence (Shaw *et al.*, 2001; Kramer *et al.*, 2008). The high prevalence observed at older ages is as a result of the cumulative acquisition of HSV-2 virus with increasing age. Antibodies to HSV-2 are produced on exposure to the virus through sexual contact. Depending on age at exposure, antibodies tend to persist in a lifetime with the virus undergoing latency and active stages.(Ozouaki *et al.*, 2006; Bollen *et al.*, 2008) At each specific age, more cases are added to the already infected women exposed earlier, accounting for the high prevalence seen in a cohort of older women.

Detailed analysis of prevalence showed that HSV-2 seropositivity highly varied among different categories of women. It differed between the DSS (32%) and VCT (44%) and between HIV seronegative (33%) and HIV seropositive (65%). The HIV positive women and Research VCT women are presumed to be a high risk group, with high risk sexual practices due to the observed high HSV-2 sero-prevalence. This is supported by the differences in sociodemographic characteristics of the women between the two study groups.Women from the VCT were more likely to have had casual sexual partners in the previous year or had multiple sexual partners. These

observations are similar to findings in other prevalent studies for other populations of women (Weiss *et al.*, 2001; Xu *et al.*, 2007). Comparing the trend of HIV infection and that of HSV-2 infection across the age classes, it was observed that the two infections parallel each other and trend for infection was similar. However, HIV-1 seroprevalence was increasing with increasing age thereafter it declined after age of 28 years. The decline for HIV seroprevalence above 28 years could be attributed to deaths due to progression of HIV disease. On the other hand HSV-2 infection is not a life threatening disease among adults therefore no decline to seroprevalence was observed.

5.2: RISK FACTORS AND PREDICTORS OF HSV-2 INFECTION

Analysis of factors associated with HSV-2 infection suggests a small but significantly higher proportion of women with HSV-2 seeking VCT services compared with women in the general population selected through the DSS. However, the nature of the risk factors for infection in multivariate analysis appeared largely the same between the two groups. There was no difference in the risk factors observed for HSV-2 prevalence with cut off index values 1.5 and >3.5.

There was a cumulative increase of HSV-2 sero-prevalence with age. Age was strongly associated with HSV-2 infection (*p value <0.001*). Women of older age group (30-34 years) were more likely to test HSV-2 positive compared to women of young age group (15-19 years). These results are consistent with findings that HSV-2

infection persists for life, therefore the prevalence increases with age throughout the sexual active years (Kramer *et al.*, 2008; KAIS, 2009).

In this study, HSV-2 infection was strongly associated with marital status of the woman by univariant analysis (*p value <0.001).*Women who were widowed, separated or divorced were 7 times more likely to test HSV-2 positive compared with those who were never married. A possible explanation for the high prevalence of HSV-2 infection among widows is that widows are a vulnerable group. In the absence of spouse they are likely to get exposed to new sexual partners increasing the risk of getting sexually transmitted infections (Shaw *et al.*, 2001).

It was also noted that women in polygamous marriages were more likely to be HSV-2 infected, odds ratio 5.4 (95% confidence interval 3.0-10). It is possible that women in a polygamous marriage have extra marital sex resulting into transmission of sexually transmitted infections within the marital circle. It is also possible that men in polygamous marriage will put their women at risk by their behavior. These results are very similar with those observed during the 2007 Kenya Aids Indicator Survey (KAIS, 2009).

History of genital ulcers was also strongly associated with HSV-2 infection. Women with genital ulcers were about 3 times more likely to test HSV-2 seropositive than those without genital ulcers. Herpes simplex virus type 2 is a main cause of genital ulcer disease. In this study 67% of those who reported having genital ulcers were

HSV-2 sero-positive. Confirming that, HSV-2 is the main cause of genital ulcer disease, accounting for almost 70% of all genital ulcer disease reported in this study and symptoms of genital ulcer disease would predict a possible HSV-2 infection. On the other hand 79% of all women who were HSV-2 sero-positive did not report symptoms of genital ulcers. These results confirm that, most HSV-2 infections are asymptomatic (Mertz *et al.*, 1992) and unnoticed by majority of those infected. Other studies have observed that, fewer than 10% of HSV-2 seropositive individuals report a history of genital ulcers (Fleming *et al.*, 1997). As a result, those infected are unaware, do not seek medical care and become potential reservoirs for transmission (Anuradha *et al.*, 2008; Chawla *et al.*, 2008; Kramer *et al.*, 2008).

A positive HIV-1 status was the strongest independent predictor of HSV-2 seropositivity, as reported in other studies (Kapiga *et al.*, 2007; Watson-Jones *et al.*, 2007). Women who were HIV positive were more likely to test HSV-2 seropositive than those who were HIV negative. Indeed, HIV-1 and HSV 2 epidemics parallel each other in Africa (Serwadda *et al.*, 2003; Corey, 2007; Watson-Jones *et al.*, 2007; Abu-Raddad *et al.*, 2008; Anuradha *et al.*, 2008; Glynn *et al.*, 2008). Previous in vitro studies have shown a negative synergy between HIV and HSV-2 infections in the female genital mucosa (Scott *et al.*, 2002; Rebbapragada *et al.*, 2007). It has also been observed that HIV infection impairs HSV-2 mucosal immune control and local HSV-2 reactivation enhances both HSV-2 and HIV transmission (McClelland *et al.*, 2002; Rebbapragada *et al.*, 2007).

Education beyond primary school (greater than year 8 in Kenyan schools), with a prevalence of 45% in this sample, was found to be associated with ~1.5 fold reduced risk of seropositivity. Similarly, not finishing primary school was a significant predictor of HSV-2 prevalence among 469 women enrolled for an at-risk cohort of HIV-1 infection from nearby coastal areas (Okuku *et al.*, 2010 (under review)). Although educational attainment has been inconsistently associated with HSV-2 sero-prevalence in prior studies (Kebede *et al.*, 2004; Watson-Jones *et al.*, 2007; Tobian *et al.*, 2009), knowledge of HSV-2 infection among women in similar parts of East Africa has been very low (Tassiopoulos *et al.*, 2007), and the development of programs to keep young women from rural areas in schools and encourage them to pursue higher education and delay early sexual debut is an intervention activity worth undertaking.

5.3: PREDICTORS OF HSV-2/HIV CO-INFECTION

A logistic model was used to identify predictors of co-infection with all risk factors which had *p values <0.05.* The predictors of co-infection as found in this study were presence of other sexually transmitted infections and genital ulcers. HIV and HSV-2 share same portal entry with all other sexually transmitted infections.

Sexually transmitted infections (STIs) are strongly associated with HIV acquisition. These include ulcerating STIs such as *Hemophilus ducreyi* and *Treponema pallidum*,

herpes simplex virus (HSV) as well as nonulcerating STIs – gonorrhea, Chlamydia and trichomoniasis (Fleming & Wasserheit, 1999). There is biological plausibility that cells susceptible to HIV infection such as CD4 T cells and macrophages assemble in genital areas affected by STIs. Furthermore, STIs anatomically disrupt mucosal barriers, which can increase the exposure of HIV susceptible cells to HIV. The synergistic role of STIs in HIV acquisition has led to systematic screening and treatment of many STIs in at-risk groups in an effort to decrease the risk of HIV acquisition (Wawer *et al.*, 1999; Cohen, 2004; Gupta *et al.*, 2004; Baeten *et al.*, 2008; Celum *et al.*, 2008; Watson-Jones, 2008; Celum *et al.*, 2010). Thus presence of STI's would strongly predict a HIV/HSV-2 co-infection. This suggests that women who have symptoms of STI's infection should also test for HIV infection and seek medical care.

