MOLECULAR CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS INFECTIONS AND ASSOCIATED CELL-MEDIATED IMMUNE RESPONSES AMONG FISHERMEN CO-INFECTED WITH HIV AND HSV-2 IN KISUMU, KENYA

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Molecular Characterization of Human Papillomavirus Infections and Associated Cell-Mediated Immune Responses among Fishermen Co-Infected With HIV and HSV-2 in Kisumu, Kenya

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A thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in Molecular Medicine in the Jomo Kenyatta University of Agriculture and Technology

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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

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LIST OF ABBREVIATIONS AND ACRONYMS

AF	Attributable fraction
AIDS	Acquired immune deficiency syndrome
aIRR	Adjusted incidence risk ratio
aOR	Adjusted odds ratio
ART	Antiretroviral therapy
ATCC	American Type Culture Collection
CBC	Complete blood count
CDC	US Centers for Disease Control and Prevention
CI	Confidence interval (at 95%)
CIN	Cervical intraepithelial neoplasia
CT	*Chlamydia trachomatis*
CTL	Cytotoxic T lymphocytes
ERC	Ethical review committee
FACES	Family Aids Care and Education Services
GUD	Genital ulcer disease
HIV	Human immunodeficiency virus
HPRT	Hypoxanthine-guanine phosphoribosyl transferase
HPV	Human papillomavirus
HR-HPV	High risk human papillomavirus
HSV-2	Herpes simplex virus type 2
IATA	International air transport association
ICC	Invasive cervical cancer
IFNγ	Interferon-gamma
IgG	Immunoglobulin G
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-10	Interleukin-10

IL-13	Interleukin-13
IRB	Institution review board
IRR	Incidence risk ratio
KAIS	Kenya AIDS Indicator Survey
LR-HPV	Low risk human papillomavirus
LSIL	Low squamous intraepithelial lesions
MAPs	Most at-risk populations
MFI	Median fluorescent intensity
MSM	Men who have sex with men
OR	Odds ratio
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PrEP	Pre-exposure prophylaxis
QA	Quality assurance
QC	Quality control
RCTP	Research Care and Training Program
RPM	Rotations per minute
RR	Relative risk
SOP	Standard operating procedure
STD	Sexually transmitted disease
STI	Sexually transmitted infection
SIL	Squamous intraepithelial lesions
Th1	T helper 1
Th2	T helper 2
TNF-α	Tumor necrosis factor – alpha
VLP	Virus like particle
VMMC	Voluntary medical male circumcision
WHO	World health organization

DEFINITION OF TERMS

Attributable fraction:	The proportion of disease resulting from a specific causative/etiological agent
HPV/HIV/HSV-2/ Prevalence:	Baseline burden of HPV/HIV/HSV-2 infections
HIV Incidence:	Detection of HIV at study exit among participants who tested negative for HIV at baseline
HSV-2 Incidence:	Detection of HSV-2 at study exit among participants who tested negative for HSV-2 at baseline
HPV Incidence:	Detection of new genotype of HPV that was not detected at any previous study visit.
High-Risk HPV:	Class of HPV types associated with increased risk of causing cancer.
Low-Risk HPV:	Class of HPV types associated with decreased risk of causing cancer.
HPV persistence:	Detection of HPV infection after a period of ≥6 months from the initial HPV DNA positive status.
High-Risk HPV persistence:	Detection of any high-risk HPV genotype at subsequent visits that are ≥6 months apart from the initial infection with a high-risk genotype.
Low-Risk HPV persistence:	Detection of any low-risk HPV genotype at subsequent visits that are ≥6 months apart from the initial infection with a low-risk genotype.

Genotypic HPV persistence:	Detection of a specific HPV genotype at subsequent visits that are ≥6 months apart from the initial detection of the same high-risk HPV genotype.
Genotypic HPV clearance:	Testing HPV DNA negative for a specific genotypes at two or more subsequent visits ≥3 months apart from the initial detection of that specific HPV genotype.
High-Risk HPV clearance:	Testing HPV DNA negative for any high-risk HPV genotype at two or more subsequent study visits ≥3 months apart from the initial detection of the high-risk HPV genotype(s).
Low-Risk HPV clearance:	Testing HPV DNA negative for any low-risk HPV genotype at two or more subsequent study visits ≥3 months apart from the initial detection of the low-risk HPV genotype(s).
Most at risk population:	These are characterized sub-populations that have higher levels of particular infections than are distributed in the general population. It is believed that there could be a common factor which increases their risk to infection
Natural immune response:	This is the activation of the body's immune response to an infectious agent results from a natural infection.
HPV Seropositivity:	This is the detection of antibodies against natural HPV infection in serum. It signifies prior exposure to HPV infection that resulted in successful induction of humoral immune response.

Sexually high-risk behavior:	These are sexual behaviors characterized by engaging in sexual practices that increase their risk of acquiring sexually transmitted infections (STIs) e.g. multiple sexual partners (sexual concurrency), high number of sexual partners, no condom use, transactional sex, sex work, anal sex, among sexual other practices.
Risk factors:	Variables that increase the risk of being infected with any of the three viruses
2vHPV:	Two-valent HPV vaccine (Cervarix® bivalent HPV vaccine) targeting two HPV genotypes (HPV-16 and HPV-18),
4vHPV:	Four-valent HPV vaccine (Gardasil® quadrivalent HPV vaccine) targeting four HPV genotypes (HPV-6, HPV-11, HPV-16 and HPV-18)
9vHPV:	Nine-valent HPV vaccine (Gardasil-9® nonavalent HPV vaccine) targeting nine HPV genotypes (HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58).

ABSTRACT

Persistent HPV infection is associated with cervical, vulvar, anal and penile cancers. High incidence rates of these cancers are observed in Africa compared to other parts of the world, with HIV co-infection exacerbating the natural course of HPV infections, and HSV-2 infection linked to HIV acquisition. Although, these three viral infections are highly prevalent in the fishing communities, there is limited data on the incidence of HPV, HIV and HSV-2 among fishermen in Kisumu, Kenya. Additionally, HPV genotypic interactions (type competition) and associated cell-mediated immune responses have not been evaluated among fishermen. This study sought to carry out molecular characterization of genotypic HPV infections, cytokine immune responses against HPV infection, HIV/HSV-2 co-infections, and the associated risk factors among fishermen around Lake Victoria in Kisumu, Kenya. Three hundred fishermen were followed-up every three months for 12 months. Socio-demographic data was collected using a structured questionnaire. Genital swabs for HPV testing as well as blood for immune response assays and HIV/HSV-2 serology were collected. HPV infection was determined using PCR, cell-mediated cytokine responses evaluated by Luminex assay and statistical analyses performed using Stata version 12. Baseline HPV prevalence was 49.7% and incidence was 53.5%. Factors associated with HPV prevalence were: lack of circumcision aOR=2.38 (95% CI: 1.18 – 4.77), history of STI (aOR=3.25; 95% CI: 1.63–6.14) and HIV infection (aOR=2.43; 95% CI: 1.10–5.37). Consistent condom use and being single were protective. Factors associated with HPV incidence were: baseline HPV infection (aRR=9.35; 95% CI: 3.03–28.90) and multiple partners (aRR=14.50; 95% CI: 1.70–31.58). Condom use was protective. HPV persisted in 86% of fishermen and factors associated with HPV persistence were: marital status (married); aOR=3.00 (95% CI: 1.15–7.83) and baseline HIV infection (aOR=4.30; 95% CI: 1.83–10.11). Baseline HIV prevalence was 23.3% (95% CI: 18.5 – 28.1) and risk factors were: older age (aOR=2.13; 95% CI: 1.25–5.07), history of STI (aOR=4.21; 95% CI: 2.07–9.34), baseline HPV infection (aOR=2.13; 95% CI: 1.05–4.77), number of lifetime sexual partners (>5) aOR=5.76 (95% CI: 1.41–13.57) and transactional sex (aOR=10.98; 95% CI: 1.86–19.34). Consistent condom use was protective. HIV incidence was 4.2 (95% CI: 1.3–7.1) per 100 person-years (pyr) and being single **(**aIRR=8.32; 95% CI: 1.27–54.67) was a risk factor while consistent condom use was protective. Baseline HSV-2 prevalence was 56.3% (95% CI: 50.7–62.0) and associated factors were: older age (aOR=1.96; 95% CI: 1.16–2.85), history of STI (aOR=2.12; 95% CI: 1.19–3.91), HIV (aOR=2.22; 95% CI: 1.17–4.22), ever married (aOR=3.80; 95% CI: 1.42–11.90), most recent sexual act with sex worker/casual partner (OR=3.56; 95% CI: 1.49–8.62) and inconsistent condom use (aOR=6.34; 95% CI: 2.24–13.04). HSV-2 incidence was 23.6 (95% CI: 15.4–31.8) /100 pyr and risk factors were: persistent high-risk (HR) HPV (aIRR=3.35; 95% CI: 1.21–11.37), multiple partners (aIRR=4.77; 95% CI: 1.12–11.38) and inconsistent condom use (OR= 3.03; 95% CI: 1.17 – 8.58). Molecular characterization assessment revealed a total of 19 genotypic associations, of which two were negative. HPV-70 was negatively associated with HPV-52 (p=0.018) and HPV-58 (p=0.011). Men infected with multiple HPV genotypes were at increased odds of being detected with a vaccine relevant HPV genotype compared to men with single HPV genotype infection (OR=8.17; 95% CI: 3.77–18.01). T-helper type-1 (Th1) cytokines IFN-γ (5.1-fold; mean fold increase) and IL-2 (4.2-fold) were significantly ($p<0.0001$) upregulated among men with HPV clearance and not in men with HPV persistence, compared to HPV uninfected men. In the three groups of men,

there were no significant changes in Th1 cytokine TNF-α, and Th2 cytokines IL-4, IL-6, IL-10 and IL-13. In conclusion, Fishermen in Kisumu, Kenya had a high burden of HPV, HIV and HSV-2 infections and were at increased risk for incident infection with these three viruses. Key risk factors included: unsafe sex, history of STI, age and marital status. These three viruses were co-factors to each other forming a triad of viral infections. Negative associations between HPV genotypes were rare among fishermen, suggesting lack of HPV genotype competition and potential for type replacement among men. Th1 cytokine response was associated with HPV clearance among these men.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Human papillomavirus (HPV) is associated with cervical cancer as well as anogenital cancers in both men and women (Rubin *et al.,* 2001; Daling *et al.,* 2004; Parkin *et al.,* 2006b; Backes *et al.,* 2009; Forman *et al*., 2012; De Vuyst *et al*., 2013). Cervical cancer is the leading cause of cancer related mortalities among females in Africa, with an incidence rate of 42.7% and a mortality rate of 27.6% in Eastern Africa: the highest rates worldwide (Ferlay *et al*., 2015; Torre *et al*., 2015). Although HPV is a sexually transmitted infection (STI) with heterosexual transmission accounting for the high HPV burden in Africa, some studies found that the use of male condom is not as efficacious in protecting against HPV infection as it is for other STIs (Manhart *et al.,* 2002; Smith *et al.,* 2010a). However, there are highly effective HPV vaccines available for protection against infections with some HPV genotypes (Petrosky *et al*., 2015).

HPV is the most common STI in both genders, with an estimate of over 6 million new infections per year in the United States (Cates *et al.,*1999; Weinstock *et al.,* 2004). Several studies have reported HPV prevalence among women in Africa ranging from 3% to 48% (Keita *et al*., 2009; Salih *et al*., 2010; De Vuyst *et al*., 2013; Ferlay *et al*., 2015) and that among men ranged from 14% to 58% (De Vuyst *et al*., 2013). Over 200 HPV types have been reported and approximately 40 types have been shown to infect the genital tract (Wiley *et al.,* 2002; Schiffman *et al.,* 2003). Infection with anogenital HPV is the leading cause of cervical cancer (Bosch *et al.,* 2003; Bosch *et al.,* 2008), vulvo-vaginal cancer (Al-Ghamdi *et al.,* 2002; Daling *et al.,* 2002), penile cancer (Rubin *et al.,* 2001), anal cancer (Frisch *et al.,* 1997) and other malignancies (Syrjanen *et al.,* 2005). HPV must be persistent within the host epithelial cells as a preliminary step toward advanced neoplastic changes (Moscicki *et al.,* 2006).

In 2008, fishermen in Kisumu, Kenya [a sexually high-risk group of men for HIV and STIs (Kwena *et al.,* 2010)], had a prevalence of HPV infection estimated at 58%

(Ng'ayo *et al.,* 2008a). However, there is limited data on HPV incidence rates. Cross-sectional studies among men who have sex with men (MSM) or women have shown that HIV infection is associated with HPV persistent infection and disease (Moscicki *et al.,* 2004a; Moscicki *et al.,* 2004b; Palefsky *et al.,* 2006), while HSV-2 infection is associated with incident HIV infection (del Mar Pujades *et al.,* 2002; Reynolds *et al.,* 2003; Brown *et al.,* 2006; Freeman *et al.,* 2006; Delany-Moretlwe *et al.,* 2009).

The high prevalence of HPV among sexually high-risk populations is partially explained by HPV being one of the infections for which the male condom is not a highly effective prevention tool (Manhart *et al.,* 2002; Smith *et al.,* 2010a). This is because HPV transmission requires skin to skin contact and genital HPV infects the entire genitals including the scrotum, vulva, anal and perianal anatomic sites, which the male condom may not protect (Manhart *et al.,* 2002). The elusiveness of HPV therapy, and the challenge with successful treatment of HPV-related diseases (Wright *et al*., 2007; Castle *et al*., 2009; Taylor *et al*., 2010), leaves prevention strategies as the only feasible way to fight the HPV epidemic. HPV virus-like particle (VLP) vaccines have been shown to be safe and efficacious against HPV and HPV-related diseases (Garland *et al*., 2007; NCT00543543, 2007; Giuliano *et al*., 2011a; Naud *et al*., 2014; Joura *et al*., 2015; Lopalco, 2016; Signorelli *et al*., 2017).

However, the three licensed HPV vaccines provide protection against only two, four or nine HPV genotypes, respectively, among the over 40 important HPV genotypes associated with anogenital cancer (Wiley *et al.*, 2002; Schiffman *et al.*, 2003; Lopalco, 2016). Only a few studies have evaluated cross-protection ability of these vaccines against non-vaccine HPV genotypes yielding conflicting results (Wideroff *et al*., 1999; Marais *et al*., 2000; Combita *et al*., 2002; Rositch *et al*., 2012b). Additionally, few studies have explored the potential of vaccine genotype-replacement that could neutralize the gains of HPV vaccination (with 4vHPV vaccine) in preventing HPV related disease (Chaturvedi *et al*., 2011; Rositch *et al*., 2012a; Rositch *et al*., 2012b; Vaccarella *et al*., 2013).

Studies on HPV disease among women have demonstrated protective adaptive immune response in women with neoplastic changes, effective at slowing or

eliminating disease progression (Malejczyk *et al*., 1997; Moscicki *et al*., 1998; Bosch *et al*., 2008; Wentzensen *et al*., 2011; Franceschi *et al*., 2014). However, the natural history of HPV infections among men has not been explored (Giuliano *et al.,* 2008a) and thus there is limited knowledge on the incidence and persistence of HPV infections among men. Moreover, the host-pathogen interaction and the kinetics of immune response to HPV infections among men is not well understood (Svare *et al.,* 1997; Hagensee *et al.,*. 2000; Slavinsky *et al.,* 2001; Giuliano *et al.,* 2008a). Few prospective studies that evaluated HPV persistence among men never explored factors associated with HPV persistence (Van Doornum *et al.,* 1994; Wikstrom *et al.,* 2000; Kjaer *et al.,* 2005; Lajous *et al.,* 2005).

Studies on HPV-related disease (cervical intraepithelial neoplasia - CIN) among these women have confirmed the role of T-cell immune response among women with neoplastic changes and clearance of persistent HPV infection (Nakagawa *et al.,* 1996; van Poelgeest *et al.,* 2006). Observed increased persistence of HPV infection and progression to HPV disease among immunosuppressed women, illustrates the critical role of the CD4 T cell mediated immune response in the resolution and control of HPV infections (Benton *et al.,* 1996; Palefsky *et al.,* 2006). Multiple recurrences of cervical HPV infections as well as an increased incidence of both cutaneous and genital warts that appears to reflect an increased risk of progression from sub-clinical to clinical disease have been shown among HIV infected patients (Fennema *et al.,* 1995; Fruchter *et al.,* 1996). Additionally, prolonged persistence of high risk HPV genotypes and a high incidence of cervical intraepithelial neoplasia (CIN) 2/3 have been observed in HIV infected young girls who are otherwise healthy (Moscicki *et al.,* 2004a; Moscicki *et al.,* 2004b). Therefore, HIV infected patients are at increased risk for persistent HPV infection and related sequelae (Fennema *et al.,* 1995; Fruchter *et al.,* 1996). Given the African HIV pandemic, the inaccessibility of HPV vaccine in low-resource countries and unavailability of interventions for men, worsen the African HPV epidemic.

Based on 2012 global estimates, the incidence of cervical cancer is 2-fold higher in low-income countries than those with high income, with highest rates observed in

Eastern Africa (43 per 100,000) compared to less than six per 100,000 in Australia and Western Asia (Serrano *et al.,* 2018). Similarly, mortality rates from cervical cancer are 3-fold in higher with highest rates documented in Eastern Africa (28 per 100,000) compared to less than two per 100,000 in Western Europe, Australia and Western Asia (Serrano *et al.,* 2018). Although, global rates of other anogenital cancers attributable to HPV infection are much lower than those for cervical cancer (1/100,000 vs 8/100,000), rates of anal cancer in MSM are over 3-times higher (35 per 100,000) than cervical cancer in women (8 per 100,000) and even double in HIV positive MSM, at 75 per 100,000 (Hoots *et al.*, 2009).

Infection with HPV has been found to be the most important risk factor globally for cancers associated with infectious agents, and responsible for approximately 5% of world cancer burden: with the highest attributable fraction (AF) of 7% observed in developing countries compared to about 2% in developed countries (Parkin, 2006a; Forman *et al*., 2012; De Vuyst *et al*., 2013). Sub-Saharan Africa and India bear the greatest burden with AF of 14% and 16%, respectively (Forman *et al*., 2012). Concerning HPV-associated cancer sites, cervical and anal cancers documented leading AF: cervical (AF; 100%), anal (AF; 90%), penile (AF; 40%) and vulva-vaginal (AF; 40%) (Parkin, 2006a, Forman *et al*., 2012).

Persistent high-risk HPV infection is required for progression from normal cytology to HPV-associated malignancies in both sexes (Parkin, 2006a; Forman *et al*., 2012 Moscicki *et al.,* 2012). However, the distribution of HPV-related diseases is evidently more weighted among women compared to men (Parkin, 2006a; Forman *et al*., 2012; De Vuyst *et al*., 2013; Torre *et al*., 2015). For instance, HPV related cervical cancer is one of the leading causes of cancer related deaths among women in the developing world, whereas HPV related penile and anogenital cancers are among the least causes of cancer related deaths among men (Forman *et al*., 2012; De Vuyst *et al*., 2013; Torre *et al*., 2015). It is tempting to conclude that the natural course of HPV infection and subsequent development of HPV disease differ significantly by gender.

Sexually high-risk men are important drivers of HIV/STIs in the general population and interventions aimed at reducing transmission must include men as one of the

targets. Therefore, understanding the dynamics of genotypic HPV infection, its biological co-factors/risk factors and cellular immune response memory among men, remains an important research question to inform development of an efficacious prevention strategy against HPV infection. However, HPV is one of the most difficult pathogens to study. Its detection relies on high expertise molecular techniques, its pathogenesis and immunobiology is complicated by existence of many genotypes, and its management remains elusive.

1.2 Statement of the Problem

Eastern Africa experiences high incidence rate of HPV-related cancers and mortality. With the high prevalence of HIV infection, these low resource countries remain overburdened by morbidity and mortality from HPV related malignancies. Although, rates of incident HPV infection, persistence and genotypic HPV infections in relevance to HPV vaccine genotypes are not well-characterized in men, tailored interventions against these infections and associated precancerous lesions that include sexually high-risk men are needed to reduce these cancers.

1.3 Justification of the Study

Persistent HPV infection is an important cause of cervical and anogenital cancers. Men with high-risk sexual behaviors are important in the transmission dynamics of HPV and other STIs at population level. Fishermen in Kisumu, Kenya, form a strong core group for HIV/STI transmission dynamics in the general population. For interventions aimed at controlling HPV among women – the greatest bearers of the HPV disease burden, it is essential that these men must be involved. Therefore, it is crucial to develop intervention strategies that target men especially those in sexually high-risk groups.

The prevalence of genital HPV among fishermen in Kisumu, Kenya, is high but rates of HPV incidence and persistence, currently remain unknown. Moreover, other important viral infections that share the same mode of transmission with HPV, such as HIV and HSV-2 have not been evaluated as biological co-risk factors for HPV infection/persistence among fishermen in Kisumu, Kenya. Several studies that have documented these associations between HIV infection and HPV persistence/ disease

as well as HSV-2 infection as a risk factor for HIV acquisition, were cross-sectional in nature and limited to MSM or women.

Although, natural history and immunobiology of HPV infections among women has been widely studied, the dynamics of HPV infection, associated immune responses and subsequent development of HPV disease among men remains unexplored. Indeed, there is limited knowledge on the role of cellular adaptive immune response in the clearance or persistence of HPV infections among men and also the host-pathogen interaction in men is not well understood. Additionally, there is no data on the relevance of the 9-valent HPV vaccine among men, despite having prevalent multiple HPV infections, whose rates do not decline with age.

No study has evaluated interactions between vaccine and non-vaccine HPV genotypes as well as potential of vaccine genotype-replacement among fishermen in Kisumu, Kenya. Molecular studies evaluating genotypic interactions among 9-valent HPV vaccine genotypes and the remaining non-vaccine HPV genotypes including potential for genotype replacement are needed to inform future HPV vaccine studies. Therefore, genotypic characterizations of HPV infections in this population would provide useful information on the relevance of HPV vaccination among fishermen. Therefore, evaluating HPV genotype associations in relevance to HPV vaccine genotypes, cellular immune responses to natural HPV infections among fishermen as well as co-factors for HPV infection/persistence was needed to provide useful information that could facilitate development of intervention strategies aimed at preventing HPV infection and persistence among men.

Additionally, estimating the rates of HIV and HSV-2 incidence among fishermen will inform public health intervention programing targeted to this population underserved with health services. Additionally, lack of knowledge on the characteristics of genotypic HPV infections, type-specific competition and natural cellular immune responses among men limits the required policies, planning and programing of HPV vaccination among men. Therefore, this study was designed to evaluate the epidemiology of HPV, HIV and HSV-2 infections, associated risk factors, characterize clinically relevant genotypic HPV infections and assess natural cell-

mediated immune responses against nine HPV genotypes (included in the 9vHPV vaccine), among fishermen around the shores of Lake Victoria in Kisumu, Kenya.

The key findings from this study provide insightful knowledge on the burden and risk of HPV, HIV and HSV-2 among fishermen as well as the potential role of these men with high-risk sexual behavior in fueling these parallel epidemics. The study also highlights immunological factors contributing to the natural course of HPV infections among men. This information is essential in the development of effective programs targeted at reducing HPV and HIV burden in both men and women. For instance, it will be useful in the context of exploring topical male microbicides against HPV or targeted antiretroviral therapy (ART) against HIV as interventions to limit male acquisition of HPV/HIV and subsequent reduction of transmission to their female partners who bear the greatest burden of HPV associated diseases.

1.4 Research Questions

1) What is the epidemiology (prevalence, incidence and associated risk factors) of HPV, HIV and HSV-2 infections among fishermen along Lake Victoria, Kisumu, Kenya?
2) What is the genotypic characteristics of HPV infections in relation to nonavalent HPV vaccine genotypes among these fishermen?
3) What natural cell-mediated cytokine immune responses are involved in the clearance or persistence of HPV infections among men?

1.5 Null Hypotheses

1. The epidemiology (prevalence, incidence and associated risk factors) of HPV, HIV and HSV-2 infections among fishermen along Lake Victoria in Kisumu, Kenya, is not different from those observed in the general population.
2. There are no genotypic associations between nonavalent HPV vaccine genotypes and prevalent HPV genotypes detected among fishermen along Lake Victoria in Kisumu, Kenya.
3. Cytokine immune responses are not involved in the clearance or persistence of natural HPV infections among fishermen in Kisumu, Kenya.

1.6 Objectives

1.6.1 General Objective

To characterize clinically relevant genotypic HPV infections and associated natural cell-mediated immune responses among fishermen co-infected with HIV and HSV-2 in Kisumu, Kenya.

1.6.2 Specific objectives

1. To estimate the prevalence, incidence and associated risk factors for HPV, HIV and HSV-2 infection among fishermen in Kisumu, Kenya
2. To determine prevailing clinically relevant HPV genotypes and their association with nonavalent HPV vaccine genotypes among these fishermen
3. To evaluate cytokine immune responses against HPV infections among the fishermen

CHAPTER TWO

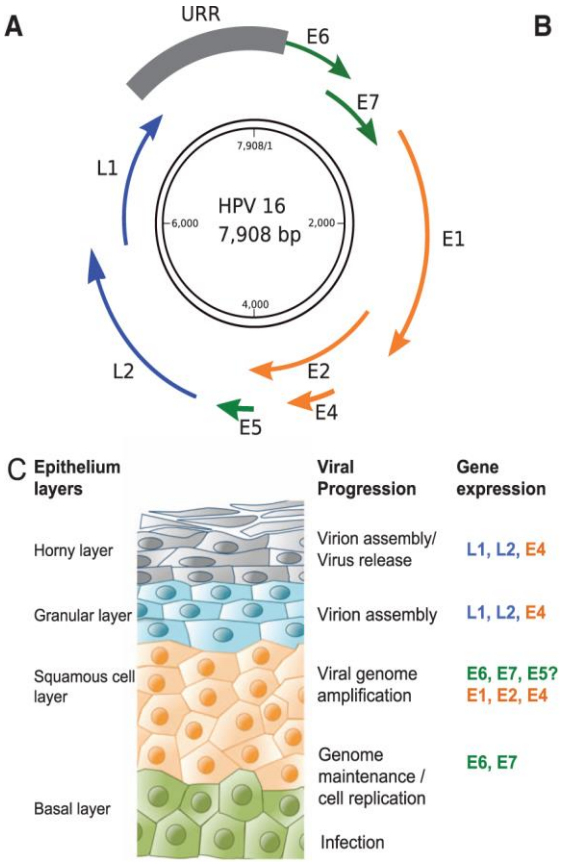
LITERATURE REVIEW

2.1 HPV Infections

2.1.1 Natural history and Pathogenesis of HPV infection

Human papillomavirus is a small DNA virus with a genome of approximately 8000 base–pairs (Scheurer *et al.,* 2005). HPV has a double stranded DNA genome and up to 10 open reading frames (ORFs). The ORFs within the early genes (E) region code for proteins involved in the regulation of viral replication and the viral life cycle (Figure 2.1), whereas the two ORFs within the late genes (L) region encode proteins that form viral capsids (Scheurer *et al.,* 2005). The "E" and "L" designations indicate that these genes are expressed early and late respectively, in the HPV life cycle (Scheurer *et al.,* 2005). In addition, the viral genome contains a region that does not direct the synthesis of viral proteins, but contains regulatory DNA elements that act as binding sites for viral and cellular proteins that regulate viral replication and transcription (Howley *et al.,* 2001). HPV do not express the entire complement of enzymes required for genome replication (Howley *et al.,* 2001). Therefore, HPVs re-program the host cell's DNA replication machinery to replicate their own genomes.

HPV infects both mucosal and cutaneous undifferentiated keratinocytes of the epithelial basal layer (Figure 2.1) probably through hair follicles or micro wounds resulting from skin abrasion (Egawa *et al.,* 2003; Kines *et al.,* 2009). The molecular process of virion attachment, cellular surface receptors and actual entry into host cells is not well understood, although a few studies have suggested that primary viral attachment is mediated through HPV capsid proteins binding heparin sulfate proteoglycan cellular surface molecule (Giroglou *al.,* 2001; Shafti-Keramat *et al.,* 2003) or through clathrin-dependent pathway (Day *et al.,* 2003). After entry into the host cell, the viral genome is incorporated into the host cell nucleus and replicated by cellular polymerase (Doorbar *et al.,* 2012). Expression of early gene-6 (E6) and early gene-7 (E7) HPV proteins lead to disrupted cell differentiation process resulting to uncontrolled proliferation of keratinocytes (Cheng *et al.,* 1995; Sherman *et al.,* 1997).



B

Locus	Function
URR	-Harbours transcription factor-binding sites -Controls gene expression
E6	-Promotes cellular immortalization by degradation of p53 -Modifies cell adhesion and differentiation by degradation of TAp63 and p73
E7	-Promotes pRb degradation, permitting cell progression to S-phase of cell cycle -Induces chromosomal instability
E5	-Induces cell proliferation -Contributes to evasion of apoptosis -Downregulates MHC expression
E1	-ATP-dependent DNA helicase activity -Role in viral DNA replication
E2	-Coactivator of viral DNA replication -Transcrition repressor of HPV E6 and E7 -Regulates cell cycle and apoptosis -Interacts with chromatin for segregation of viral genome
E4	Binds cytoskeletal proteins and disrupts cytoskeletal structure of the G2 arrested cell
L2	-Minor capsid protein -Recruits L1 protein for virus assembly
L1	-Major capsid protein -Can autoassemble into VLPs

A. HPV DNA genome; B. Function of HPV genes; C. Life cycle of HPV. (Bravo *et al.*, 2015)

Figure 2.1: Life Cycle and Genomic organization of HPV

Early gene-6 and E7 are the two primary oncogenes of high-risk HPV types (Moscicki *et al.*, 1998). The products of these two genes alter host-cell metabolism to favor neoplastic development (Dyson *et al.*, 1989; Cheng *et al.*, 1995; Sherman *et al.*, 1997). E6 binds to and degrades the host-cell protein p53 (Sherman *et al.*, 1997). An effect of this targeted degradation is to prevent apoptosis of the infected host epithelial cells. Telomerase is also activated, further augmenting oncogenic changes (Scheurer *et al.*, 2005). The E7 protein has a similar effect on cell metabolism by binding to retinoblastoma protein, inhibiting its function. This leads to disruption of the cell cycle (Scheurer *et al.*, 2005). In addition, E6 and E7 proteins may cause chromosomal

destabilization, and inhibit cyclin-dependent kinase inhibitors and host interferons (zur Hausen *et al.,* 2000).

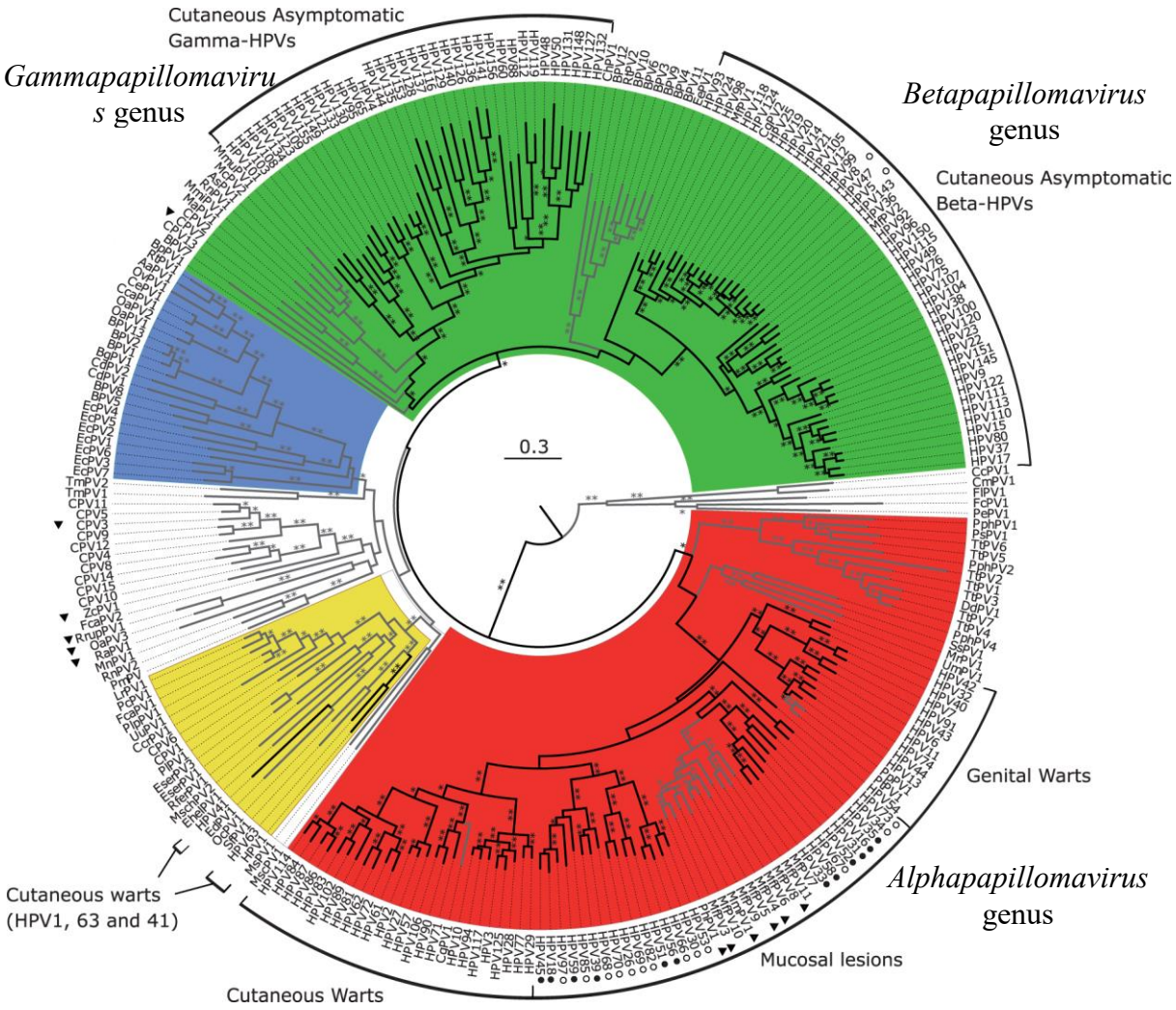
The degree of expression of HPV E6 and E7 is highly correlated with the type of cervical lesion: in low-grade lesions, E6 and E7 are expressed at low levels in the basal cells and higher levels in the upper layers of the epithelium, whereas in high-grade lesions E6 and E7 are expressed at high levels throughout the epithelium (Scheurer *et al.,* 2005). In low-grade lesions, HPV is in episomal form, whereas in higher grade lesions and cancer, the HPV DNA is more likely to have been integrated into the host-cell chromosome. The integration of HPV DNA into the host DNA increases cellular proliferation and the chance of malignancy (Scheurer *et al.,* 2005).

Over 200 HPV genotypes of the family *Papillomaviridae* have been recognized. To date more than 150 different HPV genotypes associated with infections in humans have been described and clustered into three genera: *Alphapapillomavirus*, *Betapapillomavirus* and *Gammapapillomavirus* as shown in Figure 2.2 (Bravo *et al*., 2015).

Heterogeneity of HPV was confirmed by restriction fragment length polymorphism analysis and recently by an extensive and clear definition of HPV types based on DNA sequencing of different genes and the long control region (LCR) (de Villiers *et al.,* 2004). HPV types are defined as a viral genome with a late capsid gene-1 (L1) nucleotide sequence that is at least 10% dissimilar from that of any other HPV type. In addition, the Papillomavirus Nomenclature Committee has established that HPV genomes be classified into molecular variants when they present more than 98% of similarity to the prototype sequence in the L1 capsid gene (Bernard *et al.,* 2002; de Villiers *et al.,* 2004).

Approximately 60 of these genotypes that have been found to cluster in *Alphapapillomavirus* genus have been shown to preferentially infect the oral or genital tract and anogenital mucosa (Wiley *et al.,* 2002; Schiffman *et al.,* 2003; Harari *et al*., 2014). This genus has been further classified into species (Alpha-1 to Alpha-14) based on L and E gene regions of the genome (Harari *et al*., 2014). About 40 genotypes

within this genus are associated with cancer: 20 high-risk (HR) HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 55, 56, 58, 59, 66, 68b, 73, 82, and 83) and 15 low-risk (LR) HPV genotypes primarily found in genital warts and low-grade cervical lesions (6, 11, 40, 42, 43, 44, 54, 61, 70, 71, 72, 81, 83, 84, and CP6108) (Wiley *et al.*, 2002; Schiffman *et al.*, 2003).



The three main HPV genera (Bravo *et al*. 2015)

Figure 2.2: Phylogeny of *Papillomaviridae* Family

HPV targets the basal cells in the stratified squamous epithelium and the metaplastic cells at the squamocolumnar junction of the cervix (Moscicki *et al.,* 1998). Additionally, HPV may infect the glandular epithelium of the endocervix, resulting in glandular neoplasms, such as adenocarcinoma *in-situ* or invasive adenocarcinoma (Longworth *et al.,* 2004). Steps that occur from initial infection to the development of cancer include; overcoming host immune resistance, possible integration of HPV DNA into the host genome, and accumulation of additional mutations within the infected host cell (zur Hausen *et al.,* 2000).