An explanation for the association between genital ulcers and HIV/HSV-2 co-infection as observed in this study is that, genital ulcers play an important role in acquisition of both HIV and sexually transmitted infections. They provide micro-crevices for direct entry of pathogens in addition to disrupting the genital mucosal surfaces making them susceptible to HIV or HSV-2 receptor cells. Genital herpes on the other hand is suggested to have played a more substantial role for HSV-2 in fueling HIV spread in Sub Saharan Africa than any other sexually transmitted infections (Abu-Raddad *et al.*, 2008).

5.4: EVIDENCE OF POTENTIAL RISK OF VERTICAL TRANSMISSION

The incidence of vertical transmission of HSV-2 was estimated by using age specific seroprevalence data and fitting it into a catalytic model.

The estimated rate of acquisition of HSV-2 specific antibodies of 6.0 (95%CI 5.3-6.8) cases per 100 per year among women of the general DSS population from this study would have been estimated as 4% per person per year, if a higher assay cut off value of >3.5 was used. Whereas, validation studies (Morrow *et al.*, 2003; Ashley-Morrow *et al.*, 2004; Laeyendecker *et al.*, 2004) recommend the use of a higher cut off index value to improve the performance of the Focus assay, it is likely to underestimate the true HSV-2 incidence of a population.

This study estimated an incidence of maternal HSV-2 infection of 2.1% per year for pregnant women at Kilifi through the catalytic model. Results are similar to findings reported elsewhere (Brown *et al.*, 1997) in a prospective study of 7046 HSV-seronegative pregnant women followed throughout pregnancy where more than 2% of the women acquired HSV-2 infection during pregnancy (Brown *et al.*, 1997). In this study, the catalytic model was computed on the assumptions that HSV-2 shedding during delivery as a result of primary HSV-2 infection has a 50% risk of neonatal HSV-2 acquisition (Ades *et al.*, 1989), and the risk of a baby acquiring HSV-2 from an infected mother occurs at primary infection within 11 days (Corey *et al.*, 1983) (the average duration of viral shedding) of delivery (Brown *et al.*, 1991; Brown *et al.*,

2003; Brown *et al.*, 2005). Kilifi has a birth rate of 8000 live births per year (Bauni *et al.*, 2007), hence it is estimated that about 3 cases of neonatal herpes will occur per year among pregnant women in this population.

Earlier studies in Kilifi (English *et al.*, 2003; Bauni *et al.*, 2007), assumed that the large number of perinatal deaths, especially those occurring at home, where cause could not be established, may have been related to HIV and sexually transmitted infections (English *et al.*, 2003; Bauni *et al.*, 2007). However, this data suggests that the burden of neonatal HSV-2 disease from maternal infection is low and contributes insignificantly to the observed neonatal deaths in this region. Interestingly, the estimate of the incidence of neonatal herpes is similar to the estimate of 1 case per 3200 live births from the USA (Brown *et al.*, 2003). However, most births in Kilifi district occur at home and options for appropriate interventions while mothers experience acute HSV-2 infections prior to birth are very limited. The high rate of maternal HSV-2 incidence estimated in this population may also potentially contribute to an increased risk of intra-partum mother to child transmission of HIV-1 (Cowan *et al.*, 2008). Where possible, prevention programmes aiming to reduce the burden of HIV-1 infections in women need to include information on HSV-2 infection. Pregnant women should be counseled to use condoms and avoid unprotected oral-genital contact during the last trimester of pregnancy and be advised to report to a referral health facility when signs of genital herpes infection are noted prior to delivery (Mindel *et al.*, 2000).

5.5: STUDY LIMITATIONS

There are several potential limitations to this study. First, during the 2004 HIV-1 survey, records of women refusing participation in the HIV-1 survey were not kept. Therefore, it was difficult to establish whether individuals who refused and those who participated in the study differed in characteristics. Second, this was a cross sectional study restricted to women only. Hence, the analysis of predictors of prevalent HSV-2 infection only established associations and could not make causal inferences. Third, the HIV-1 study did not involve conducting of any biological measurements of other common sexually transmitted diseases (e.g. syphilis) or perform visual inspection for the presence of genital ulcers. Forth, the catalytic model was based on assumptions that HSV-2 incidence is independent of age (or time), even though, the risk for HSV-2 infection increases with age. In this study, including age-dependence in the rate of infection did not significantly improve the model fit. Furthermore, the data appear to follow a linear cumulative hazard from which it was deduced that there was no good evidence for age (or time) dependence in HSV-2 incidence. Fifth, the burden of neonatal herpes infection may also have been underestimated as HSV-2 acquisition due to reactivated HSV-2 infections were not included in the model (considered to have less than 5% risk of neonatal herpes infections transmission), and HSV-1 infections in this study population were not measured. Lastly, there was no gold standard to compare with the specificity and sensitivity of the Focus assay. The HSV-2 prevalence observed could not be ascertained to HSV-2 only or as a result of a cross

reactivity with or presence of HSV-1. However, this limitation was overcome in some part by raising the cut off value for the assay to >3.5 other than using the recommended manufacturers' cut off assay value of 1.1.

In summary, the existing burden of HSV-2 infection in the Kilifi semi-rural population is similar to national HSV-2 prevalence estimates in Kenya. Women infected with HSV-2 have an increased risk of acquiring HIV-1, and HSV-2 infection may remain a driving force behind the HIV-1 epidemic in this semi-urban population. Mother to child herpes transmission is unlikely to be a significant public health problem in this population.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The serological prevalence of HSV-2 in the general population of women in Kilifi is similar to the national prevalence for women. Majority of HSV-2 occurrence is asymptomatic. HIV is a predictor of HSV-2 infection. HSV-2 seropositivity is associated with age, HIV status, history of genital ulcers and education level. HIV/HSV-2 co-infection is associated with presence of genital ulcers and presence of other sexually transmitted infections. There is evidence for potential risk of HSV-2 vertical transmission though it is currently unlikely to be a significant public health problem in this population.

6.2 RECOMMENDATION

- As a strategy to reduce transmission of sexually transmitted infections like HSV-2 among young women, interventions that keep the girl child at school and or reduce early sexual debut may be useful.
- Prevention of STI, especially technology that prevents multiple sexually transmitted infections may be useful for this population.

- Though the risk of vertical transmission is low, all women visiting antenatal care clinics should be counseled and be advised to report to a health facility when signs of genital herpes infection are noted prior to delivery.

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APPENDICES

APPENDIX I: HERPES SELECT 2 ELISA IgG SOP

PURPOSE / INTRODUCTION:

The purpose of this SOP is to give guidance on the Herpes Select 2 ELISA assay procedure by Enzyme Immuno-assay and the preparation of reagents, samples and controls involved.

2.0 SCOPE / RESPONSIBILITY:

Following this SOP is the responsibility of the trained Laboratory staff working in the Serology Laboratory.