Therefore, HPV must be persistent within the host epithelial cells as a preliminary step toward advanced neoplastic changes. The traditional view has been that this process takes years, if not decades, to occur after initial HPV infection. Recent studies suggest that these changes may develop more quickly than previously thought. Winer *et al.* (2005), followed women after initial HPV infection for the development of CIN 2/3 and demonstrated that approximately 27% of women with an initial HPV 16 or 18 infections progressed to CIN 2/3 within 36 months (Winer *et al.,* 2005). A second study of a large health maintenance cohort found that approximately 20% of women 30 years of age or older who were initially infected with HPV 16 developed CIN 3 or cervical cancer within 120 months (Khan *et al.,* 2005). Women who had an initial HPV 18 infection had approximately 15% risk of developing CIN 3 or cervical cancer at 120 months (Khan *et al.,* 2005).

HPV infection, unlike many genitourinary infections, is not usually associated with immediate symptoms such as itching, burning, ulceration and genital discharge (Mao *et al.,* 2003). Rather, the majority of those infected with HPV will not develop clinical disease or symptoms because it is thought that the host immune system resolves most infections. In one study, only 24.8% of women infected with HPV 6 or 11 actually developed genital warts (Forslund *et al.,* 1993). A large, prospective 10-year cohort study of more than 20,000 women enrolled in a health maintenance organization found that the incidence of CIN 3 or cancer was approximately 7% in HPV-positive women for the duration of the study (Sherman *et al.,* 2003). Thus, only the minority of patients with HPV infections develop serious clinical complications. The exact

mechanism by which HPV infection is cleared by the host immune system is currently unknown.

A number of factors are associated with an increased risk of initial infection and/or clinical sequelae such as; genital warts, CIN or invasive cancer (Castellsague *et al.,* 2003). Individuals who smoke are more likely to develop cancer, and it is thought that smoking increases the likelihood of developing squamous intraepithelial lesions (SIL) (Castellsague *et al.,* 2003). Herpes simplex virus (HSV) and *Chlamydia trachomatis* (CT) infection are suggested to be associated with cervical cancer among HPV infected women. Smith *et al.* (2002) performed a pooled analysis that found prior exposure to HSV-2 was associated with 2-fold increased risk of squamous cell carcinoma of the cervix in patients with HPV (Smith *et al.,* 2002). Notably, CT infection is associated with a similarly increased risk of squamous cell carcinoma (Anttila *et al.,* 2001). CT infection is also associated with more persistent HPV infection, which may contribute to the increased risk of clinical complications of HPV infection in individuals co-infected with CT (Samo *et al.,* 2005). Unlike HPV detection among 98-99 % of women with cervical cancer, only about 48% of men with penile cancer were detected with HPV DNA (Backes *et al.,* 2009). However, HPV 16, 18 and 6 (in this order) remain the most common HPV types associated with penile cancer (Backes *et al.,* 2009).

2.1.2 HPV Transmission

HPV infection is commonly spread by skin-to skin sexual contact. Few studies have evaluated HPV transmission in both men and their female sexual partners, most of which only evaluated cross-sectional concordance between sexual partners as a surrogate marker for HPV transmission (Hippelainen *et al.,* 1994; Baken *et al.,* 1995; Bleeker *et al.,* 2005; Nicolau *et al.,* 2005). A recent cross-sectional study of heterosexual couples found that 37% of partners were infected with the same HPV type. Notable findings were that overall viral loads were much lower in penile-scrape specimens than in cervical-scrape specimens. Higher loads of HPV-16 were associated with having a partner who was also positive for HPV-16 (Bleeker *et al.,* 2005). Another study that assessed HPV by DNA detection or peniscopy found that

76% of male sex partners of women with HPV were positive for HPV infection; however, this study did not evaluate type-specific concordance (Nicolau *et al.,* 2005). A study of 50 couples found that HPV type–specific concordance between sex partners was more common than can be predicted by chance alone (Baken *et al.,* 1995). Another study found that only 22.7% of couples had the same HPV type in genital specimens (Hippelainen *et al.,* 1994).

Only one study has evaluated longitudinal transmission of HPV among heterosexual couples. Hernandez *et al.* (2008a) followed up 25 heterosexual monogamous couples for an average of 7.5 months and demonstrated that transmission from female to male was significantly higher than transmission from male to female. Overall transmission rate from penis to cervix was 4.9/100 person-months compared to 17.4/100 person-months transmission rate from cervix to the penis (Hernandez *et al.,* 2008a). Recent modeling efforts seeking to match transmission characteristics to available incidence and prevalence data estimated the median probability per sex act of HPV transmission to be about 40%, range 5%–100%; (Burchell *et al.,* 2006).

2.1.3 Epidemiology of HPV infections

Human papillomavirus (HPV) infection is the most common STI with an estimate of over 6 million new infections every year in the United States (Cates *et al.,* 1999; Weinstock *et al.,* 2004; US Centers for Disease Control and Prevention [CDC], 2006). Infection with anogenital HPV is the main cause of cervical cancer (Bosch *et al.,* 2003), vulvo-vaginal cancer (Al-Ghamdi *et al.,* 2002; Daling *et al.,* 2002), penile cancer (Rubin *et al.,* 2001), anal cancer (Frisch *et al.,* 1997) and other malignancies (Syrjanen *et al.,* 2005).

2.1.3.1 HPV infection among women

The natural history of HPV infection is well-characterized in women, and most female infections are acquired through sexual contact with men (Winer *et al.,* 2003). The importance of the 'male factor' in the etiology of cervical cancer (Skegg *et al.,* 1982) was suggested years before the identification of sexually transmitted HPV as the principal cause of these tumors (Du¨rst *et al.,* 1983; Mun˜oz *et al.,* 1992).

Although genital HPV infection is the most common STI, only about 10% of people in US have active HPV infection, with 4% having cytological abnormalities and 1%

showing evidence of genital warts. Koutsky *et al.* (1997), further observed that although 1% of Americans have clinically visible genital warts, as many as 13% of those attending STD clinics have genital warts (Koutsky *et al.,* 1997). Registered HPV prevalence among women in Africa differ significantly between countries (De Vuyst *et al*., 2013) with lowest prevalence of 3% documented in Sudan (Salih *et al*., 2010) and highest prevalence of 48% reported in Guinea (Keita *et al*., 2009). Most HPV infections are asymptomatic or subclinical and transient in nature often becoming undetectable over time (Serrano *et al*., 2018). The greatest risk factors for HPV infection are: gender, age, and sexual activity with the highest rates being consistently found in sexually active women less than 25 years of age (Serrano *et al*., 2018).

It is estimated that at least 75% of all sexually active individuals will acquire HPV at some point in their lives (Aral *et al*., 1999) and almost everyone will acquire HPV infection in their life (CDC, 2017). In a cohort of mid-adolescent women followed for 24 months, Brown *et al.,* (2005) showed that, 82% of the 60 women studied got infected with HPV during the study period (Brown *et al.,* 2005). In another cohort of 148 female university students followed for 2 years after initiating sexual activity, Winer *et al.* (2003) demonstrated a cumulative HPV incidence of 38.9%. At the 24 months, HPV 16 and HPV 18 had a cumulative infection rate of 10.4% and 4.1% respectively; thus HPV 16 emerged the most common HPV type. Higher prevalence of HPV 16 has been of special interest because, HPV 16 alone is linked to more than 50% of all cervical cancers (Stone *et al.,* 2002).

2.1.3.2 HPV infections among men

Several studies evaluating HPV infections among men have demonstrated a prevalence ranging from as low as 1.3% to as high as 72.9 % in studies in which multiple anatomic sites or specimens were evaluated (Hippelainen *et al.,* 1993; Forslund *et al.,* 1993; Lazcano-Ponce *et al.,* 2001; Shin *et al.,* 2004; Lajous *et al.,* 2005; Dunne *et al.,* 2006; Partidge *et al.,* 2006; Nielson *et al.,* 2007; Smith *et al.,* 2007; Ng'ayo *et al.,* 2008a; Smith *et al.,* 2010a). The observed variance in HPV prevalence in men could be explained by variability in the study populations, specimen collection

method, anatomic sites of specimen collection, type of specimens used, and laboratory HPV detection methods (Giovannelli *et al.,* 2007). The most common anogenital HPV types detected in men were similar to the types commonly detected in women with HPV type 16 consistently being among the most common; however, other types (6, 11, 18, 31, 33, 42, 52, 53, 54, 56, 59, and 84) were also reported (Van Doornum *et al.,* 1994; Castellsague *et al.,* 1997; Svare *et al.,* 2002; Baldwin *et al.,* 2003; Bleeker *et al.,* 2005; Lajous *et al.,* 2005; Nielson *et al.,* 2007; Smith *et al.,* 2007; Ng'ayo *et al.,* 2008a; Smith *et al.,* 2010a). One study showed that undetermined HPV types may be found more frequently in men than in women (Bleeker *et al.,* 2005). Infection with multiple HPV types is common among men.

2.1.3.3 Specimen collection for HPV Diagnosis among men

HPV prevalence estimates are bound to vary depending on the technique used in HPV detection (Giovannelli *et al.,* 2007). These comprise of the sampling techniques, anatomic site(s) sampled, the specimen types and specimen handling, specimen processing, and genotyping methods. Penile and urethral brushing (swabbing) have been shown to be the best sampling techniques for HPV testing in men (Giovannelli *et al.,* 2007). Previous studies have varied sampling methods that involved rubbing or rotating a swab or brush, either dry or moist, on the genital epithelium. One study that evaluated 3 different sampling techniques (10 men for each method) found that using a saline-wetted sterile Dacron swab after rubbing the sampling site with a small piece of emery paper was superior for specimen adequacy rather than using the swab alone or using a saline-wetted sterile cytobrush (Weaver *et al.,* 2004). One study found that self-collection yielded a greater proportion of adequate specimens than physician-collected specimens (Hernandez *et al.,* 2006).

Several studies have compared HPV detection in individual anatomic sites or specimens with most studies evaluating the glans, corona, prepuce, or shaft of the penis. These studies reported HPV prevalence of 6.5%–65.4% (Wikstrom *et al.,* 1991; Van Doornum *et al.,* 1994; Lazcano-Ponce*et al.,* 2001; Fife *et al.,* 2003; van der Snoek *et al.,* 2003; Weaver *et al.,* 2004; Nicolau *et al.,* 2005; Hernandez *et al.,* 2006; Giuliano *et al.,* 2007). Four studies further evaluated the penile shaft and reported

HPV prevalence of 5.6%–51.5% (Fife *et al.,* 2003; Weaver *et al.,* 2004; Hernandez *et al.,* 2006; Giuliano *et al.,* 2007). Four studies sampled the prepuce, and the reported HPV prevalence was 24.0%–50.0% (Wikstrom *et al.,* 1991; Weaver *et al.,* 2004; Nicolau *et al.,* 2005; Hernandez *et al.,* 2006). Giuliano *et al* (2007) examined the glans penis/coronal sulcus reporting a prevalence of 35.8% second to the penile shaft (Giuliano *et al.,* 2007); however, according to studies by Fife *et al* (2003), and Lazcano-Ponce *et al* (2001), glans penis/coronal sulcus was the site with the highest detectable HPV prevalence. HPV prevalence was 7.1%–46.2% in 6 studies that evaluated the scrotum (Fife *et al.,* 2003; Weaver *et al.,* 2004; Nicolau *et al.,* 2005; Aguilar *et al.,* 2006; Hernandez *et al.,* 2006; Giuliano *et al.,* 2007). Seven studies evaluated the distal 1–3 cm of the urethra, and the reported HPV prevalence was 8.7%–50% (Wikstrom *et al.,* 1991; Forslund *et al.,* 1993; Van Doornum *et al.,* 1994; Astori *et al.,* 1995; Lazcano-Ponce *et al.,* 2001; Nicolau *et al.,* 2005; Aguilar *et al.,* 2006).

The few studies that evaluated the perianal area, anus, or rectum found an HPV prevalence of 0%–32.8% (Van Doornum *et al.,* 1994; Fife *et al.,* 2003; van der Snoek *et al.,* 2003; Nicolau *et al.,* 2005; Giuliano *et al.,* 2007). Semen was evaluated in three studies that compared multiple anatomic sites and specimens (Astori *et al.,* 1995; Rintala *et al.,* 2002; Giuliano *et al.,* 2007) and in some studies that evaluated semen as a single specimen (Wikstrom *et al.,* 1991; Van Doornum *et al.,* 1994; Fife *et al.,* 2003; Vaeteewoottacharn *et al.,* 2003; Nicolau *et al.,* 2005; Aguilar *et al.,* 2006; Hernandez *et al.,* 2006; Giuliano 2007); HPV was detected in 2.2%–41.3% of men evaluated and in up to 82.9% of specimens. Most studies that evaluated urine reported an HPV prevalence of <7% (Forslund *et al.,* 1993; Astori *et al.,* 1995; Lazcano-Ponce *et al.,* 2001; Rintala *et al.,* 2002; Fife *et al.,* 2003; Weaver *et al.,* 2004). Seven studies reported sample adequacy by the evaluation of b-globin (Forslund *et al.,* 1993; Astori *et al.,* 1995; Lazcano-Ponce *et al.,* 2001; Fife *et al.,* 2003; Weaver *et al.,* 2004; Aguilar *et al.,* 2006; Hernandez *et al.,* 2006; Giuliano *et al.,* 2007). Samples collected from the prepuce, shaft, glans, corona, and scrotum were the most likely to have adequate DNA; 70%–98.5% were b-globin positive. Therefore optimal sampling anatomic site for estimating HPV prevalence among heterosexual men includes the penile shaft,

glans, penis/coronal sulcus and scrotal or perianal sites. For HPV testing in this study genital swabs were collected by swabbing a combination of all these anatomic sites: penile shaft, glans, penis/coronal sulcus and scrotal perianal sites.

2.1.3.4 Risk factors for HPV infection among men

Several studies have evaluated the risk factors associated with HPV infection in men in a cross-sectional analysis (Baken *et al.,* 1995; Castellsague *et al.,* 1997; Lazcano-Ponce *et al.,* 2001; Castellsague *et al.,* 2002; Franceschi *et al.,* 2002; Svare *et al.,* 2002; van der Snoek *et al.,* 2003; Baldwin *et al.,* 2004; Shin *et al.,* 2004; Lajous *et al.,* 2005; Nielson *et al.,* 2007; Smith *et al.,* 2007; Hernandez *et al.,* 2008b; Ng'ayo *et al.,* 2008a; Smith *et al.,* 2010a). Some of these studies reported factors statistically significantly associated with infection or acquisition or persistence, while considering multivariate analyses to evaluate factors independently associated with HPV infection (Svare *et al.,* 2002; Hogewoning *et al.,* 2003; Baldwin *et al.,* 2004; Kjaer *et al.,* 2005; Lajous *et al.,* 2005; Smith *et al.,* 2007; Hernandez *et al.,* 2008b; Ng'ayo *et al.,* 2008a; Smith *et al.,* 2010). Only 4 studies (with varied duration of follow-up) prospectively evaluated HPV infection and associated risk factors in men (Van Doornum *et al.,* 1994; Wikstrom *et al.,* 2001; Kjaer *et al.,* 2005; Lajous *et al.,* 2005). However, most of these studies targeted men having sex with men (MSM) and therefore further investigations among heterosexual men is needed.

2.1.3.5 Anogenital and cervical HPV disease

Even though majority of HPV infections are asymptomatic or self-limiting, acquisition of specific types of HPV can result in clinically significant disease. Most notable among the high-risk oncogenic types of HPV are types 16 and 18, responsible for approximately 70% of all cervical cancer (Parkin *et al.,* 2006b; Wiley *et al.,* 2006) as well as a lower proportion of cancer of the vagina, vulva, penis, anus, mouth and oropharynx (Parkin *et al.,* 2006b); and HPV 6 and 11, responsible for up to 90% of anogenital warts and virtually all cases of recurrent respiratory papillomatosis (JORRP) (Lacey *et al.,* 2006; Wiley *et al.,* 2006). Collectively, HPV 6, 11, 16, and 18 impose a substantial disease burden and affect both quantity and quality of life (Fitzmaurice *et al.,* 2015).

Cervical cancer is the second most common cancer in women worldwide (Parkin *et al.,* 2005; Torre *et al*., 2015) and infection with carcinogenic HPV is necessary for the development of this type of cancer. Despite carcinogenic HPV types being detected in virtually all invasive cervical cancer cases, HPV, a common infection in young women becomes undetectable within 1-2 years among most of these women (Burd *et al.,* 2003; Mun˜oz *et al.,* 2003). Therefore, persistence of HPV infection is considered to drive progression of cervical neoplasia to invasive cervical cancer (Moscicki *et al.,* 2006). Several studies have suggested that detection of the same carcinogenic HPV type over time is particularly important for cervical carcinogenesis (Wallin *et al.,* 1999; Schiffman *et al.,* 2005).

Annual estimates of incident anogenital warts are imprecise and range from 250,000 to 1,000,000 (Chesson *et al.,* 2004) with about 90% of all anogenital warts being caused by HPV 6 or 11 (Insinga *et al.,* 2005). In 2003, it was estimated that there were 4,000 new cases of anal cancer in the U.S. (Jamal *et al.,* 2003). Over 82.8% of all anal cancer is attributable to HPV types 16 and 18 (Frisch *et al.,* 1999; Daling *et al.,* 2004; Parkin *et al.,* 2006b). It is estimated that 1,145 new cases of penile cancer occurred in the U.S. in 2003 (Jamal *et al.,* 2003, U.S. Cancer Statistics Working Group 2006) and 25% of all penile cancers are attributable to HPV 16 & 18 (Parkin *et al.,* 2006b; Rubin *et al.,* 2001). The worldwide prevalence of HPV among men with penile carcinoma is estimated be to 48%, and ranges from 22% to 66% (Backes *et al.,* 2009).

2.1.4 Management and prevention of HPV infections

There are no recommended anti-viral therapies against HPV infections. To manage HPV associated precancerous lesions from progressing to invasive cancer, several ablative and excisional methods are employed. These include: freezing HPV-associated growths (cryotherapy), excision of HPV-associated growths using methods such as loop electrosurgical excision procedure (LEEP), laser conization, laser ablation, surgical conization/cold-knife conization or alpha-interferon (Wright *et al*., 2007). Some of these treatments have been associated with reduced risk of new oncogenic HPV infections (Castle *et al*., 2009; Taylor *et al*., 2010).