3.0 DEFINITIONS:

3.0.1 HSV----------Herpes Simplex Virus

3.0.2 HPC ---------- High Positive Control

3.0.3 NC ---------- Negative control

3.0.4 LPC ---------- Low Positive Control

3.0.5 EDTA----------Ethylene diamine tetra acetic acid

3.0.6 EIA----------Enzyme Immuno Assay

3.0.7 SOP----------Standard Operating Procedure

3.0.8 TMB----------Tetramethylbenzidine

4.0 SPECIMEN:

Serum

Plasma

5.0 EQUIPMENT / MATERIALS/ REAGENTS:

5.1 EQUIPMENT

5.1.1 Single channel pipette (10 - 200µl)

5.1.2 Multi channel pipette (50 -200µl)

5.1.3 Graduated cylinder

5.1.4 Reagent reservoir

5.1.5 Plate washer

5.1.6 Plate reader with 450nm filter nm

5.1.7 Timer

5.1.8 Vortex machine

5.1.9 Single channel pipette (100 - 1000µl)

5.2 MATERIALS

5.2.1 Disposable pipette tips (10,200 & 1000µl)

5.2.2 Disposable gloves

5.2.3 Absorbent tissue

5.2.4 Adhesive plate sealers

5.2.5 15mls and 50mls sterile centrifuge tubes

5.2.6 Apex tubes (2ml)

5.3 **REAGENTS**

5.3.1 Negative Control

5.3.2 High Positive Control

5.3.3 Low Positive Control

5.3.4 IgG Cut-Off Calibrator

5.3.5 HerpeSelect sample diluent

5.3.6 Wash buffer concentrate

5.3.7 Distilled water

5.3.8 TMB solution (substrate reagent)

5.3.9 Conjugate

5.3.10 Coated HSV microtitre strips

5.3.11 **1 M** Sulphuric Acid (analytical grade)

Caution: All kit components are stable until expiration date printed on the label. Do not use beyond expiration date. **Please use the expiry date on the outside of the box as the final expiry date.**

6.0 METHODOLOGY:

6.1 PROCEDURE

6.1.1 Enter specimen numbers and dates in the specimen-listing sheet in the Microsoft excel HSV spreadsheet blank and save in an appropriate file and name *C:\Documents and Settings\All Users\HSV 2 STUDY\Results*

6.1.2 In the same spreadsheet, go to the' Enter Data here' sheet (*appendix 7.3*) and fill in the kit details, date of assay and operator's name these will automatically appear in the plate map sheet, print the map, which will guide you through the assay.

6.1.3 Pull out plasma from –80 freezer and allow them to reach room temperature (20-25^{0}C)

6.1.4 Pull out Herpes Select 2 ELISA IgG Focus Diagnostic kit and allow all reagents to attain room temperature.

6.1.5 Make up fresh wash buffer as in (*appendix 7.1)*.

6.1.6 Dilute each specimen, controls and calibrator (*see Appendix 7.3*) 1:101 by dispensing into labeled tubes 1000µl of sample diluent and add 10 µl of specimen, controls and calibrator into each appropriate apex tube containing1000µl of sample diluent

6.1.7 Take out the number of strips needed depending on sample and controls number as shown in *(appendix 7.3)*

6.1.8 Pre-soak the strips with 1X wash buffer using the plate washer (Program10: HSV2 soak)

6.1.9 Blot the wells by tapping vigorously using a clean paper towel until residual wash buffer is removed.

6.1.10 Vortex each sample and add 100 µl of the sample as shown in the Plate map using a single channel pipette (appendix 7.3). Add 100 µl of sample diluent in the 'BLANK WELL'

6.1.11 Seal plate with adhesive cover

6.1.12 Incubate for 60 minutes at room temperature (20-25^{0}C).

6.1.13 Wash using program set in the washer (Program11: HSV 2 wash)

6.1.14 Remove residual wash buffer by blotting with absorbent tissue same as

6.1.15 Add 100ul of **Conjugate** in to each well using multichannel pipette.

6.1.16 Incubate the plate at 20 to 25^{O}C (room temperature) for 30 minutes.

6.1.17 Wash using program set in the washer (Program11: HSV 2 wash)

6.1.18 Remove residual wash buffer by blotting with absorbent tissue.

6.1.19 Add 100ul of **Substrate reagent** in to each well

6.1.20 Incubate the plate at 20 to 25^{O}C (room temperature) for 10 minutes

6.1.21 Add 100 µl of **1 M Sulfuric acid** (stop solution) to each well to stop the reaction

6.1.22 Read plate within 30 min using a spectrophotometer set at 450nm

6.1.23 Copy and paste results in the HSV 2 spread sheet in the 'Enter OD data here' sheet (*Appendix 7.2*)

6.2 RESULTS

6.2.1 Results are generated in sheet 3 of the HSV 2 spread sheet (*Appendix 7.4*)

6.2.2 Save results with the assay date in *C:\Documents and Settings\All Users\HSV 2 STUDY\Results*

6.3 Qualification of Control values

6.3.1.1 NC must be < 0.90 index value.

6.3.1.2 HPC must be > 3.5 index value.

6.3.1.3 LPC must be between 1.5 and 3.5 index value

6.3.1.4 All Cut-Off calibrators must be within 0.100 to 0.700 OD units

6.4 Cut-Off value

6.4.1 If the test is valid Cut-Off value = Mean OD of Cut-off Calibrator

6.4.2 A test sample is **positive** if sample index value is ≥ 1.10

6.4.3 A test sample is **negative** if sample index value is < 0.90

6.4.4 A test sample is **equivocal** if sample index value is ≥ 0.90 and ≤ 1.10.

6.4.5 All equivocal samples must be re-tested. If on re-testing, the result remains equivocal, a second sample should be drawn 4-12 weeks later and testing repeated.

6.4.6 Report all positive and equivocal results to the clinicians for action

6.4.7 **Index Value**

6.4.7.1 Index value is calculated by dividing OD of the controls and samples by the Mean OD of the Cut-Off calibrator

6.4.8 Booking of results

6.4.8.1 Results are saved in the appropriate folder (C:\Documents and Settings\All Users\HSV 2 STUDY)

6.4.8.2 Printed results are filed in Assay results file (HSV 2 RESULTS F1)

7.0 APPENDICES:

7.1 **Preparation of Wash Buffer (1:10 in distilled water)**

# of wells	Wash Buffer Concentrate	dH_2O	Total
96	70 ml	630ml	700ml
60	50 ml	450ml	500ml
30	25 ml	225ml	250ml

7.2 Enter OD data here sheet

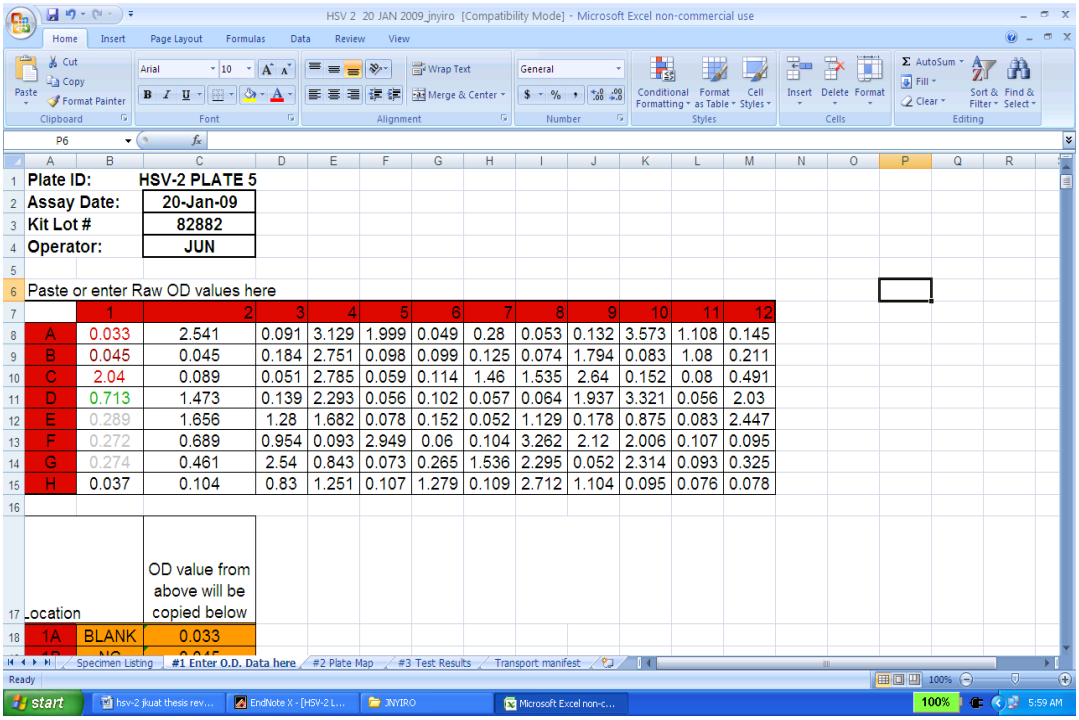


Plate ID:	HSV-2 PLATE 5	
Assay Date:	20-Jan-09	
Kit Lot #	82882	
Operator:	JUN	

Paste or enter Raw OD values here

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.033	2.541	0.091	3.129	1.999	0.049	0.28	0.053	0.132	3.573	1.108	0.145
B	0.045	0.045	0.184	2.751	0.098	0.099	0.125	0.074	1.794	0.083	1.08	0.211
C	2.04	0.089	0.051	2.785	0.059	0.114	1.46	1.535	2.64	0.152	0.08	0.491
D	0.713	1.473	0.139	2.293	0.056	0.102	0.057	0.064	1.937	3.321	0.056	2.03
E	0.289	1.656	1.28	1.682	0.078	0.152	0.052	1.129	0.178	0.875	0.083	2.447
F	0.272	0.689	0.954	0.093	2.949	0.06	0.104	3.262	2.12	2.006	0.107	0.095
G	0.274	0.461	2.54	0.843	0.073	0.265	1.536	2.295	0.052	2.314	0.093	0.325
H	0.037	0.104	0.83	1.251	0.107	1.279	0.109	2.712	1.104	0.095	0.076	0.078

Location		OD value from above will be copied below
1A	BLANK	0.033

7.3 Plate Map

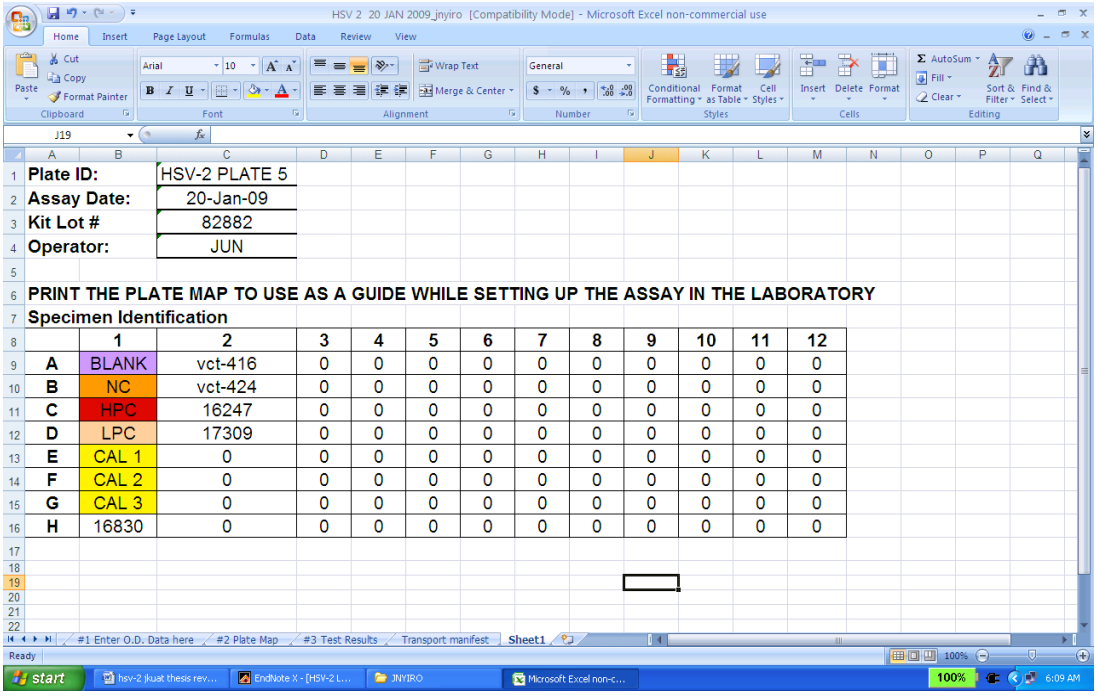


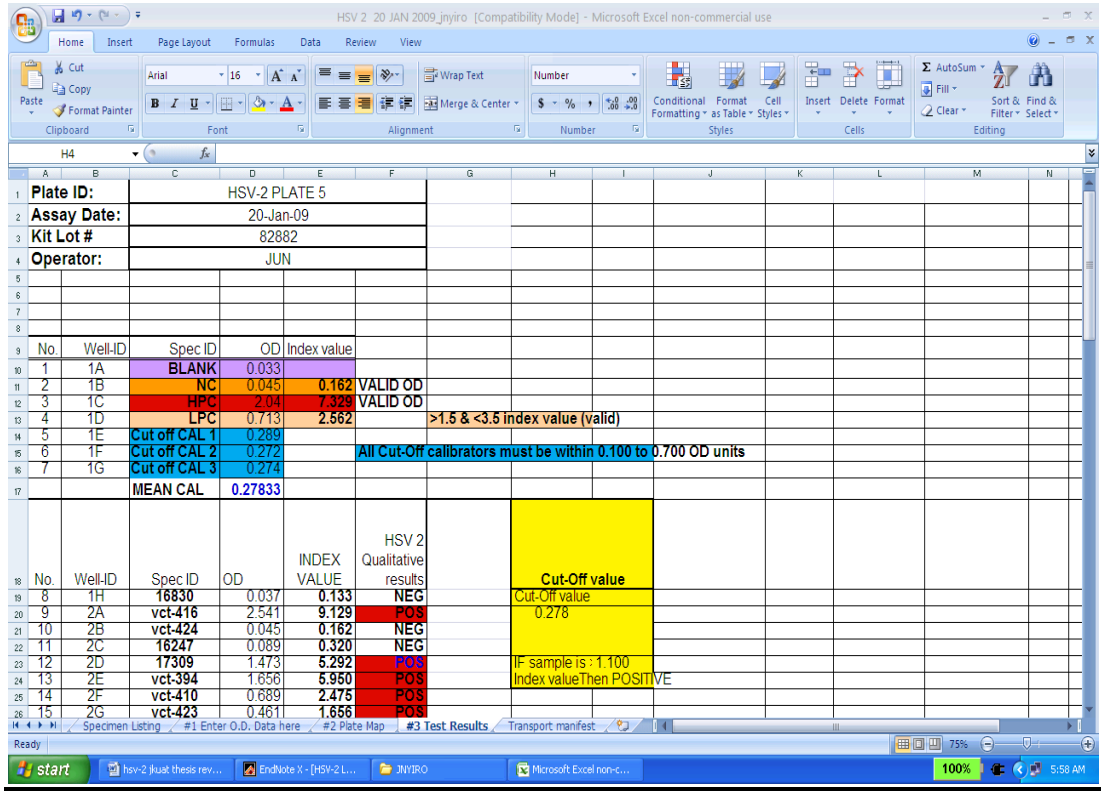
Plate ID:	HSV-2 PLATE 5	
Assay Date:	20-Jan-09	
Kit Lot #	82882	
Operator:	JUN	