HPV vaccination is one of the best interventions for preventing HPV infection and averting HPV-related diseases (Naud *et al*., 2014). There are three prophylactic HPV vaccines using L1 virus-like particles (VLP) and targeting different oncogenic HPV genotypes (Lopalco, 2016): Cervarix® bivalent HPV vaccine (2vHPV) against two HPV genotypes (HPV-16 and HPV-18), Gardasil® quadrivalent HPV vaccine (4vHPV) against four HPV genotypes (HPV-6, HPV-11, HPV-16 and HPV-18) and Gardasil-9® nonavalent HPV vaccine (9vHPV) against nine HPV genotypes (HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58). The Food and Drug Administration (FDA) licensed 9vHPV vaccine in ages 9-26 years for females and 9-15 years for males (FDA Gardasil-9, 2014). Although, there is additional data recommending use of 9vHPV vaccine in both males and females aged 9-26 years (Petrosky *et al*., 2015), the cost of these vaccines is prohibitory making them inaccessible to most low resource countries.

These three currently available HPV vaccines have been shown to be safe, immunogenic (Castellsagué *et al*., 2015; Garland *et al*., 2015; Van Damme *et al*., 2015; Vesikari *et al*., 2015; Van Damme *et al*., 2016) and efficacious (NCT00543543, 2007; Naud *et al*., 2014; Joura *et al*., 2015; Signorelli *et al*., 2017) in preventing HPV infections and associated diseases among healthy young males and females (Garland *et al*., 2007; Giuliano *et al*., 2011a; Joura *et al*., 2015, Lopalco, 2016; Signorelli *et al*., 2017). Safety and immunogenicity among young PLWH including adolescents and young adults was demonstrated in two of these vaccines: Cervarix® and Gardasil® (Levin *et al*., 2010; Wilkin *et al*., 2010; Weinberg *et al*., 2012; Kahn *et al*., 2013; Giacomet *et al*., 2014; Kojic *et al*., 2014; Toft *et al*., 2014; Rainone *et al*., 2015; Levin *et al*., 2017). However, there is limited data the on efficacy of these HPV vaccines among PLWH and no data is available on the safety, immunogenicity and efficacy of Gardasil-9® in this subpopulation (PLWH).

2.1.5 Molecular characterization of vaccine-relevant HPV infections

To establish a persistent infection, human papillomaviruses need to overcome several major barriers: first, the virus needs to create a cellular milieu that supports replication of the viral genome in differentiated squamous epithelial host cells; second, host

defense mechanisms that limit viral survival such as clearance of infected cells by apoptosis, immune responses of the host, and eventually replicative senescence of infected and abnormally proliferating host cells need to be abolished (Zur *et al.*, 1999).

One mechanism that pathogens use to circumvent the host immune response is through genetic variation that alters products of gene expression with the potential of enabling the pathogen to evade the immune system priming or response. This partially explains the many HPV genotypes that have been characterized so far based on nucleotide sequence variability in the L1 capsid gene. This played a major role in HPV vaccine development strategy. An effective HPV vaccine capable of preventing majority of carcinogenic HPV infections must contain immunogens from multiple HPV genotypes (Schiller *et al.*, 2015). Currently, Gardasil-9® (9vHPV) vaccine covers the highest number of HPV genotypes.

With little or no cross-protection, HPV genotype replacement has been thought to be a key concern following HPV vaccination. The prevalence of non-vaccine genotypes negatively associated with vaccine HPV genotypes will likely flare-up after successful prevention of respective vaccine genotypes through vaccination, thus reversing the expected gains of an HPV vaccination program. Several studies that evaluated genotypic competitions relevant to HPV vaccine genotypes (for 2vHPV and 4vHPV vaccines) yielded mixed results (Wentzensen *et al*., 2009; Chaturvedi *et al*., 2011; Vaccarella *et al*., 2011; Rositch *et al*., 2012a; Palmroth *et al*., 2012; Rositch *et al*., 2012b; Querec *et al*., 2013; Mollers *et al*., 2014; Nie *et al*., 2016). No study has evaluated HPV type competition against 9vHPV vaccine genotypes.

In this study among fishermen in Kisumu, Kenya, molecular characterization of HPV genotypic infections in relation to 9vHPV genotypes was performed. Additionally, negative and positive associations between 9vHP vaccine genotypes and other non-vaccine HPV genotypes detected among these men were computed to assess potential genotypic competition (type replacement) in relevance to 9vHPV vaccine genotypes.

2.1.6 Immune responses to natural HPV infections

The exclusively intra-epithelial life cycle of HPV is central to understanding the host immune response. The virus infects basal keratinocytes probably via micro-abrasions of the epithelial surface that leave the basal lamina intact (Roberts *et al.,* 2007) after which all subsequent events in the viral life cycle are tightly linked to the differentiation programme of the keratinocyte as it progresses up through the epithelium (Doorbar *et al.,* 2005).

Acquisition of human papillomavirus (HPV) results in an infection of variable duration which may or may not be associated with clinically apparent pathologic effects. Lesions caused by skin-tropic HPV types generally manifest as cutaneous warts and most often resolve over a period of months to years. On the other hand, anogenital infections are more likely to remain clinically unapparent. The time taken to clearance for the high-risk HPV particularly HPV 16 seems on average to be 6-16 months, considerably longer than the 4-8 months reported for the low-risk HPV (Trottier *et al.,* 2006). However if the immune response fails to clear or control the infection then a persistent infection, often with focally high levels of high-risk HPV DNA is established and it is this cohort of individuals that have an increased probability of progression to high grade cervical intra-epithelial neoplasia (CIN 2/3) and invasive carcinoma (Ho *et al.,* 1998; Liaw *et al.,* 2001; Moscicki *et al.,* 2006).

HPV induce chronic infections that may have no apparent systemic sequellae and rarely kill the host, but periodically shed large amounts of infectious virus for transmission to naïve individuals (Moscicki *et al.,* 2006). Therefore, persistent HPV types have devised mechanisms that enable them to either bypass or negotiate the immune defense systems of the host: innate immunity and adaptive immunity (Moscicki *et al.,* 2006). The interaction between the host's innate immunity and HPV is largely un-documented. Studies evaluating the role of innate immune response during acute HPV infection are needed. Several studies have reporter adaptive immune response to HPV infection among women suggesting that the human host mounts an adaptive immune response to HPV infection (Benton *et al.,* 1996; de Jong *et al.,* 2002; Welters *et al.,* 2003; Palefsky *et al.,* 2006).

2.1.6.1 Humoral immune response against HPV infection

Several studies have explored humoral (antibody) immune response against type-specific HPV infection. Serological studies using HPV virus like particles (VLPs) have shown that infection with a genital HPV among women is followed eventually by sero-conversion and type-specific antibody to the major viral coat protein L1; antibody to the minor viral coat protein L2 is not detectable in natural infections in animals or humans (Dillner *et al.,* 1999). Sero-conversion most frequently occurs between 6 and 18 months after the first detection of HPV DNA in subjects with persistent HPV infection i.e. detection of HPV DNA of the same type on two occasions at least 6 months apart (Carter *et al.,* 1995; Wideroff *et al.,* 1995; Carter *et al.,* 1996) and rarely in subjects with incident HPV infections i.e. detection of HPV DNA on only one occasion (Carter *et al.,* 2000). Stone *et al.* (2002) utilized an experimental serological test to determine the presence of antibodies to HPV 16, signifying prior exposure to HPV, instead of the more commonly assessed viral DNA which is indicative of active infection. This study showed that women were more likely to be seropositive for HPV16 (17.9%) than were men (7.9%). However, this methodology may in fact underestimate the true prior exposure to HPV 16, because <60% of women infected with HPV 16 develop type-specific antibodies (Stone *et al.,* 2002).

Among studies that evaluated HPV-16 seroprevalence (Wikstrom *et al.,* 1992; Widero *et al.,* 1996; Hagensee *et al.,* 1997; Svare *et al.,* 1997; Hisada *et al.,* 2000; Carter *et al.,* 2001; Slavinsky *et al.,* 2001; Stone *et al.,* 2002; Kreimer *et al.,* 2004; Thompson *et al.,* 2004) HPV-16 seropositivity differed by study population. Most studies in sexually transmitted disease (STD) clinics reported a higher rate of seropositivity than studies in other populations. The range of reported seropositivity in male STD clinic attendees was between 18.7% and 48% in the United States (Hagensee *et al.,* 1997; Thompson *et al.,* 2004). The seropositivity to HPV-16 in males in the general population was 7.9% in the United States (Stone *et al.,* 2002). HPV-16 seropositivity was similar in male STD clinic attendees in Denmark, Greenland, and Jamaica (Svare *et al.,* 1997; Strickler *et al.,* 1999); however, a lower seroprevalence was noted in China, and in contrast higher seroprevalence in Sweden (Wikstrom *et al.,* 1992;

Widero *et al.,* 1996). Eight of nine studies that compared seroprevalence in men and women reported a higher seroprevalence in women than in men (Eisemann *et al.,* 1996; Svare *et al.,* 1997; Strickler *et al.,* 1999; Carter *et al.,* 2001; Slavinsky *et al.,* 2001; Stone *et al.,* 2002; Kreimer *et al.,* 2004;Thompson *et al.,* 2004).

Comparison studies of HPV-6, 11, and HP-18 seropositivity were more difficult, because most studies of HPV-6 and -11 were conducted among STD clinic attendees, and the study of HPV-18 was conducted in clinics or community centers. HPV-6 or -11 seroprevalence ranged from 26.4% (Slavinsky *et al.,* 2001) to 41% (Wikstrom *et al.,* 1992; ; Carter *et al.,* 1995; Eisemann *et al.,* 1996; Hagensee *et al.,* 1997; Wikstrom *et al.,* 1997; Slavinsky *et al.,* 2001). The estimate of HPV-18 seroprevalence in one study was 18.8% (Kreimer *et al.,* 2004).

Risk factors for HPV seropositivity

Several studies have evaluated risk factor for HPV seropositivity. However, only six studies included multivariate analyses to identify factors independently associated with HPV seropositivity (Hagensee *et al.,* 1997; Svare *et al.,* 1997; Strickler *et al.,* 1999; Slavinsky *et al.,* 2001; Stone *et al.,* 2002; Thompson *et al.,* 2004). Three of these studies showed that age (generally, being >20 years old) was associated with increased likelihood of detection of HPV-16 antibodies (Strickler *et al.,* 1999; Slavinsky *et al.,* 2001; Thompson *et al.,* 2004). There was no observable association between age and HPV-6 or HPV-11 seropositivity in studies that evaluated age as a risk factor for the two HPV types (Hagensee *et al.,* 1997; Slavinsky *et al.,* 2001).

Sexual behavior was evaluated in six studies (Hagensee *et al.,* 1997; Svare *et al.,* 1997; Strickler *et al.,* 1999; Slavinsky *et al.,* 2001; Stone *et al.,* 2002; Thompson *et al.,* 2004) and was found to be independently associated with HPV seropositivity, including number of lifetime sex partners (Svare *et al.,* 1997) and recent number of sex partners (Hagensee *et al.,* 1997; Strickler *et al.,* 1999; Slavinsky *et al.,* 2001; Thompson *et al.,* 2004). Having had 11 occasional sex partners within the preceding year was associated with an increased risk of HPV seropositivity in one study (Thompson *et al.,* 2004). Younger age (<18 years) at first sexual intercourse was evaluated in two studies (Svare *et al.,* 1997, Stone *et al.,* 2002) and was a significant

risk factor in one study (Svare *et al.,* 1997). One study found an increased risk of HPV-16 seropositivity in men who had sex with men (Stone *et al.,* 2002).

Only two studies have evaluated condom use and HPV seropositivity by multivariate analyses (Strickler *et al.*, 1999; Slavinsky *et al.,* 2001) and one study found that HPV-16 seropositivity was more likely to occur with less-frequent condom use during the preceding year (never vs. more than one-half the time) (Strickler *et al.*, 1999). One study examined consistent condom use in a bivariate analysis and found no significant association between condom use and the detection of HPV-16 or -6 and -11 antibodies (Slavinsky *et al.,* 2001).

2.1.6.2 Cell-mediated immune response against HPV infection

Infection with persistent high-risk HPV is a leading risk factor for development of cervical cancer (Walboomers *et al*., 1999) and other anogenital malignancies (zur Hausen *et al*., 1996). Although HPV is a common STI, majority of infected individuals eliminate the virus in 12 – 24 months after acquisition (Evander *et al*., 1995; Ho GY *et al*., 1998, Moscicki *et al*., 1998; Schiffman *et al*., 2007). Increased incidence and progression of HPV infections in immunosuppressed individuals illustrates the critical importance of the CD4 T–cell regulated cell-mediated immune response in the resolution and control of HPV infections (Benton *et al.,* 1996; Palefsky *et al.,* 2006). HIV infected women show multiple recurrences of cervical HPV infections (Fruchter *et al.,* 1996) and an increased incidence of both cutaneous and genital warts (Fennema *et al.,* 1995) that appears to reflect an increased risk of progression from sub–clinical to clinical disease. Prospective studies show prolonged persistence of high risk HPV DNA in HIV infected 13-18 year old girls who are otherwise healthy (Moscicki *et al.,* 2004a) and a high incidence of CIN2/3 in this group (Moscicki *et al.,* 2004b). Importantly the risk for incident CIN in these HIV infected girls appeared to be primarily due to the persistence of low grade squamous intra-epithelial neoplasia (LSIL), rather than the persistence of high risk HPV DNA without a detectable lesion, implying that consistent viral gene expression in a persistent active infectious cycle is important in clinical progression (Moscicki *et al.,* 2004a).

CD4 T cell responses to L1 are essential for prevention of HPV infection while E2 and E6 are important in the control of clinical progression associated with high–risk HPV infections. A non-intervention follow-up study of women with cytological evidence of low grade CIN, showed that HPV-16 E2-specifc T cell responses, as measured by specific IL-2 release *in-vitro*, occurred frequently at the time of lesion clearance (de Jong *et al.,* 2002). Cell-mediated Th1 HPV type-specific immunity against the E2 and E6 protein has been detected in healthy individuals with no clinical signs of HPV-16 infection (Welters *et al.,* 2003). In a longitudinal study extending over 12 months of women with histologically diagnosed CIN 1 systemic CD4+ responses to E2 were detected in HPV 16+ histological repressors but were absent in HPV 16+ histological progressors (de Jong *et al.,* 2002). These data suggest that a hall mark of effective immune control of HPV 16 infection in the cervix is the generation of CD4+ cells specific for E2 cytotoxic effectors.

Cell-mediated cytotoxicity is the most important effector mechanism for the control and clearance of viral infections and is implemented by a range of cells both antigen specific cytotoxic T cells (CTL) and the so called "innate lymphocytes" a heterogeneous group that includes natural killer (NK) cells and invariant natural killer T cells (iNKT) (Alexander *et al.,* 1996). HPV specific CTL can be detected in patients with previous (Nakagawa *et al.,* 1997) or ongoing HPV infection (Alexander *et al.,* 1996; Evans *et al.,* 1996; Evans *et al.,* 1997). Both CD4+ and CD8+ cytotoxic effectors have been shown to be involved in these responses (Nakagawa *et al.,* 1999). In a longitudinal study of women with polymerase chain reaction (PCR) determined cervical HPV 16 infection lack of CTL response to E6 but not E7 correlated with persistent HPV infection suggesting that a CTL response to HPV 16 E6 is important for viral clearance and, by implication, neoplastic progression (Nakagawa *et al.,* 2000).

NK cells are key components of the innate immune response to viral infections (Alexander *et al.,* 1996). They are a sub-set of lymphocytes that kill virally infected or tumor cells lacking surface expression of MHC Class I molecules and there is evidence that they are important in HPV infections. Peripheral blood mononuclear

cells (PBMC) from patients with active HPV-16 neoplastic disease display a reduced NK cell activity against HPV-16 infected keratinocytes (Cho *et al.,* 2001; Lee *et al.,* 2001).

Therefore, natural host immune response among women is believed to play a vital role in HPV clearance or provide some level of protection against future infections with the same HPV genotypes (Malejczyk *et al*., 1997, Bosch *et al*., 2008, Safaeian *et al*., 2010, Wentzensen *et al*., 2011, Castellsagué *et al*., 2014, Beachler *et al*., 2016). Moreover, several studies among women have provided empirical evidence on the role of cell-mediated immune response in HPV clearance or persistence (Scott *et al*., 1999, Farhat *et al*., 2009, Chan *et al*., 2010, Scott *et al*., 2013). However, knowledge on natural immunity against HPV infection among men has not been documented (Franceschi *et al* 2014). Additionally, immunological factors contributing to the natural course of HPV infection among men are elusive. To further our understanding of cell-mediated immune priming during natural HPV infection in men, we evaluated a panel of eight cytokines in PBMCs collected from men with or without HPV infection. Interleukin-2 (IL-2), IL-4, IL-5, IL6, IL-10, IL-13, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) were selected based on previous literature (Scot *et al*., 1999; Scott *et al*., 2001; Pinto *et al*., 2003; Azar *et al*., 2004; Pinto *et al*., 2006; García-Piñeres *et al*., 2007; Farhat *et al*., 2009; Chan *et al*., 2010; Scott *et al*., 2013).

In summary, it is clear that HPV disease burden is much higher in women than men, yet the prevalence of HPV is presently comparable across the two genders, and data on natural history of HPV infections among men is not available. Women have been observed to mount a better antibody immune response against HPV infections compared to men. Consequently the prevalence of HPV among women has been observed to generally decline with age, unlike among men whose prevalence remain relatively constant across different age categories (Moscicki *et al*., 2006). Notably, all studies documenting cell-mediated immune response to natural type-specific HPV infections are among women. Therefore, studies examining risk factors for HPV incidence, persistence and relevance of HPV vaccination among men are needed.

Exploring immunologic and biological factors modulating HPV infections among men could help explain the higher burden of HPV disease in women as opposed to among men. In this prospective study of fishermen; epidemiology (prevalence, incidence and associated risk factors) of HPV infections was determined and HPV genotypes characterized. Moreover, cell-mediated cytokine immune responses against HPV infection were assessed.

2.1.7 HPV and Human immunodeficiency virus (HIV) co-infections

Immunosuppressed people living with HIV (PLWH) are at a high risk of both HPV infection and HPV-associated disease compared to those negative for HIV infection (Harris *et al*., 2005; Clifford *et al*., 2006; Denny *et al*., 2008; De Vuyst *et al*., 2008; Singh *et al*., 2009; Veldhuijzen *et al*., 2011; Denny *et al*., 2012). High HIV RNA levels and CD4 < 200 cells per mm^3 counts are associated with both incident and persistent HPV infection, although the association with incident infection is stronger (Strickler *et al.,* 2005). Among women with oncogenic HPV, HIV positive women with low CD4 cell counts are more likely than either HIV-negative women or HIV-positive women with high CD4 cell counts to develop SIL (Harris *et al.,* 2005). HIV infected men are at increased risk of contracting HPV and possibly with multiple HPV types (Muller *et al.,* 2010). In multivariate analysis, HIV positive status has been found to be independently associated with multiple HPV infections among men (Ng'ayo *et al.,* 2008a; Muller *et al.,* 2010). In a prospective study evaluating HIV acquisition among HPV infected men, Smith *et al.* (2010b) demonstrated an increased risk of HIV among HPV positive men.

2.2 Epidemiology of HIV infection among Fishermen

The HIV/AIDS pandemic is currently in its fourth decade of existence since the first case was reported in 1981 (UNAIDS-WHO, 2009). However, the global distribution of the virus in the population is highly variable geographically, by age, gender, race, and occupation (Coovadia *et al.,* 2005; Velayati *et al.,* 2007; UNAIDS-WHO, 2009). Socio-economic, behavioral characteristics and cultural practices are also thought to contribute to the spread of HIV (UNAIDS-WHO, 2009; Agot *et al.,* 2010). Generally, HIV prevalence is highest in countries located in Sub-Sahara Africa (UNAIDS-WHO, 2009). In the US and Europe, high HIV rates are observed among injection drug users (IDUs) and young men who have sex with men (MSM), particularly among young African–American MSM (UNAIDS-WHO 2009; Likatavicius *et al.,* 2011).

In Sub Saharan Africa, heterosexual transmission accounts for the majority of HIV infection (Dunkle *et al.,* 2008; Lingappa *et al.,* 2008; Anand *et al.,* 2009). Therefore, disparities in the distribution of HIV across different populations (and subpopulations) suggest existence of variable risk factors predisposing individuals within these sub-populations to varying levels of risk for HIV infection. Similarly, sex workers and long distance truck drivers have been reported to have higher prevalence of HIV when compared to the general population (Pandey *et al.,* 2008; Stefan *et al.,* 2012; Delany-Moretlwe *et al.,* 2013). This observation may largely be due to modified sexual practices or /and rapidly expanding sexual networks associated with these occupations.

HIV prevalence of 20% to 28% among fishing communities is higher compared to the general population (Entz *et al.,* 2000; Allison *et al.,* 2004a; Allison *et al.,* 2004b; Kwena *et al.,* 2010). This has posed a serious threat to the fishing industry (Allison *et al.,* 2004b). Notably, access to care from public health facilities remains a challenge for this underserved population (Seeley *et al.,* 2005). The fishing industry therefore, presents an occupational risk factor for HIV infection, explained partially by the migratory nature of the fishing and fish trade activities (Seeley *et al.,* 2005). Several studies and national surveys have documented high

prevalence of HIV infection among the fishing communities including among fishermen (Kwena *et al.,* 2010; Kenya AIDS Indicator Survey [KAIS], 2014). However, HIV incidence and associated risk factors among this population remain largely unavailable. Quantification of HIV acquisition rates and exploring risk factors related to HIV incidence are crucial steps in characterizing the HIV epidemic among fishermen and designing specific intervention strategies suited to this highly migratory population.

In this prospective cohort study, the incidence rate of HIV infection among fishermen working on beaches around Lake Victoria in Kisumu County, Kenya was established. Additionally, factors associated with prevalence and HIV acquisition risk were explored in this subpopulation. Potentially, these would rationalize and inform development of well-targeted interventions against new HIV infections among fishermen and the fishing communities at large.

2.3 Epidemiology of HSV-2 infection among Fishermen

Genital ulcer disease (GUD) is clearly associated with increased risk of HIV acquisition and transmission. The genital ulcerations facilitate increased shedding and easier entry of HIV into the host (Dickerson *et al.,* 1996; Schacker *et al.,* 1998; O'Farrell *et al.,* 2007). Therefore, it is biologically plausible that presence of herpetic lesions would increase the risk of HIV acquisition. Herpes simplex virus type-2 (HSV-2) is one of the most prevalent STI worldwide and the important leading cause of GUD a head of syphilis and chancroid (Mertz *et al.,* 1998; Paz-Bailey *et al.,* 2007; Looker *et al.,* 2008). Epidemiological studies have established a strong association between prevalent HSV-2 and incident HIV infection (del Mar Pujades *et al.,* 2002; Reynolds *et al.,* 2003; Brown *et al.,* 2006; Freeman *et al.,* 2006; Sobngwi-Tassiopoulos *et al.,* 2007; Tobian *et al.,* 2009b; Delany-Moretlwe *et al.,* 2009; Tambekou *et al.,* 2009). Recent studies further suggest that incident HSV-2 infection increase the risk for new HIV infection (Reynolds *et al.,* 2003; Freeman *et al.,* 2006; Sobngwi-Tambekou *et al.,* 2009; Okuku *et al.,* 2011; Mehta *et al.,* 2012). In summary, underlying HSV-2 infection substantially elevates the risk of incident HIV infection.

Fishing communities are believed to be composed of a sub-population of men and women with high-risk sexual behavior (Kissling *et al.,* 2005) biologically demonstrated by HIV and HSV-2 prevalence rates that are reportedly much higher than the general population (Ng'ayo *et al.,* 2008b; Kwena *et al.,* 2010; Asiki *et al.,* 2011; Seeley *et al.,* 2012; Kiwanuka *et al.,* 2013; Opio *et al.,* 2013). Although previous studies have evaluated risk factors for incident HSV-2 infection among individuals with high-risk sexual behavior (Freeman *et al.,* 2006; Tassiopoulos *et al.,* 2007; Chohan *et al.,* 2009; Okuku *et al.,* 2011), there is limited information on HSV-2 incidence among fishermen and generally the fishing communities. Knowledge of HSV-2 incidence rate and associated risk factors is needed in the design of targeted interventions to limit new HSV-2 infection, potentially lowering rates of new HIV infection. It is therefore important to understand the risk factors for incident HSV-2 infection among men in the fishing communities in Sub-Saharan Africa; the epicenter of HIV epidemic. In this study, we report the results of the first study to evaluate risk factors for incident HSV-2 infection among fishermen on the shores of Lake Victoria around Kisumu Kenya.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

A prospective cohort study was conducted among fishermen on the shores of Lake Victoria in Kisumu, Kenya. Study participants were recruited and followed up every three months for a period of one year. The study clinic was based at Ober Kamoth Health Center located close to beaches on Lake Victoria for easy access by study participants. Administrative office and clinical laboratory activities were based at RCTP – KEMRI CMR – Research Centre, Lumumba Health Centre in Kisumu. Laboratory testing was performed at the KEMRI's research laboratories and University of California, San Francisco (UCSF) research laboratories in the USA.

3.2 Study Population

This study was conducted among fishermen working on Lake Victoria beaches in Kisumu County (covering Kisumu East and Kisumu West Districts), Kenya. The inclusion criteria were: Fishermen aged 18 – 64 years, sexually active (had sex at least once within three months prior to study participation); Able and willing to give informed consent for study participation; Willing to return to the clinic after every 3 months (a total of 12 months) for follow up clinical procedures and laboratory testing.

3.3 Sample size Determination

The main outcome of this study was to assess immune response to type-specific HPV infection among men with HPV clearance and persistent HPV infection compared to men without HPV infection. For a study in which two proportions were compared with a *z*-test based on the normal approximation to the binomial distribution, the samples size was determined by the equation (Fleiss *et al*., 1980)

$$N = \frac{2\left(z_{1-\alpha/2}\sqrt{2\bar{p}(1-\bar{p})} + z_{1-\beta}\sqrt{p_c(1-p_c)+p_a(1-p_a)}\right)^2}{(p_c - p_a)^2}$$

Where; p_c and p_a are pre-study estimates of the two proportions to be compared, p_c - p_a (i.e. the minimum expected difference). The two groups comprising N were

assumed to be equal in number, and a two-tailed statistical analysis used. Based on this proposal, the proportion of men with immune response against HPV among men clearing any of the 9-valent HPV vaccine genotypes was estimated to be p_C=48% and for a 2.7-fold reduction (p_a=18%) in prevalence of immune response among men without HPV infection (Hagensee *et al.*, 1997; Thompson *et al.*, 2004). For a significance criterion of α=0.05 and a power of 80%, using the formula above, with:

p_c=0.48, p_a = 0.18, $p_c - p_a$ = 0.30, $\bar{p}$ = (0.48+0.18)/2=0.33, $Z_{1-\alpha_{/2}}$ =1.960, and $Z_{1-\beta}$ =0.842, it was estimated that the required total sample size N required was 38 per group.

$$N = 2\frac{\left[1.96\sqrt{2 * 0.33(1-0.33)} + 0.842\sqrt{0.48(1-0.48)+0.18(1-0.18)}\right]^2}{(0.48-0.18)^2} = 75$$

Or 38 fishermen per group

For the three groups: men with HPV clearance, HPV persistence and men without HPV infection each having 38 men, a total of 114 fishermen were the required sample for this study. Due to the migratory nature of fishermen, it was estimated that an attrition rate of 23% will occur over the 1 year follow-up period and based on estimated HPV prevalence of 50% in the proposed study population (Ng'ayo *et al.*, 2008a; Smith *et al.*, 2010a), a corrected prospective sample size of 298 fishermen was needed. Therefore, to achieve the objectives of this study, a cohort of 300 fishermen was enrolled and followed up for a period of 12 months.

3.4 Study Sampling

This study recruited eligible fishermen from 23 active beaches out of 33 on Lake Victoria in Kisumu County (covering Kisumu East and Kisumu West Districts), Kenya (Figure 3.1). The remaining 10 beaches of 33 were inactive during study sampling and recruitment period.

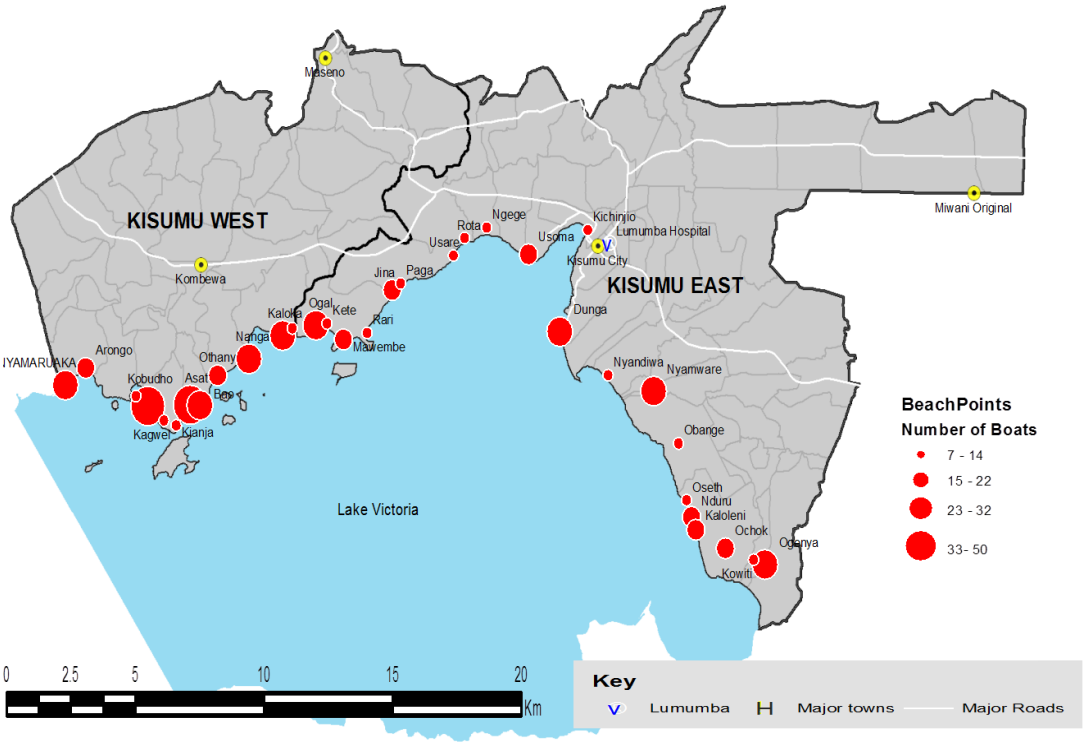


Figure 3.1: Relative size of beaches along Lake Victoria in Kisumu from which participants were recruited

The study sample of 300 fishermen was randomly selected from these 23 fish landing beaches proportionate to their size as per the beach management unit registry, with larger beaches contributing higher proportions of the study sample. Simple random sampling technique was employed for actual study participant recruitment to achieve the respective sample proportions from the 23 beaches (Table 3.1).

Table 3.1: Beach specific sample size computed as proportionate to population size of registered Fishermen

S/No.	Beach Name	Estimated Fishermen Population size	Sample Population proportion (%)	Proportionate sample size
1	Achuodho	23	1.7	5
2	Asat	151	11.2	34
3	Bao	54	4.0	12
4	Jina	5	0.4	1
5	Kagwel	32	2.4	7
6	Kaloka	62	4.6	14
7	Kete	36	2.7	8
8	Kianja	29	2.1	6
21	Kichinjio	26	1.9	6
10	Kobudho	59	4.4	13
11	Mawembe	24	1.8	5
12	Nanga	136	10.1	30
13	Nduru	90	6.7	20
14	Nyamware	183	13.5	41
15	Nyandiwa	36	2.7	8
16	Obange	99	7.3	22
17	Ogal	68	5.0	15
18	Oseth	54	4.0	12
19	Paga	50	3.7	11
20	Rare	41	3.0	9
21	Rota	9	0.7	2
22	Usare	18	1.3	4
23	Usoma	68	5.0	15
	Total	**1353**	**100.0**	**300**

3.5 Ethical Considerations

This study was conducted in full conformity with the most current version (7th version) of the Helsinki Declaration (2013) and adherence to the three principles guiding research on human subjects – Respect of person, Justice and Beneficence. Prior to study initiation, study protocol, study procedures and study instruments (Appendix I–VI) that included informed consents and questionnaire, were approved by KEMRI Ethical Review Committee (ERC) as Study protocol # SSC2014 (Appendix VII–X) and UCSF institution review board (IRB) as Study protocol UCSF-IRB# 12-08590 (Appendix XI and XII). Permits authorizing shipment of specimens to UCSF laboratories for specialized testing was sought from KEMRI ERC (Appendix XIII) and CDC (Appendix XIV). Specimens were shipped in accordance to IATA regulations. Left over samples were stored for two years after the end of the study to address any issues that required retesting or additional testing and discarded thereafter.

Informed consent process was initiated prior to the individual's agreement to participate in the study and continued throughout the individual's study participation. Consent forms (Appendix I and II) describing the study in detail, the study procedures, and the risks involved with participation in the language the participant was comfortable with were administered to each eligible participant. Enrollment informed consent was a requirement for every participant prior to any study specific procedures being performed. Study tools including the consent forms and questionnaires were approved by KEMRI Ethical Review Committee.

During consenting process, the participants were asked to read and review these documents. Upon reviewing the document, the research study was explained to the participant and any questions that arose were answered. The participants were given the opportunity to discuss the study with their surrogates, ask questions and/or allowed to think about the study prior to agreeing to participate. The participants were informed that they may withdraw from the study at any time throughout the course of the study if they so wished**.** A copy of the informed consent documents was given to the participants for their records. The rights and welfare of the participants were

protected by emphasizing to them that the quality of their medical care will not be affected if they declined to participate in this study. Every effort was made to protect participants' privacy and confidentiality.

To ensure confidentiality, each participant was assigned a unique Participant Identification Number (PTID) that was indicated on all study documents and specimens (instead of their names as a de-identification process) except for the consent form which only had the names of the participant and not the PTID. The participant names and corresponding PTID was recorded on a study link-log. This link-log was securely stored separately to minimize chances of inadvertent identification of participants' personally identifying information. All hard paper copies of study documents were stored in a locked filing cabinet separately from consent forms and the link-log. Electronic files and computer hosting the data were password-protected.

3.6 Study Procedures

3.6.1 Clinical and Pre-analytical Procedures

Enrolment Visit procedures: The study visit procedures were as summarized in Figure 3.2 below. At the initial visit for each person seeking participation in the study, all those meeting the inclusion criteria and those willing to participate in this study, were enrolled. At this visit enrollment PTID was assigned to each participant providing written informed consent and a detailed demographic questionnaire administered (Appendix III), followed by a baseline general examination and a detailed genital examination that included examination of penile epithelium and urethral meatus with a magnifying glass.

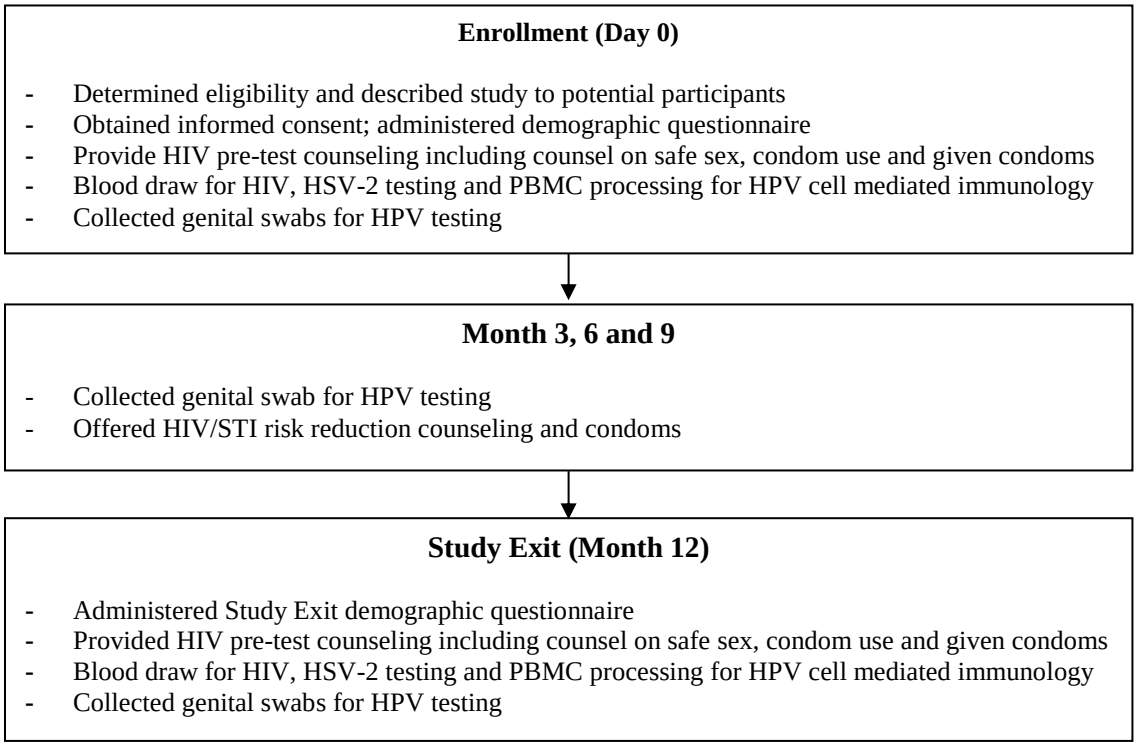


Figure 3.2: Flow chart showing the sequence of study procedures throughout the study

The following was sought during the examination: Itchiness, burning, pain, rash, open sore, and/or cracked skin. Those with signs or symptoms of STI including clinical HPV were referred appropriately for treatment and syndromic STI management. Consenting participants were given risk reduction counseling and condoms. At this visit, participants provided blood (by venipuncture) for PBMC processing, CD4/CD8 count, complete blood count (CBC) and serologic testing for HIV and HSV-2 infection, as well as penile swabs (of penile shaft, glans penis/coronal sulcus and scrotum) for HPV infection testing. The bulk of the collected blood was used for processing PBMCs required for immunological assays.