PRINT THE PLATE MAP TO USE AS A GUIDE WHILE SETTING UP THE ASSAY IN THE LABORATORY

Specimen Identification

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	vct-416	0	0	0	0	0	0	0	0	0	0
B	NC	vct-424	0	0	0	0	0	0	0	0	0	0
C	HPC	16247	0	0	0	0	0	0	0	0	0	0
D	LPC	17309	0	0	0	0	0	0	0	0	0	0
E	CAL 1	0	0	0	0	0	0	0	0	0	0	0
F	CAL 2	0	0	0	0	0	0	0	0	0	0	0
G	CAL 3	0	0	0	0	0	0	0	0	0	0	0
H	16830	0	0	0	0	0	0	0	0	0	0	0

7.4 Test results sheet



1	**Plate ID:**		HSV-2 PLATE 5											
2	**Assay Date:**		20-Jan-09											
3	**Kit Lot #**		82882											
4	**Operator:**		JUN											
5														
6														
7														
8														
9	No.	Well-ID	Spec ID	OD	Index value									
10	1	1A	**BLANK**	0.033										
11	2	1B	**NC**	0.045	**0.162**	VALID OD								
12	3	1C	**HPC**	2.04	**7.329**	VALID OD								
13	4	1D	**LPC**	0.713	**2.562**		>1.5 & <3.5 index value (valid)							
14	5	1E	**Cut off CAL 1**	0.289										
15	6	1F	**Cut off CAL 2**	0.272		All Cut-Off calibrators must be within 0.100 to 0.700 OD units								
16	7	1G	**Cut off CAL 3**	0.274										
17			**MEAN CAL**	0.27833										
18	No.	Well-ID	Spec ID	OD	INDEX VALUE	HSV 2 Qualitative results		**Cut-Off value**						
19	8	1H	**16830**	0.037	**0.133**	NEG		Cut-Off value						
20	9	2A	**vct-416**	2.541	**9.129**	POS		0.278						
21	10	2B	**vct-424**	0.045	**0.162**	NEG								
22	11	2C	**16247**	0.089	**0.320**	NEG								
23	12	2D	**17309**	1.473	**5.292**	POS		IF sample is : 1.100						
24	13	2E	**vct-394**	1.656	**5.950**	POS		Index valueThen POSITIVE						
25	14	2F	**vct-410**	0.689	**2.475**	POS								
26	15	2G	**vct-423**	0.461	**1.656**	POS								

8.0 REFERENCES:

- HerpeSelect 2 ELISA IgG Focus Diagnostics kit package insert

APPENDIX II: DETERMINATION OF A CUT OFF VALUE FOR HSV-2 PREVALENCE

Validation studies on the performance of ELISA kits for IgG2 specific antibodies to Herpes Virus have suggested different options to adopt as a quality control measure while using the HerpeSelect IgG2 ELISA assay. This is due to its low specificity against various Gold standards and also with variation in its performance as observed on sera from African populations to that of the Western populations.(Morrow *et al.*, 2003; LeGoff *et al.*, 2008; Smith *et al.*, 2008)

Different cut off index values are recommended for use while determining HSV-2 prevalence in the absence of a Gold standard. A cut off index value of 1.1 is the recommended cut off value by the manufacturer for diagnostic purposes. A cut off index value of 2.2 has been recommended as appropriate for use in describing prevalence in the absence of a Gold standard and for results to compare with those obtained by the Kalon kit assay. Another cut off index value of greater than 3.4 is recommended for use if the assay is intended for HSV-2 sero-epidemiological surveys only (Laeyendecker *et al.*, 2004).

According to the manufacturer's specifications on performance of the HerpeSelect kit, all index values > 1.5 should be considered as sero-positive results while values <0.8 should be considered as sero-negative. A cut off index value of 1.1 can be used to

maximize sensitivity of the assay (Diagnostics, 2007). In order to determine HSV-2 prevalence of this population, a detailed analysis of the serological status of the study participants was explored based on the index values and their log transformation values.

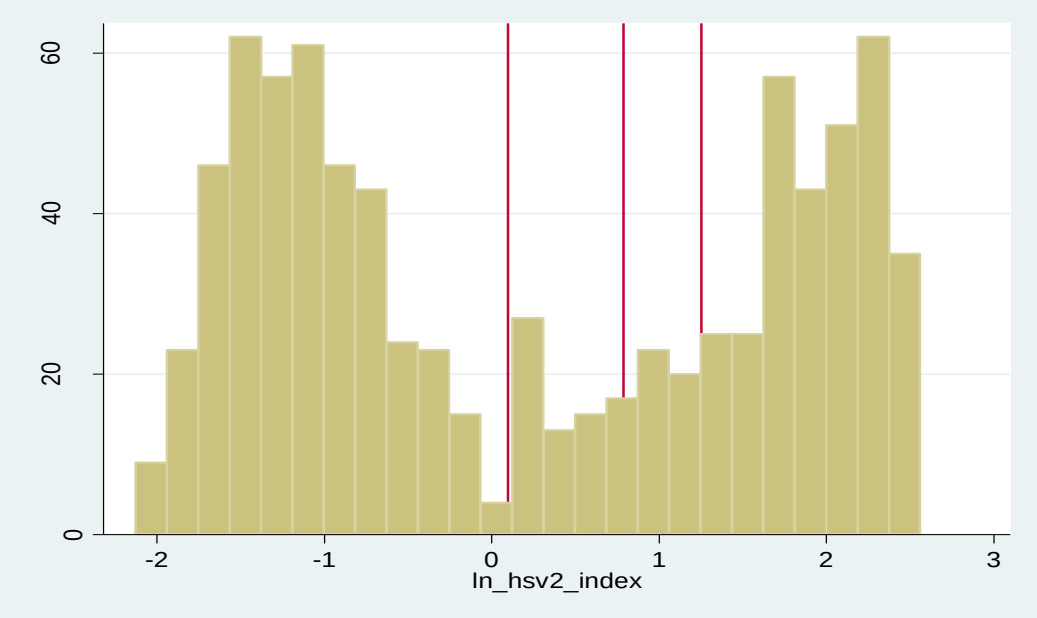


Figure 1: A graph of log transformed index value at cut off 1.1, 2.2 &3.5

A cut off index value of 1.1, shows a clear separation between the sero-positive and sero-negative results. Similar results were observed in a scatter plot of ln(index values) against age.

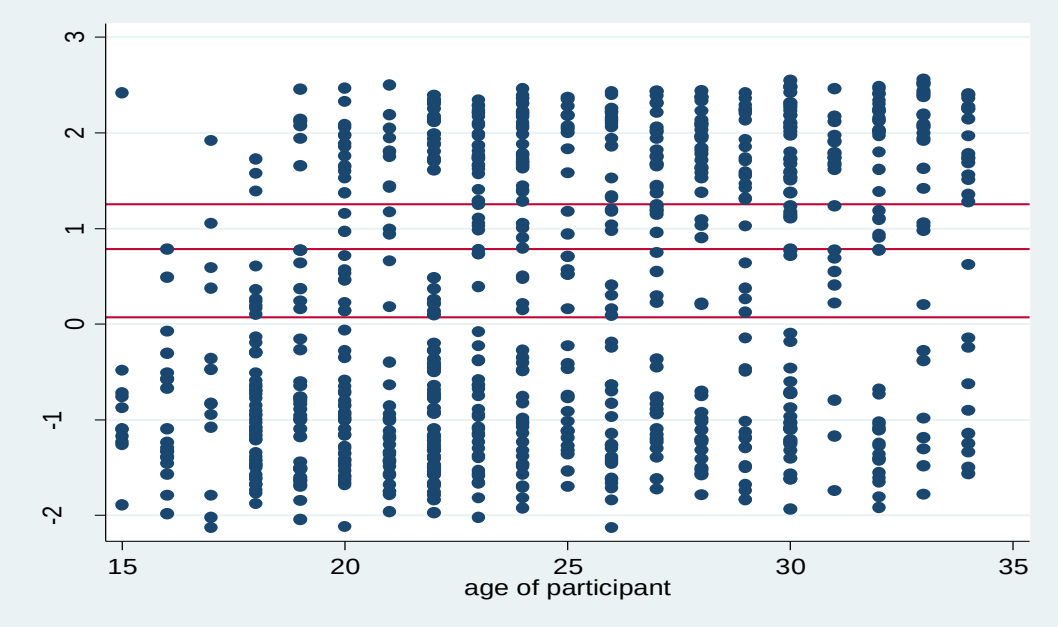


Figure 2: A dot plot of ln index values against age (cut off 1.1, 2.2 &3.5)

If ln index values are distributed against age in a scatter plot, at cut off of 1.1, the sero-positive and sero-negative results clearly separate out especially at older ages.