Before processing, samples were keyed into Fishermen HPV study laboratory sample reception book with PTID number, sample type/appearance and date of collection. The serum and plasma samples were stored in the freezer at –70 °C or below until

they were required to be processed for testing, HPV penile swabs were stored at –70 °C until testing, and PBMC were harvested from whole blood using density gradient technique, stored at –80 °C overnight and transferred to liquid nitrogen storage until testing (Appendix XV). After processing, the samples were recoded into the sample storage inventory in the order of enrollment in an ascending order and stored appropriately. Individual specimens were labeled with PTID, sample type and stored in 10x10 cryoboxes labeled with a serial cryobox number for easy tracking of individual samples. Frozen specimens at –70 °C were shipped on dry ice, while those kept in liquid nitrogen were transported in a dry shipper to UCSF laboratories for specialized testing upon securing appropriate ethical clearance and export/import permits.

3.6.2 Laboratory Procedures

3.6.2.1 HIV, HSV-2, CD4/CD8 and CBC testing

HIV antibody testing was conducted using standardized algorithms (Abbott Determine HIV1/2, Abbot Park, IL; and Unigold Rapid Assay, Trinity Biotech plc, Bray, Ireland). If a participant was double positive or discordant on two rapid tests, a confirmatory ELISA (BioMerieux Vironostika HIV Uni-Form II Antigen/Antibody ELISA, Marcy l'Etoile, France) test was performed. Baseline sera from HIV negative participants who seroconverted in the course of study follow (tested positive at exit) were subjected to confirmatory ELISA testing to rule out possibility of acute infection at baseline. CBC and CD4/CD8 count were evaluated using the Beckman Coulter ACT™ 5diff Cap Pierce (CP) Hematology Analyzer (Beckman Coulter, Fullerton, CA, USA) and BD FacsCount flow cytometer (Becton Dickinson, San Jose, CA,USA) respectively. HSV-2 antibody testing was conducted at the end of the study on stored sera using Kalon HSV-2 IgG ELISA (Kalon Technologies, Guildford, UK).

3.6.2.2 HPV Molecular Testing

Molecular detection of HPV was performed on the collected genital swabs. The HPV genotypic detection occurred in three stages: extraction of HPV DNA, amplification of the HPV DNA and genotypic detection of the amplified HPV DNA.

Extraction of HPV total genomic DNA: Genital swabs were incubated in 1 mL of phosphate buffered normal saline at 37 ^{o}C overnight and vortexed vigorously to dislodge all cellular materials into solution. Genomic DNA was extracted by QIAamp MinElute media kit (Qiagen, Valencia, CA) as per manufacturer's instructions. In summary, cells were digested using proteinase K and released DNA captured in a DNA binding column, precipitated and cleaned using Isopropanol and ethanol. The purified DNA was then eluted using an elution buffer into a clean tube ready for amplification by PCR.

Amplification of L1 HPV Fragment: Using biotinylated primer pools (Table 3.2) of PGMY09 and PGMY11 plus HMBO1 mixture of consensus primers sets (Gravitt *et al.,* 2000) manufactured by Roche Molecular Biochemicals, amplification of 450 bp of the viral L1 ORF was carried out as described by Farhat *et al*. (2015). Briefly, 100 µL of PCR mixture containing final concentrations of 0.06 µM PGMY09, PGMY11 primer sets, 1.2 µM HMBO1, 200 µM deoxyribonucleoside triphosphate mix (Thermo Fisher Scientific, Waltham, MA), 1X buffer, 4 mM $MgCl_2$, and 7.5 U of Gold-Taq polymerase (Applied Biosystems, Grand Island, NY). Two denaturation steps (2-min at 50 ^{o}C and 9-min at 95 ^{o}C) were followed by 40 cycles of amplification with a PCR thermo cycler (AB 2720, Applied Biosystems, Grand Island, NY). Each cycle included a denaturation step at 95 ^{o}C, an annealing step at 55 ^{o}C and an elongation step at 72 ^{o}C for one minute each. The final elongation step was prolonged for further five minutes. Hypoxanthine-guanine phosphoribosyltransferase (HPRT1) gene primer set (IDT, Corralville, Iowa) at 0.6 µM final concentration was used to confirm specimen adequacy through amplification and detection of human DNA.

Detection of the PCR product: The amplified PCR product was detected using multiplex Luminex xMAP platform (Luminex Corp., Austin, TX), where the PCR product was hybridized on oligonucleotide probes coupled on polystyrene beads. The Luminex xMAP suspension array technology is based on polystyrene beads with a diameter of 5.6 µm that are internally dyed with various ratios of two spectrally distinct fluorophores, creating an array of 100 different bead sets with specific absorption spectra. Different molecules, such as individual oligonucleotide probes,

can be coupled to different bead sets. These sets are combined to a suspension array and, due to their unique absorption spectra, allow up to 100 different probes to be measured simultaneously in a single reaction.

Table 3.2: PGMY Primer Sequences (Gravitt *et al*. 2000)

Primer designation	Primer sequence (5′-3′)
PGMY11-A	GCA CAG GGA CAT AAC AAT GG
PGMY11-B	GCG CAG GGC CAC AAT GG
PGMY11-C	GCA CAG GGA CAT AAT GG
PGMY11-D	GCC CAG GGC CAC AAC AAT GG
PGMY11-E	GCT CAG GGT TTA AAC AAT GG
PGMY09-F	CGT CCC AAA GGA AAC TGA TC
PGMY09-G	CGA CCT AAA GGA AAC TGA TC
PGMY09-H	CGT CCA AAA GGA AAC TGA TC
PGMY09-I[a]	G CCA AGG GGA AAC TGA TC
PGMY09-J	CGT CCC AAA GGA TAC TGA TC
PGMY09-K	CGT CCA AGG GGA TAC TGA TC
PGMY09-L	CGA CCT AAA GGG AAT TGA TC
PGMY09-M	CGA CCT AGT GGA AAT TGA TC
PGMY09-N	CGA CCA AGG GGA TAT TGA TC
PGMY09-P[a]	G CCC AAC GGA AAC TGA TC
PGMY09-Q	CGA CCC AAG GGA AAC TGG TC
PGMY09-R	CGT CCT AAA GGA AAC TGG TC
HMB01[b]	GCG ACC CAA TGC AAA TTG GT

[a]PGMY09-I and PGMY09-P are 18 bp in length.
[b]HMB01 is shifted 3′ to avoid internal priming and formation of secondary structure

Coupling of oligonucleotide probes to beads: The sequences of 5′-amine modified probes used in the PGMYLX assay were as presented in Table 3.3. A second probe was included to increase specificity for some selected HPV genotypes (Table 3.3). A probe: 5'-ACTTGTATTTGCATGCCCAA-3' was included in the mixture of probes to detect the amplified HPRT human gene for specimen adequacy. The synthesized probes (IDT, Coralville, Iowa) were coupled to 5'carboxylated beads (Luminex Corp) through a carbodiimide base coupling procedure as manufacturer's instructions for coupling to beads. Briefly, approximately 10 million carboxylated beads were suspended in 50 µL of 0.1 M 2-(N-morpholino) ethanesulfonic acid at pH 4.5 (MES). Probe oligonucleotides (0.5 nM) and 50 µg of N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) were added and mixed thoroughly with the beads. Incubation was carried out in the dark under agitation for 30 min and repeat agitation for another 30 min with additional 50 µg EDC. After the addition of EDC and a repeat incubation step, the beads were washed once with 0.5 mL of 0.02% Tween 20 and once with 1 mL of 0.1% of sodium dodecyl sulfate (SDS) before being stored in 100 µL of TE buffer at 4 °C in the dark until use.

PCR product hybridization : Ten µL of PCR amplified product was mixed with 20 µL of probe-coupled beads (10,000 bead/µL of each bead) in 7 µL of 1X TE and 33 µL of 0.15M TMAC, in a 96 well PCR plate (Fisher Scientific, Waltham, MA), denatured at 95 °C for 10 minutes in AB 2720 thermocycler, kept on ice for two minutes and hybridized at 41 °C for 90 minutes on Eppendorf Thermomixer (Fisher Scientific, Waltham, MA) shaking at 600 RPM. After hybridization, the beads were transferred to a 96-well filter plate (Millipore, Billerica, MA), pre-wetted with 100 µL wash buffer and using a vacuum manifold, washed twice with wash buffer, then incubated with 100 µL of diluted Strep-R-PE (Invitrogen, Grand Island, NY) in 2M TMAC staining buffer (1:1600) and incubated on Thermomixer at 25 °C for another hour of shaking at 600 RPM.

Table 3.3: HPV type specific probe sequences used in the PGMYLX assay

SNo.	HPV genotype	Risk	Probe 1 Sequence	Probe 2 Sequence
1	6	LR	TGGAAGATGTAGTTACGGATG	
2	11	LR	TGTAGCAGATTTAGACACAGA	
3	16	HR	GATATGGCAGCACATAATGAC	CAATTGCCTGGGATGTTAC
4	18	HR	CTTAAATTTGGTAGCATCATATTG	TCAGCCGGTGCAGCATCC
5	26	HR	GCTGACAGGTAGTAGCAGAGTT	GCCATAACATCTGTTGTAAGTG
6	31	HR	GATCTTCCTTGGGCTTTTGG	AATTGCAGCACAAACAGACA
7	33	HR	CTGTCACTAGTTACTTGTGTGCAT	TTTGGAGGTACTGTTTTTTGA
8	35	HR	ATCATCTTTAGGTTTTGGTGC	CTGTCACTAGAAGACACAGCAG
9	39	HR	GTAGAAGGTATGGAAGACTCTA	AGATGGTGGAGGAGCTACAG
10	40	LR	TGGGGTTGGTGTGGGGGACTGTGTGGCAGC	
11	42	LR	GCGTTGTTACCTTAGCCTGA	ATCACCAGATGTTGCAGTG
12	45	HR	GCACAGGATTTTGTGTAGAG	GCTTTTCTGGAGGTGTAGTAT
13	51	HR	CATCCTCCAAACTAGCAGAC	CAGCAGTGGCAGTGCTAATA
14	52	HR	ACAAGTTATAGCAGTAGAAGTG	CCTTTCCTTTAGGTGGTGTGT
15	53	HR	CCAGTCTTCCAGTAAGGTAGAA	GACATAGACTGTGTGGTTGC
16	54	LR	TTATTAAAGCTATCCTGCGTGG	TCCTCCAAACTACTTGTAGCTG
17	55	HR	CGCATGTATTGTTTATATTCTGTA	AGACTGAGTTGTAGCAGCAC
18	56	HR	CGTGCATCATATTTACTTAACTG	CGTTGACATGTTATAGCTGTGC
19	58	HR	TCCTTTTCTTTAGGGGGTGCT	CTTAGTTACTTCAGTGCATAATGTC
20	59	HR	GGGTCCTGTTTAACTGGC	CTGGTAGGTGTGTATWCATTAGG
21	61	LR	TTCCCTAAAGCTTGTGGCTT	GCATAGCGATCCTCCTTGG
22	62	LR	CAAATTCCTCCGTGTGTCGC	TGTCATTTGCGCATACGGG
23	66	HR	AATGTGCTTTTAGCTGCATTAAT	GGCTGTTCCCTCTGACATG
24	67	LR	TCATGGTGTGTATGTATTGCA	CCTTTGCTGTTGGAGGGGATG
25	68b	HR	CTGATTGCAGATAGCGGTATG	GGTACAGCTGATTCAGTAGTAG
26	70	LR	GGCCGTTTCGGTGCAGGC	
27	72	LR	ATACAGAGGACGCTGTGGC	GTGTGGCGAAGATACTCACG
28	73	HR	GAGCTACTAGCCTGTGTACCTAC	GTTGAGGACGTTGGCAACT
29	81	LR	GCAGCAGATGTAGCTGTGC	TGTCCAAAATGACATGTCGGC
30	82	HR	TGTTTGTGCAACAGATTGAG	CTGAGGGGGGCAAGGTTA
31	83	HR	GAGGCTGTGTATTCATTAGCC	ATCAAGGCTGGTGGAAGGA
32	84	LR	TTTATATTCTGATTCGGTGTTGG	TGGAGGGAGGGGGCACAAC
33	CP6108	LR	GGCAGACTGGGAAGCAGCA	GGTTCTGGGGCAGCAGTGC

Beads were washed three times with 100 µL wash buffer and re-suspended in 100ul wash buffer for additional five minutes, shaking at 600 RPM on Thermomixer before reading. Genomic DNA isolated from Caski cells (ATCC, Manassas, VA) was used as positive control and genomic DNA isolated from K562 cell line (ATCC, Manassas, VA) was used as negative control. Roche Linear array positive control for HPV-16 (Roche Diagnostics, UK) was included in every run as the HPV positive control. The hybridized PCR product was then detected on multiplex Luminex xMAP platform (Luminex Corp).

On the Luminex xMAP platform, each HPV genotype was allocated a specified fluorescent region (Figure 3.3). Characteristic fluorescence intensity peak for each region was capture and analyzed (Figure 3.4). The bead specific fluorescence intensity was measured (Figure 3.5). This was a quality check associated with each HPV type indicating adequacy of the type specific beads in each sample. The average median fluorescent intensity (MFI) of six negative control wells on each assay run was used as background and the MFI greater than 10 times 2 SD from the background was considered positive.

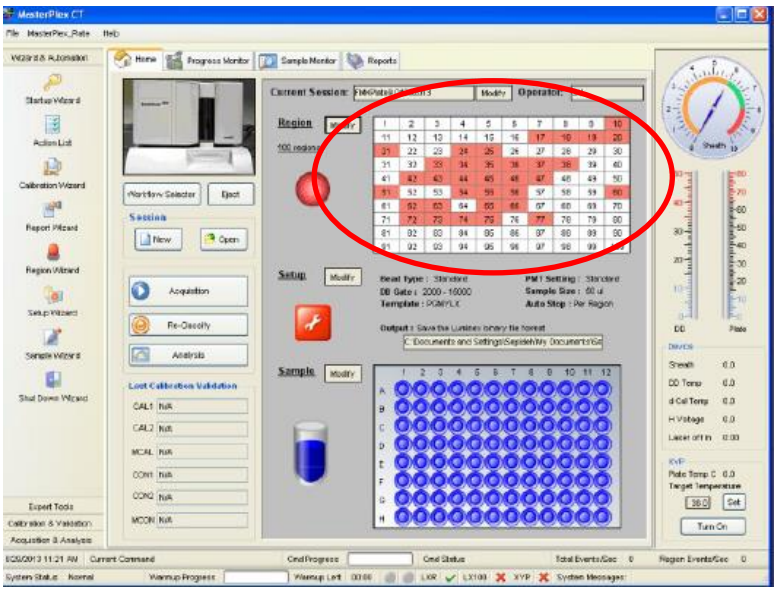


Figure 3.3: A snap shot of the Luminex xMAP plate and fluorescence region

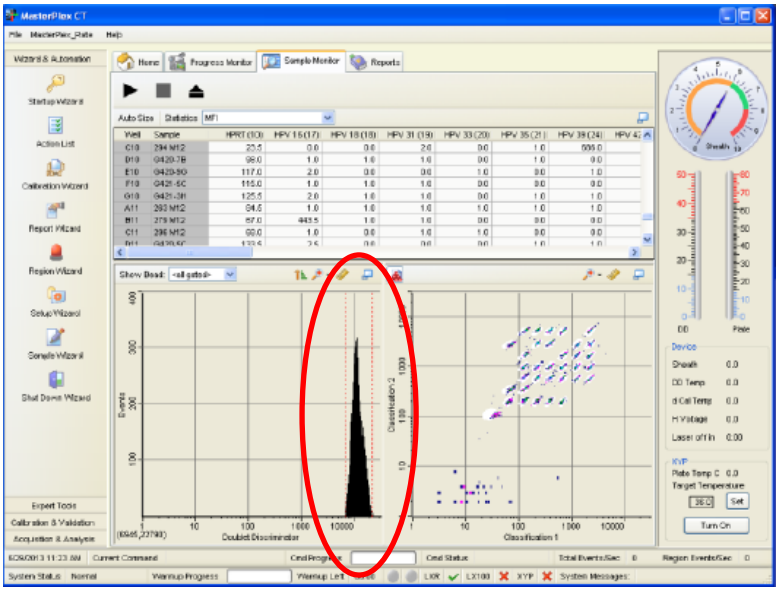


Figure 3.4: Snapshot of the fluorescent intensity acquisition during analysis

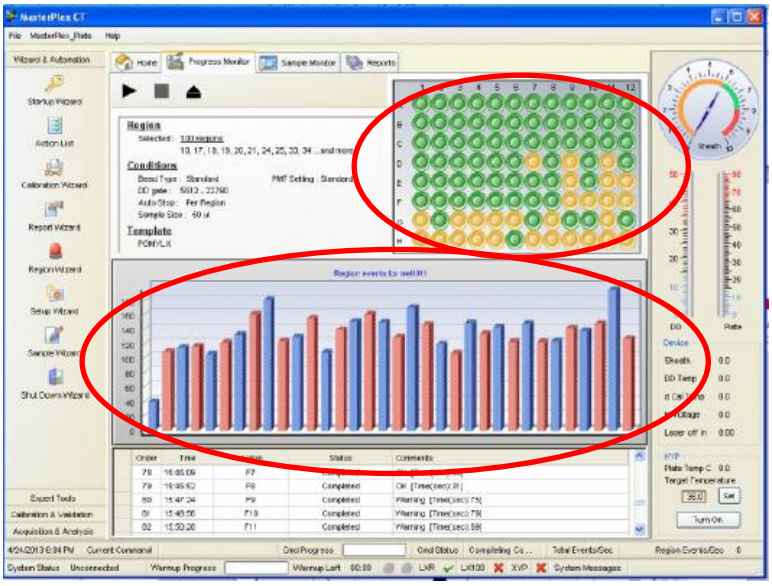


Figure 3.5: Snap shot of the Luminex xMAP 96- well plate analysis in progress

3.6.2.3 IFN-γ cellular immune response against HPV-16 by ELISPOT Assay

To explore cellular immune response among men, preliminary IFN-γ secretion by stimulated PBMCs was evaluated using IFN-γ ELISPOT Assay described in Appendix XVI. Synthetic peptides for HPV-16 E6 (Sequence ID: ref|NP_041325.1|) full length protein sequence (158 aa) individually spliced into 15-mers with 8 amino acid (aa) overlap, generating a pool of 22 peptides (Table 3.4) were used as HPV antigen. In summary, a sterile multi-screen plate was coated overnight at 4 °C with mouse anti-human IFN-γ antibody, washed and blocked using pooled human serum. Thawed PBMCs were counted, mixed with IL2 and IL7, and distributed into duplicate coated wells. Then 100 µL of antigen at a concentration of 12.5 µg/mL of each peptide from a stock of 50 µg/mL added to the test wells. After overnight incubation at 37 °C, 5% CO_2, the plate was washed and incubated with Biotinylated mouse anti- human IFN-γ monoclonal antibody (mAb) for 2 hours at 37 °C. The plate was then washed and incubated with Avidin-Peroxidase complex for 1 hour at 37 °C, 5% CO_2. The plate was then washed and incubated with freshly prepared DAB substrate for 10 minutes. The plate was then washed with deionized water and the spots enumerated after drying overnight.

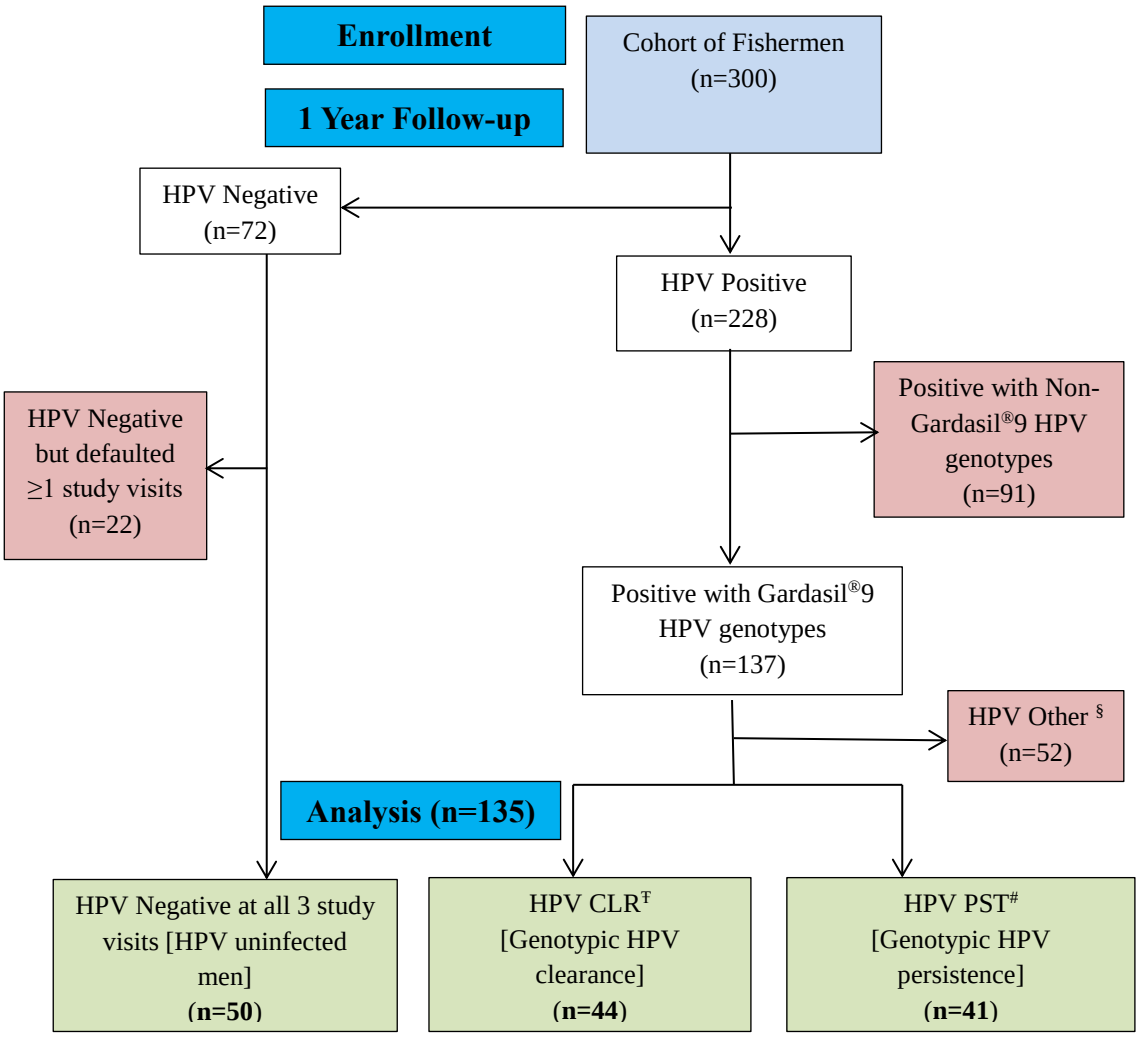
Table 3.4: HPV-16 E6 protein peptide pool of 15-mers with 8 amino acid overlap

#	Peptide Name	Sequence	#	Peptide Name	Sequence
1	HPV16_E6_15_1	MHQKRTAMFQDPQER	12	HPV16_E6_15_78	SKISEYRHYCYSLYG
2	HPV16_E6_15_8	MFQDPQERPRKLPQL	13	HPV16_E6_15_85	HYCYSLYGTTLEQQY
3	HPV16_E6_15_15	RPRKLPQLCTELQTT	14	HPV16_E6_15_92	GTTLEQQYNKPLCDL
4	HPV16_E6_15_22	LCTELQTTIHDIILE	15	HPV16_E6_15_99	YNKPLCDLLIRCINC
5	HPV16_E6_15_29	TIHDIILECVYCKQQ	16	HPV16_E6_15_106	LLIRCINCQKPLCPE
6	HPV16_E6_15_36	ECVYCKQQLLRREVY	17	HPV16_E6_15_113	CQKPLCPEEKQRHLD
7	HPV16_E6_15_43	QLLRREVYDFAFRDL	18	HPV16_E6_15_120	EEKQRHLDKKQRFHN
8	HPV16_E6_15_50	YDFAFRDLCIVYRDG	19	HPV16_E6_15_127	DKKQRFHNIRGRWTG
9	HPV16_E6_15_57	LCIVYRDGNPYAVCD	20	HPV16_E6_15_134	NIRGRWTGRCMSCCR
10	HPV16_E6_15_64	GNPYAVCDKCLKFYS	21	HPV16_E6_15_141	GRCMSCCRSSRTRRE
11	HPV16_E6_15_71	DKCLKFYSKISEYRH	22	HPV16_E6_15_144	MSCCRSSRTRRETQL

3.6.2.4 HPV Cytokine Immune response Assay

To evaluate cytokine responses against HPV among men, PBMCs were stimulated using HPV L1-VLP antigens and secreted cytokines measured using a multiplex Luminex assay. Cell-mediated immune responses against natural infection with any (or a combination) of the nine HPV genotypes (HPV genotype: 6, 11, 16, 18, 31, 33, 45, 52, and 58) included in the nonavalent HPV vaccine; GARDASIL®9 (Human Papillomavirus 9-valent Recombinant Vaccine), were evaluated. Cytokine response analysis was performed among fishermen who were: negative for HPV at all 3 study visits (controls, n=50), cleared HPV infection (HPV CLR, n=44) or had persistent HPV infection (HPV PST, n=41) as shown in Figure 3.6. Men with HPV clearance (HPV CLR) were defined as men detected with at least one GARDASIL®9 HPV genotype(s) at baseline and tested HPV DNA negative for the same GARDASIL®9 HPV genotype(s) at the two subsequent study visits (month 6 and month 12). Men with persistent HPV infection (HPV PST) were those detected with the same GARDASIL®9 HPV genotype(s) at all three study visits.

Thawed cryopreserved PBMCs diluted to a final concentration of $2x10^6$ cells/mL were cultured for 48 hrs. at 37 °C and 5% CO_2 in RPMI-1640 (Gibco, Invitrogen Life Technologies, Carlsbad, CA), supplemented with 10% heat inactivated FBS (Gibco, Invitrogen) and 1% 100 µg/ mL–100 U/mL Penicillin-Streptomycin/ 2 mM L-glutamine (Gibco, Invotrogen). To determine HPV-specific cell-mediated immune responses, cells were incubated in presence of the following antigens: Phytohaemagglutinin (PHA, at 2.5 µg/mL; Remel, Kansas, USA) as positive control, baculovirus lysate (at 2.5 µg/mL; Novavax, Inc. Maryland, USA) as antigenic control and GARDASIL®9 L1 VLPs without adjuvant (Merck & CO., Inc, Kenilworth, NJ) at: 3.8 µg/mL (HPV Type 6), 7.5µg/mL (HPV Type 16), 5 µg/mL (HPV Type 11 and HPV Type 18), and 2.5 µg/mL (HPV Type 31, HPV Type 33, HPV Type 45, HPV Type 52, and HPV Type 58). Cell cultures incubated in the absence of antigen were used as negative control. Supernatants from the cytokine induction cultures were harvested and preserved at −80 °C until analysis.



§ HPV Other: Men detected with Gardasil®9 HPV genotype(s) not meeting criteria for clearance or persistence

₸ HPV CLR (HPV clearance): Men who tested negative for a Gardasil®9 HPV genotype(s) detected at baseline, on the two subsequent follow up study visits

HPV PST (HPV persistence): Men detected with the same Gardasil®9 HPV genotype(s) at all 3 study visits

Figure 3.6: Study design for the evaluation of cytokine immune response against natural HPV infections among Fishermen in Kisumu County, Kenya

Cytokine immune responses were evaluated in thawed cell culture supernatants by Luminex 100 platform (Luminex Corp.). A panel of eight cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ and TNF-α) were included in this analysis. Cytokine concentrations (pg/mL) were estimated using Milliplex® MAP kit (EMD Millipore, Massachusetts, USA) according to instructions from the manufacturer. The lowest cytokine detection limit was set at 3.2 pg/mL – the lowest dilution of the kit's standard. Samples with cytokine concentration values below this limit (on VLP stimulated PBMCs but positive on PHA treated cells) were considered immune non-responders, assigned a score of zero and excluded from analysis. Any cytokine whose levels were below detection limit in all analyzed specimens was excluded from data analysis.

3.7 Data Management

3.7.1 Data Entry and Storage

Quality control and Quality assurance of completed study questionnaires and medical examination forms was conducted. At the end of every clinic day, completed study documents were reviewed to identify and resolve any gaps or inconsistencies on the forms before data were entered into SPSS data base. All collected data was treated with confidentiality. Paper copies were stored in locked cabinets and the electronic database was password protected to ensure controlled access. De-identified dataset (using PTIDs) was kept away from the consent forms and the link logs. The key to the cabinet was kept by the study PI.

3.7.2 Data analysis

To determine factors associated with HPV, HIV and HSV-2 prevalence and incidence among fishermen, descriptive data analysis was performed and presented in tables and graphs. Prevalence for HPV, HIV, and HSV-2 was computed and the incidence density of these infections estimated in person-years of follow-up. Chi square and t-test statistics were computed for categorical and continuous variable respectively. Univariate data analysis was performed followed by multivariate analysis by logistic regression for variables with p=0.1 in univariate analysis, to determine factors independently associated with HPV, HIV and HSV-2 infections at significance level

of $p<0.05$. Risk factors for HPV, HIV and HSV-2 infection were evaluated by comparing men with and those without these infections, whereas for HPV persistence by comparing men with persistent HPV infection and those with non-persistent HPV infection; odds ratio (OR) or risk ratios (RR) were presented with their 95% confidence interval (CI).

To characterize genotypic HPV infections and potential of type replacement among fishermen, the prevalence of HPV genotypes was computed and presented on graphs. Genotypic dendrograms for probability of co-infections with other HPV genotypes among men detected with the nine vaccine preventable HPV genotypes (HPV-6, 11, 16, 18, 31, 33, 45, 52 and 58) were plotted using R version 3.4.3 software (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2017: https://www.R-project.org). Bayesian logistic regression was performed to determine genotypic associations between the 9 vaccine preventable HPV genotypes and the other 24 HPV genotypes.

To understand cell-mediated immune response among fishermen, the numbers of men with cytokine immune responses were presented as proportions with their respective 95% confidence interval (95% CI). Cytokine concentrations among immune responders and biomarkers for baseline immunological status were presented as mean ± standard deviation (SD). Comparisons of immune response data between men with HPV clearance, HPV persistence and HPV uninfected men were performed using independent t-test, and differences with a p-value of <0.05 considered significant. For these cytokine responses, p-values were only reported for upregulated cytokine levels whose change remained statistically significant after Bonferroni's correction for multiple comparisons. Statistical analyses were conducted using Stata version 12.0 software (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of Fishermen Selected from beaches on Lake Victoria in Kisumu, Kenya

4.1.1 Study Recruitment and Retention

Three hundred fishermen aged 18 – 47 years provided written informed consent, got enrolled in to the study and were followed up every three months for a period of one year between September 2011 and December 2012. Of these three hundred participants, 255 (85%) were successfully retained for the one year study follow up and exited from study uneventfully. Of the 44 who were lost to follow-up by the time of exit procedures, three had died from non-study related causes of death (two drowned in the lake due to epilepsy and one was attacked at night), while 41 had traveled to far islands in search for fish and were untraceable. Retention at visit-2 (month 3), visit-3 (month 6) and visit-4 (month 9) were 96% (288), 88% (264) and 86% (258) respectively.

4.1.2 Baseline Characteristics of studied Fishermen in Kisumu, Kenya

Enrolled study participants were sexually active men with a mean age (SD) of 27.7 (6.40) years old and majority of these men were younger with 64.7% (194/300) being ≤ 28 years old (Table 4.1). Additionally, single men were significantly younger with a mean (SD) age of 22.6 (3.39) compared to married men who had a mean age of 29.0 (6.34); $p<0.001$. The mean (SD) age at sexual debut was 15 (3.7) years. The majority of men (57%) had attained primary education. Of the 300 men enrolled into the study, 236 (78.7%) reported ever being married, while 231 (77%) were married at the time of baseline structured interview. Among married men, 135 (58%) had multiple sexual partners with at least one new partnership formation in the year prior to the study compared to 53 (83%) among single men who were 3.6 times as likely as married men to report multiple sexual partners in this period; OR=3.60 (95% CI: 1.72–7.73).

Table 4.1: Background characteristics of fishermen in the study

Characteristic (N=300)	Category	N (%)
Age	Mean age (SD)	27.7 (6.4)
	Median age (IQR)	26.5 (21 – 32)
Marital status	single never married	64(21.3%)
	Married	231(77.0%)
	Other (divorced / widowed)	5(1.7%)
Ethnicity	Luo	283 (94.3%)
	Other	17 (5.7%)
Monthly income	Income ≤ 115US$ (10,000 KShs)	273 (91.0%)
	Income > 115US$ (10,000 KShs.)	27 (9.0%)
Religion	Mainline Protestant	75 (25.0%)
	Catholic	46 (15.3%)
	Independent African churches	131 (43.7%)
	Other	48 (16.0%)
Duration of vocational training	Less than 1 year	22 (32.4%)
	1- 2 years	33 (48.5%)
	More than 2 years	13 (19.1%)
Sexual History:		
Age of sexual debut	Mean age (SD)	13.5 (3.7)
	Median age (IQR)	13 (5)
Condom use at sexual debut	Yes	20 (6.7%)
	No	280 (93.3%)
History of HIV test:	Ever tested for HIV	243 (81.3%)
Immunologic parameters, N=287; (mean, SD):	Baseline CD4 (HIV Positive)	518.9 (297.31)cells/µl
	Baseline CD4 (HIV Negative)	922.7 (304.32)cells/µl
	Baseline CD4 (overall)	829.8 (346.82) cells/µl
	White blood cells ($x10^9$/L)	5.6 (1.53)

The study population was characterized with low level of education (with a mean of 8.8 years of school, SD 3.1) and low or no condom use: 7% reported use of condom at sexual debut, 10% and 36% reported frequent use of condom (>75% of the time) with regular and new sexual partners respectively (Table 4.2).

Table 4.2: Baseline sexual characteristics of studied fishermen

CHARACTERISTIC	CATEGORY	N (%)
Sexual History:		
Circumcised	Yes	72(24.0%)
	No	228(76.0%)
Age of sexual debut	Mean age (SD)	13.5(3.7)
	Median age (IQR)	13(5)
Condom use at sexual debut	Yes	13(6.7%)
	No	280(93.3%)
Condom use with regular sexual partner	>75% of the time	31 (10%)
	25-75% of the time	68 (23%)
	<25% of the time	201 (67%)
Condom use with new sexual partner	>75% of the time	128 (43%)
	25-75% of the time	65 (22%)
	<25% of the time	107 (35%)
Sexual partners last one month	1 partner	217(72.0%)
	2 - 3 partner	74(25.0%)
	More than three partners	9(3.0%)
Sexual partners last six month	1 partner	152(50.7%)
	2 - 3 partners	120(40.0%)
	>3 partners	28(9.3%)
Sexual partners last twelve months	1 partner	112(37.3%)
	2 - 3 partner	118(39.3%)
	>3 partners	70(23.4)
Sexual partners in life time	1-3 partners	53(17.7%)
	4 - 8 partners	113(37.7%)
	>8 partners	134(44.6%)
Ever engaged in transactional sex	Yes	266(88.7%)
	No	34(11.3%)
Genital hygiene		
Wash genital during bathing		265(88.3%)
Wash genital only		44(14.7%)
Wash genitals immediately before sex		35(11.7%)
Wash genitals immediately after sex		80(26.7%)
History of STI		
Ever tested for HIV		243 (81.3%)
History of STI		77(25.8%)

Additionally, a significant number of men 183 (61%); $p<0.01$ and 118 (39%); $p<0.05$, never used condoms with frequent sexual partners and new sexual partners respectively. The proportion of men (43%) who used condoms >75% of the time with a new sexual partner was significantly higher ($p<0.001$), compared to that of men (10%) who used condom >75% of the time with regular sexual partners (Figure 4.1).