Since antibodies to HSV-2 persist in one's lifetime after infection, at older ages antibody titres are relatively high compared to young ages due to the difference in duration of exposure. An analysis of the cut off index value adjusted for age was therefore made and results shown in the figure below.

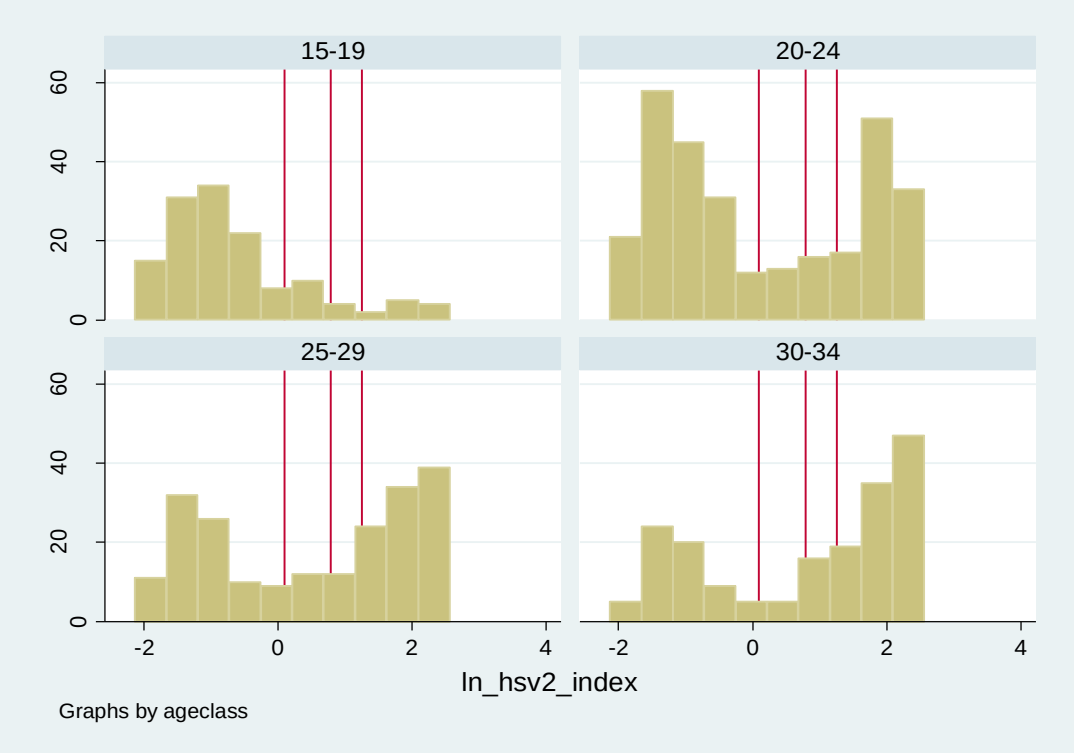


Figure 3: Variation of age with ln Index value at cut of 1.1, 2.2 &3.5

The above graph suggests that the ln(1.1) cut off might be a little too low for the youngest age group. This required a selection of an index cut off value greater than 1.1 to estimate the overall serological prevalence of this population. Therefore, cut off index value of 1.5 recommended as by the manufacturer as a low positive was assessed and results are as shown in figure 6 below. This cut off value was selected for use in describing HSV-2 incidence in this study.

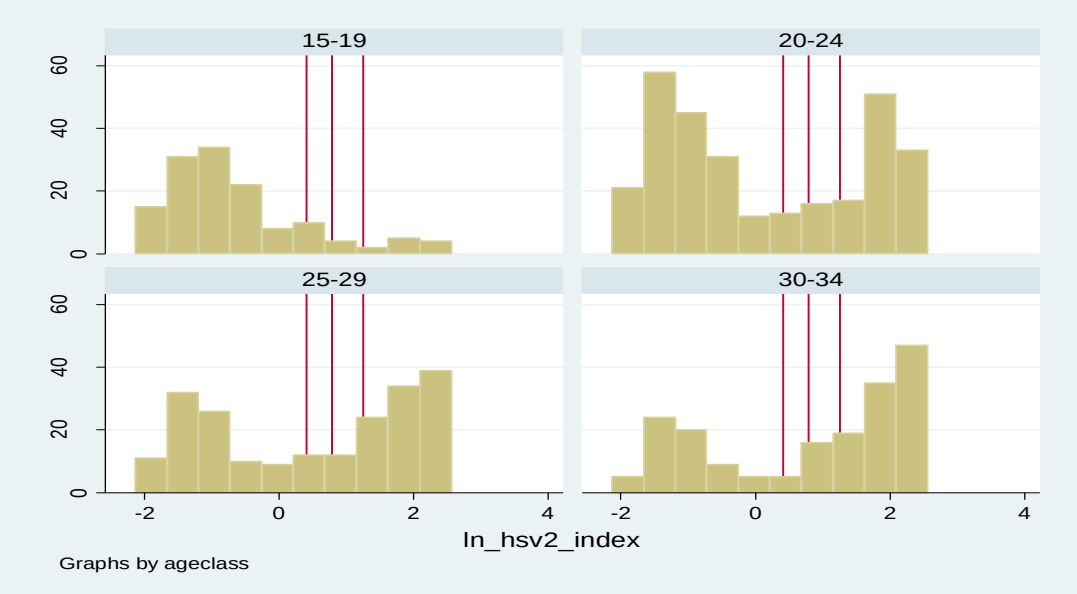


Figure 4: Variation of age with ln index values 1.5, 2.2 and 3.5

If index value of 1.5 was used for overall HSV-2 sero-status distribution, the results were as shown below.

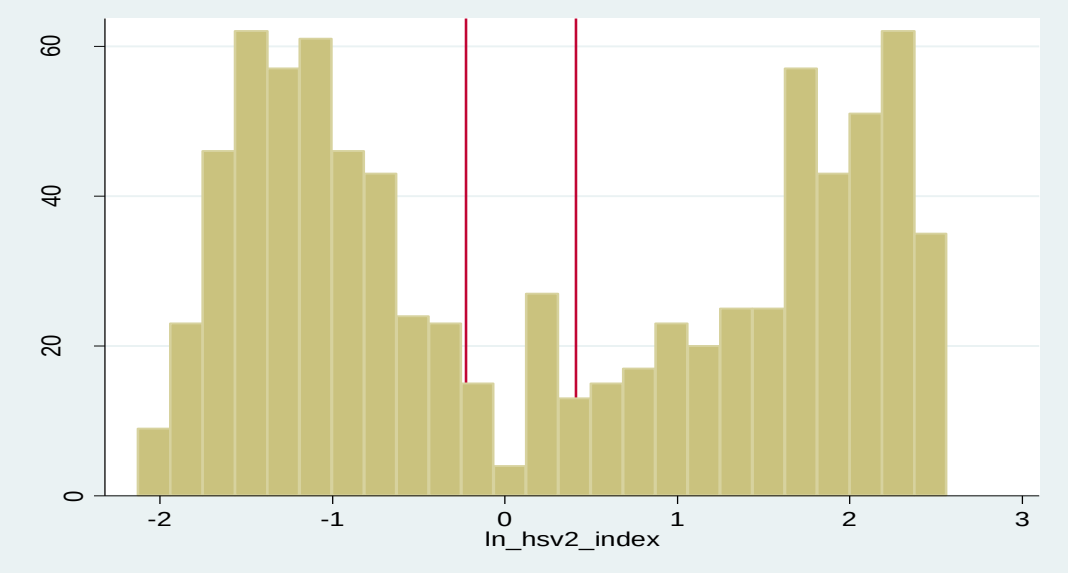


Figure 5: Distribution of HSV-2 serostatus by index values at cut off 0.9 &1.5

For this study therefore, a reference cut off index value of 1.5 was used to estimate the HSV-2 incidence based more on the general distribution of the index values and its ability to determine sero-status even at younger ages and on expectations that at this cut off, the assay would detect early seronconversions and therefore give a good estimate of maternal incidence.

In order to optimize specificity another cut off index value of >3.5 was also used to determine the HSV-2 prevalence and describe the factors associated with HSV-2 infection. This cut off value >3.5 is also the recommended for screening of HSV-2 in sero-epidemiological surveys. The index values lying between 0.9 and below 3.5 were in this case considered as intermediate sero-status.

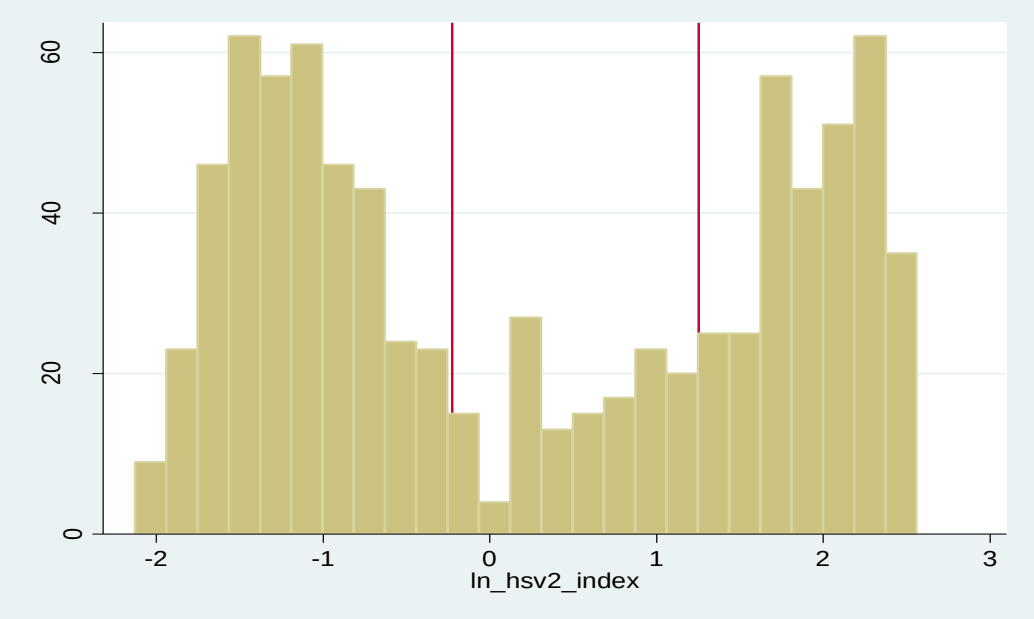
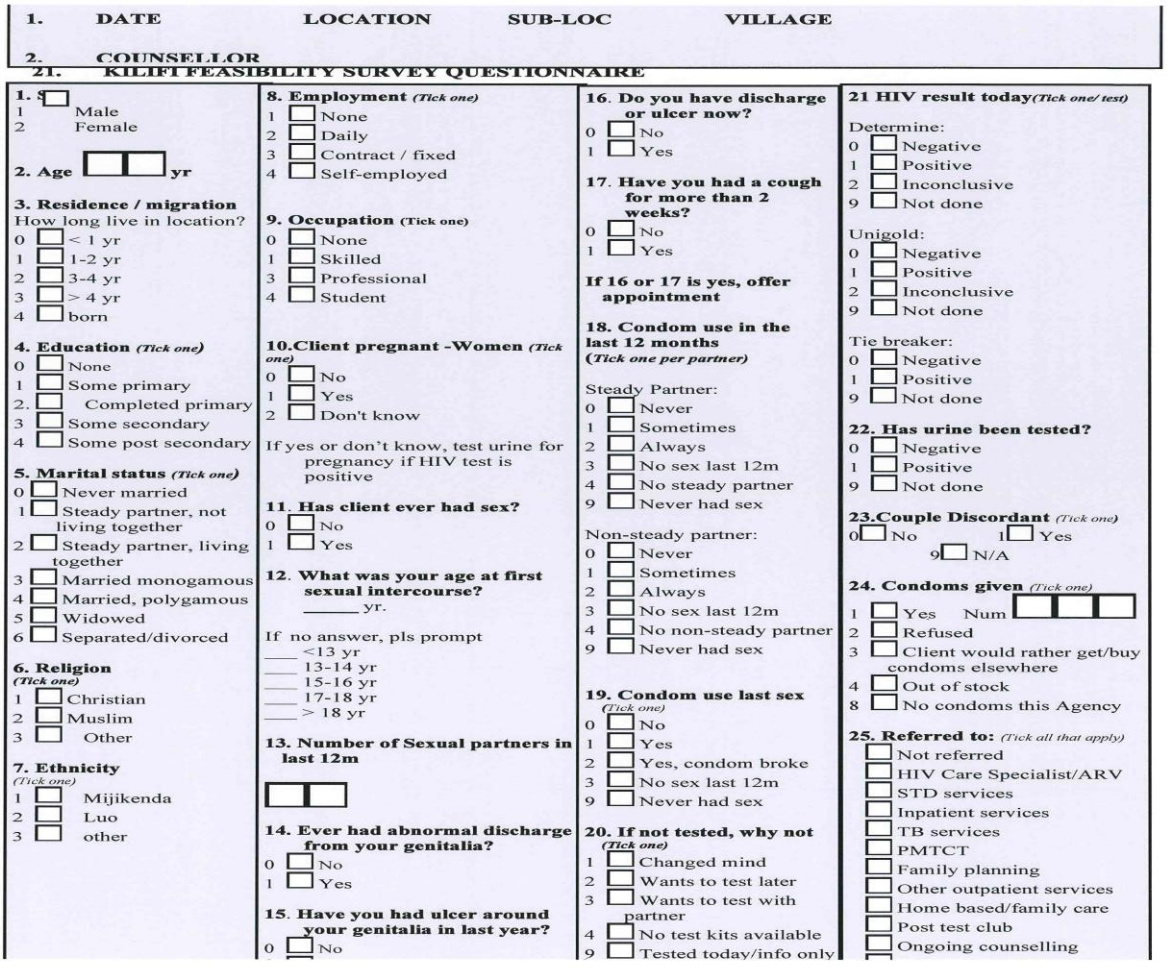


Figure 6: Distribution of HSV-2 serostatus by ln index values

APPENDIX III: HIV SURVEY QUESTIONNAIRE



1. DATE LOCATION SUB-LOC VILLAGE

2. COUNSELLOR

21. KILIFI FEASIBILITY SURVEY QUESTIONNAIRE

1. S ☐
1 Male
2 Female

2. Age ☐☐ **yr**

3. Residence / migration
How long live in location?
0 ☐ < 1 yr
1 ☐ 1-2 yr
2 ☐ 3-4 yr
3 ☐ > 4 yr
4 ☐ born

4. Education *(Tick one)*
0 ☐ None
1 ☐ Some primary
2. ☐ Completed primary
3 ☐ Some secondary
4 ☐ Some post secondary

5. Marital status *(Tick one)*
0 ☐ Never married
1 ☐ Steady partner, not living together
2 ☐ Steady partner, living together
3 ☐ Married monogamous
4 ☐ Married, polygamous
5 ☐ Widowed
6 ☐ Separated/divorced

6. Religion *(Tick one)*
1 ☐ Christian
2 ☐ Muslim
3 ☐ Other

7. Ethnicity *(Tick one)*
1 ☐ Mijikenda
2 ☐ Luo
3 ☐ other

8. Employment *(Tick one)*
1 ☐ None
2 ☐ Daily
3 ☐ Contract / fixed
4 ☐ Self-employed

9. Occupation (Tick one)
0 ☐ None
1 ☐ Skilled
3 ☐ Professional
4 ☐ Student

10.Client pregnant -Women *(Tick one)*
0 ☐ No
1 ☐ Yes
2 ☐ Don't know

If yes or don't know, test urine for pregnancy if HIV test is positive

11. Has client ever had sex?
0 ☐ No
1 ☐ Yes

12. What was your age at first sexual intercourse?
_____ yr.

If no answer, pls prompt
___ <13 yr
___ 13-14 yr
___ 15-16 yr
___ 17-18 yr
___ > 18 yr

13. Number of Sexual partners in last 12m

☐☐

14. Ever had abnormal discharge from your genitalia?
0 ☐ No
1 ☐ Yes

15. Have you had ulcer around your genitalia in last year?
0 ☐ No

16. Do you have discharge or ulcer now?
0 ☐ No
1 ☐ Yes

17. Have you had a cough for more than 2 weeks?
0 ☐ No
1 ☐ Yes

If 16 or 17 is yes, offer appointment

18. Condom use in the last 12 months
(Tick one per partner)

Steady Partner:
0 ☐ Never
1 ☐ Sometimes
2 ☐ Always
3 ☐ No sex last 12m
4 ☐ No steady partner
9 ☐ Never had sex

Non-steady partner:
0 ☐ Never
1 ☐ Sometimes
2 ☐ Always
3 ☐ No sex last 12m
4 ☐ No non-steady partner
9 ☐ Never had sex

19. Condom use last sex *(Tick one)*
0 ☐ No
1 ☐ Yes
2 ☐ Yes, condom broke
3 ☐ No sex last 12m
9 ☐ Never had sex

20. If not tested, why not *(Tick one)*
1 ☐ Changed mind
2 ☐ Wants to test later
3 ☐ Wants to test with partner
4 ☐ No test kits available
9 ☐ Tested today/info only

21 HIV result today *(Tick one/ test)*

Determine:
0 ☐ Negative
1 ☐ Positive
2 ☐ Inconclusive
9 ☐ Not done

Unigold:
0 ☐ Negative
1 ☐ Positive
2 ☐ Inconclusive
9 ☐ Not done

Tie breaker:
0 ☐ Negative
1 ☐ Positive
9 ☐ Not done

22. Has urine been tested?
0 ☐ Negative
1 ☐ Positive
9 ☐ Not done

23.Couple Discordant *(Tick one)*
0☐ No 1☐ Yes
9☐ N/A

24. Condoms given *(Tick one)*
1 ☐ Yes Num ☐☐☐
2 ☐ Refused
3 ☐ Client would rather get/buy condoms elsewhere
4 ☐ Out of stock
8 ☐ No condoms this Agency

25. Referred to: *(Tick all that apply)*
☐ Not referred
☐ HIV Care Specialist/ARV
☐ STD services
☐ Inpatient services
☐ TB services
☐ PMTCT
☐ Family planning
☐ Other outpatient services
☐ Home based/family care
☐ Post test club
☐ Ongoing counselling

APPENDIX IV: APPROVAL TO CONDUCT STUDY BY KEMRI ERC

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: kemri-hq@nairobi.mimcom.net; director@kemri.org; Website: www.kemri.org

KEMRI/RES/7/3/1 **DECEMBER 14th, 2008**

TO: **DR. EDUARD SANDERS, (PRINCIPLE INVESTIGATOR)**

THRO': **DR. NORBERT PESHU,**
CENTRE DIRECTOR, CGMR-C,
KILIFI

Forwarded 18/12/08

RE: **SSC No. 820:** A cross sectional, observational, feasibility study to assess recruitment and determine HIV sero-prevalence among volunteers

Dear Sir,

This is to inform you that during the 161st meeting of KEMRI/National Ethical Review Committee held 9th December 2008, the request ***TO USE STORED SAMPLES*** for the above referenced study was reviewed.

The letters dated 17 November and 7 October 2008 refers.

The Committee notes that the above study is a cross-section study to determine the prevalence of HSV-2 and factors associated with HSV/HSV-2 co-infection which will involve retrospective screening of all serum samples (n=850) for women aged 15-34 years for HSV-2 infection.

This will complete a survey that included cohort studies volunteers at a higher risk for HIV-1 infection (SSC 894 &1224) and now will analyse samples from a lower risk female population (SSC 820). The participants in SSC 820 were consented for storage and further studies for STIs.

We also note that it will not be possible to provide laboratory results back to the participants and as such the study has no individual benefits however, the data from the study will assess the HSV-2 burden among general women in Kilifi and concur that it will contribute knowledge to a potential public health problem.

The request to use stored samples in granted. You may continue with your study.

Respectfully,

R.C. Kithinji

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

KEMRI / WELLCOME TRUST RESEARCH PROGRAMME
18 DEC 2008
RECEIVED
P. O. Box 230-80108 KILIFI

In Search of Better Health

APPENDIX V: LETTER OF APPROVAL TO CONDUCT STUDY FROM IAVI

SAMPLE REQUEST FORM

Date: _____10/01/2008_____ Site: ___Kilifi_____

Requested By: ___Joyce Uchi Nyiro_____ E-mail: __jnyiro2005@yahoo.com___

Purpose (brief justification):

The serum samples are requested to investigate the serological prevalence of HSV-2 infection among women using a Gg-2 specific ELISA assay. Prevalence information will provide an assessment of HSV-2 infection and potential risk of sexual and vertical transmission.

Being a cohort of women that participated in the HIV-1 survey, we would like to determine the prevalence of HIV-1/HSV-2 co-infection. HSV-2 infection greatly enhances HIV-1 transmission and acquisition. Through correlation of the previous HIV-1 survey data and HSV-2 prevalence data we would determine the predictors of co-infection which will be useful to the KEMRI/IAVI research Center's program on HIV-1 vaccine efficacy trials.

Details of Samples Requested: (spreadsheet as an attachment to this form)

Serum samples from all women volunteers of the 2004 HIV-1 survey aged 15-34 years. Women should be residents of Mtondia and Kilifi town. We need 100 mcl of 850 of the IAVI protocol A study. Find characteristics of the subjects whose samples are requested in the attached spreadsheet.

Medical Affairs Approval: Sign _____ Date 09 Oct 08

Designee: Regional Clinical Programme Manager OR e mail acknowledgement attached.

Site Approval: Sign _____ Date 9 Oct 2008

Designee: Principle Investigator or Laboratory Manager

Proposed shipment date: n/a

Site Contact for follow up: E Sanders

Shipment sent: By: n/a Date: _____

Shipment Received By: _____ Date: _____