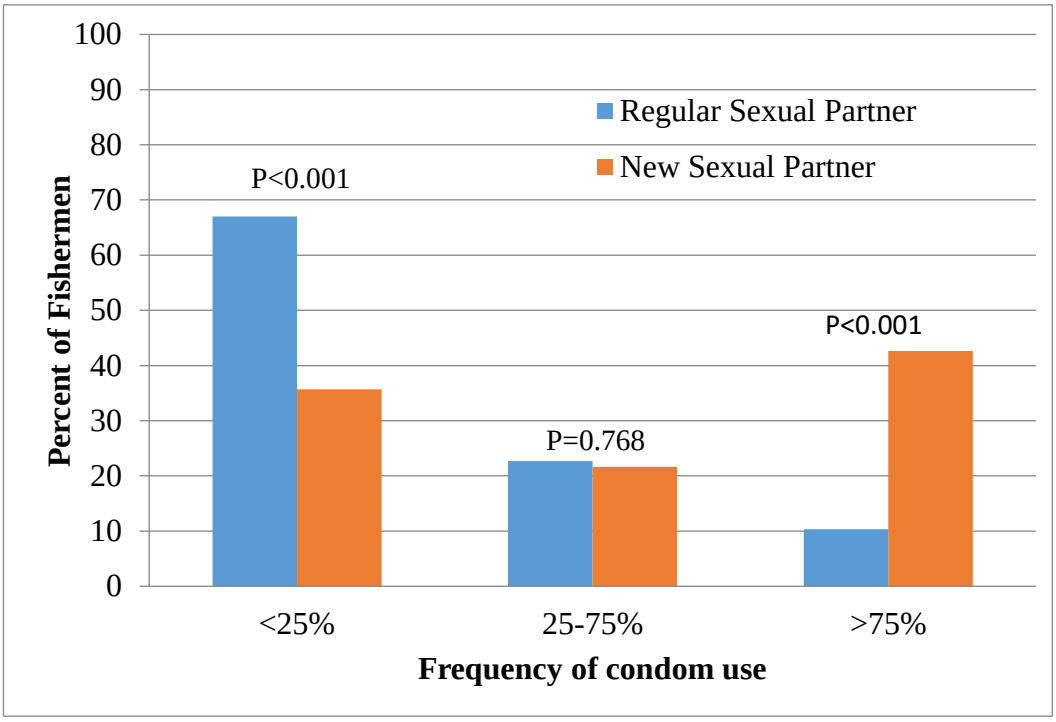


Figure 4.1: Frequency of condom use with regular and new sexual partners

Conversely, the proportion of men (35%) who used condoms <25% of the time with a new sexual partner was significantly lower (p<0.001), compared to that of men (67%) who used condom <25% of the time with regular sexual partners (Table 4.2). The majority (88%) of men reported ever washing their genitals during bathing. However, slightly over a quarter of the 300 fishermen reported washing genitals immediately after sexual intercourse and 15% reported washing their genitals immediately before sex.

Majority of married men 82% (189/231) reported that their most recent sexual act (most recent sexual partnership) was with a woman other than their wife: 61% (116/189) men had sexual intercourse with their girlfriend and 39% (73/189) with a sex worker/casual partner. Only 18% (41/231) had the most recent sexual act with their wife. This suggested that concurrent sexual partnerships were very common among married fishermen. However, among single men, 73% (47/64) reported that their most recent sexual act was with their regular sexual partner (girlfriend) while

26% (17/64) reported that the most recent sexual act was with a sex worker/casual partner. The majority (81.3%) of enrolled men had ever been tested for HIV and 77 men (25.8%) reported a history of STI symptoms. The mean (SD) baseline CD4 irrespective of HIV status was 829 (346.8) cells/ml of blood. Condom use during the most recent sexual act was significantly low (Figure 4.2). Only 14% (42/295) of men reported using a condom during this sexual act. In general, proportions of men that reported use of condom were not statistically different ($p>0.05$) across different types of sexual partnerships (regular partner or sex worker/casual partner). However, being single (never married) was significantly associated with condom use at most recent sexual act compared to being married ($p<0.05$) irrespective of type of sexual partnership.

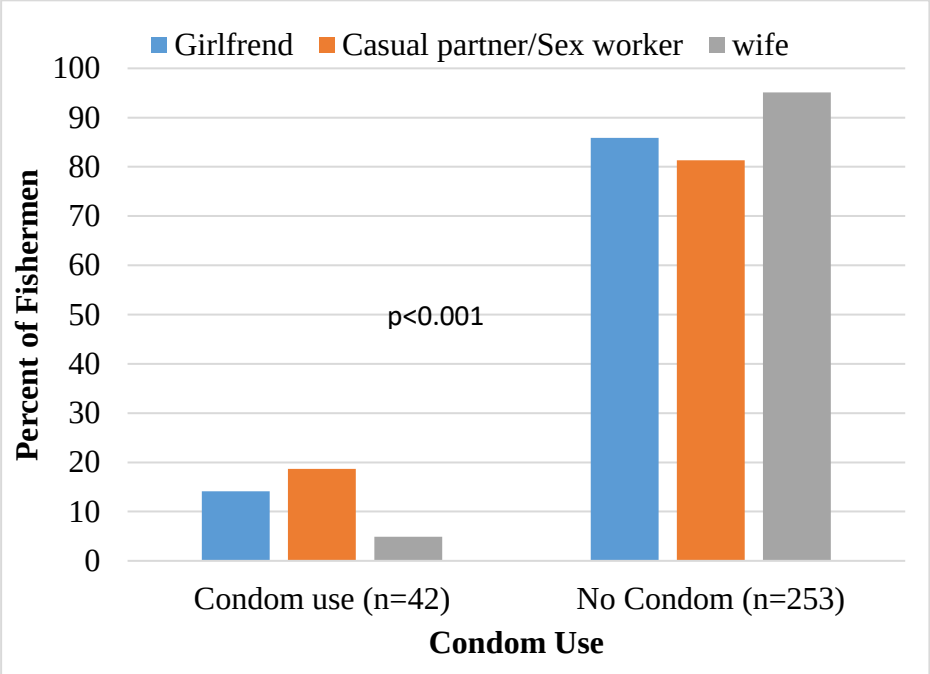


Figure 4.2: Condom use during most recent sexual act by relationship type the sexual partner

4.2 Epidemiology of HPV, HIV and HSV-2 infections among Fishermen

4.2.1 HPV infections among Fishermen

4.2.1.1 Baseline HPV infections Among Fishermen in Kisumu, Kenya

Baseline HPV prevalence was 49.7% (149/300). Of the 149 men who had HPV infections at baseline, 89 (60%) had multiple HPV types ranging from two genotypes to nine genotypes (Figure 4.3). Among those with multiple HPV types, 36 (40%) were co-infected with HIV, 61 (69%) co-infected with HSV-2, 28 (31%) co-infected with both HIV and HSV-2, and 69 (78%) co-infected with either HSV-2 or HIV (Figure 4.4). Of the 149 HPV positive men at baseline, 112 (75%) were infected with at least one HR HPV genotype.

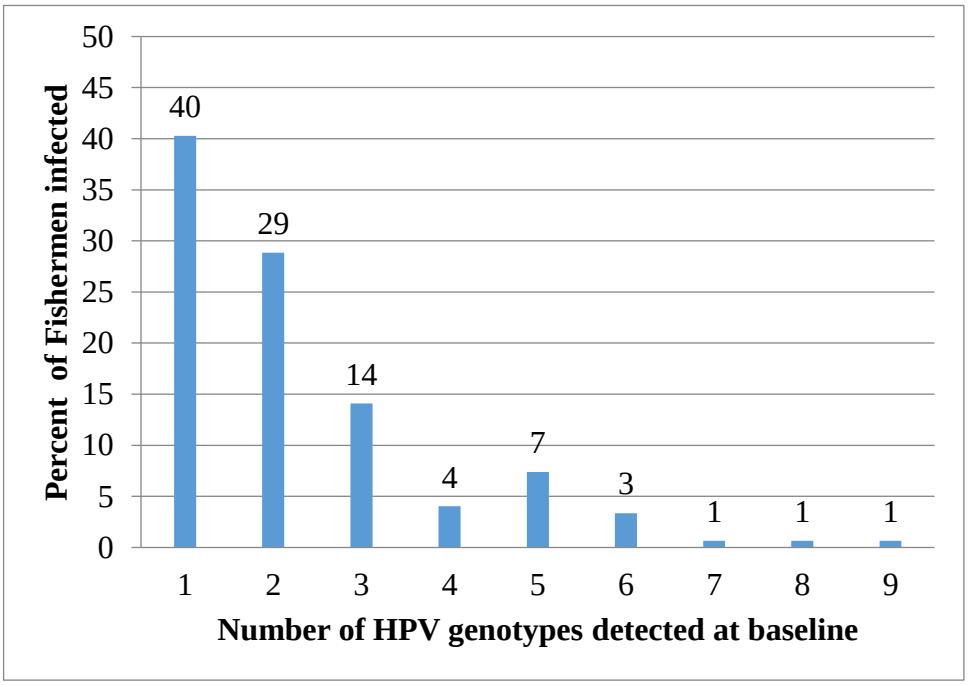


Figure 4.3: Distribution of number of HPV genotypes among HPV infected Fishermen

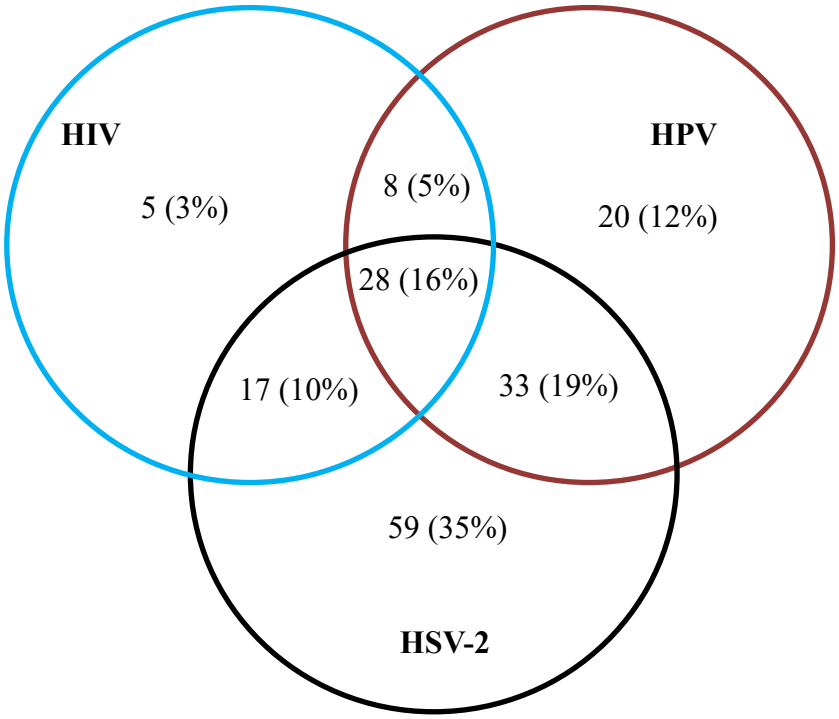


Figure 4.4: Vane diagram showing the number of men with HIV and HSV-2 co-infections among fishermen detected with multiple HPV genotypes at baseline

In bivariate analysis, there was no association between age and baseline HPV infection (p=0.817), and HPV prevalence across different age categories was similar (Figure 4.5). Men who reported belonging to the Luo traditional churches were almost 4-times more likely to have HPV at baseline compared to other religious categories (aOR=3.83; 95% CI: 1.46 – 10.03). On the other hand, men who reported being single (never married) were significantly less likely to have HPV infection at baseline compared to married men (aOR=0.33; 95% CI: 0.15 – 0.77).

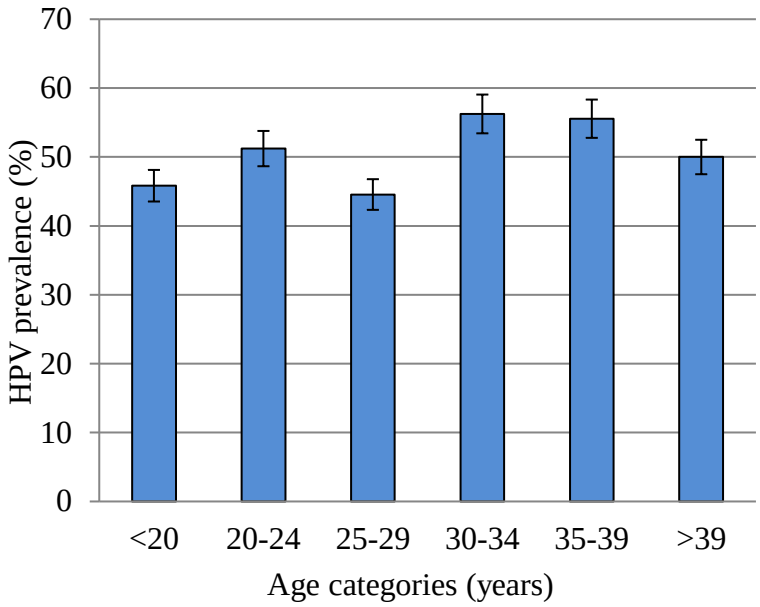


Figure 4.5: Trend of HPV prevalence across different age strata

However, men who reported the most recent sexual act to have been with their wife were significantly less likely to have HPV infection at baseline compared to those who reported girlfriend (OR=0.11; 95% CI: 0.04 – 0.29). Un-circumcised men had two times increase odds of being detected with HPV infection at baseline compared to circumcised men (aOR=2.38; 95% CI: 1.18 – 4.77). Having ≥2 sexual partners showed a trend towards association with baseline HPV infection in bivariate analysis, however, the number of sexual partners was not associated with baseline HPV infection in multivariate analysis (Table 4.3).

Table 4.3: Factors associated with baseline HPV infection among fishermen

Characteristic	Bivariate logistic regression			Multivariate logistic regression		
	OR	95% CI	p-value	aOR	95% CI	p-value
Age	1.01	0.98 – 1.05	0.491		Not included	
Ethnic group						
Luo	0.87	0.33 – 2.32	0.781		Not included	
Other	1.15	0.43 – 3.06	0.781			
Religion						
Protestant	1.00	Reference			Reference	
Catholic	0.75	0.40 – 1.40	0.363	0.86	0.37 – 2.01	0.721
Traditional	1.86	0.99 – 3.51	0.055	3.83	1.46 – 10.03	0.006
Marital status						
Ever married	2.53	1.19 – 3.47	0.035		Reference	
Single	0.65	0.38 – 0.82	0.035	0.33	0.15 – 0.77	0.009
Lack of circumcision	1.78	1.04 – 3.05	0.037	2.38	1.18 – 4.77	0.015
Sexual partners in 6 months						
< 2 partner	0.63	0.40 – 1.00	0.048		Reference	
≥ 2 partners	1.60	1.00 – 2.55	0.048	1.84	0.33 – 3.16	0.719
Sexual partners in 12 months						
< 2 partner	0.61	0.38 – 0.98	0.04		Reference	
≥ 2 partners	1.35	1.05 – 2.15	0.04	1.63	0.66 – 4.02	0.285
Sexual partners in lifetime						
< 6 partner	0.97	0.54 – 1.76	0.135		Not included	
≥ 6 partners	1.42	0.90 – 2.24	0.135			
Wash genitals before sex	0.71	0.35 – 1.45	0.348	0.42	0.18 – 0.94	0.047
Wash genitals after sex	0.92	0.55 – 1.53	0.741	0.95	0.51 – 1.77	0.875
Baseline HSV	1.64	1.03 – 2.60	0.035	1.56	0.90 – 2.73	0.116
Baseline CD4 (continuous)	0.32	0.17 – 0.62	0.001	0.45	0.19 – 0.84	0.033
Baseline CD4 (Stratified):						
CD4 > 500	1.00	Reference			Reference	
CD4 ≤ 500	3.11	1.56 – 6.29	<0.001	4.35	1.87 – 8.91	0.001
Baseline HIV	2.79	1.58 – 4.92	<0.001	2.43	1.10 – 5.37	0.028
Relation with recent partner					Not included	
Girl friend	1.64	1.03 – 2.59	0.036			
Casual partner	1.28	0.78 – 2.11	0.335			
Wife	0.11	0.04 – 0.29	<0.001			
Condom use with recent partner						
Yes	0.40	0.20 – 0.81	0.011		Reference	
No	2.49	1.24 – 5.00	0.011	2.85	1.22 – 5.76	0.015
Condom use with regular partner					Not included	
Most of the time (>75%)	0.37	0.17 – 0.77	0.008			
Some of the time (25-75%)	1.11	0.63 – 1.95	0.729			
Rarely (<25%)	1.88	1.07 – 5.74	0.048			
Condom use with new partner					Not included	
Most of the time (>75%)	0.35	0.20 – 0.64	<0.001			
Some of the time (25-75%)	0.57	0.22 – 1.49	0.253			
Rarely (<25%)	3.01	1.77 – 5.12	<0.001			
Ever had STI						
No	0.47	0.27 – 0.80	0.005		Reference	
Yes	2.14	1.25 – 3.64	0.005	3.25	1.63 – 6.14	0.009

Of the 287 men who had CD4 results, 143 (50%) had HPV infection at baseline. However, a unit increase in CD4 was protective against baseline HPV infection (aOR=0.45; 95% CI: 0.19 – 0.84). Men with a CD4 ≤500 cells/ml of blood were 4-times more likely to be infected with HPV at baseline compared to those with CD4>500 cells/ml of blood (aOR=4.35; 95% CI: 1.87 – 8.91). Baseline HIV positive status was significantly associated with HPV infection at baseline ($p<0.001$). Therefore, majority of HPV infected men with CD4≤500 cells/ml at baseline, were also HIV infected. Additionally, men who reported ever having an STI were 3-times more likely to have baseline HPV infection compared to those that reported never having had an STI (aOR=3.25; 95% CI: 1.63 – 6.14).

Despite low reported condom use among fishermen in this study, consistent condom use was generally protective against HPV infection. Fishermen who reported using condom >75% of the time with a regular partner were less likely to have HPV infection at baseline; (OR=0.37; 95% CI: 0.17 – 0.77). This was consistent among men who reported similar condom use with new partner and most recent sexual partner (OR=0.35; 95% CI: 0.20 – 0.64) and (OR=0.40; 95% CI: 0.20 – 0.81) respectively. Fishermen who rarely used condom (<25% of the time) with a new sexual partner had the highest increased odds of testing HPV positive at baseline. Lack of condom use on most recent sexual act was associated with almost a 3-fold increased likelihood for baseline HPV infection (aOR=2.85; 95% CI: 1.22 – 5.76).

In the final model of stepwise multivariate logistic regression; baseline CD4, religion, circumcision status, and marital status were found to be significant predictors of HPV status at baseline. HIV status and genital washing before sex showed a trend towards association as predictors for baseline HPV infection. The p-values and the direction of associated with HPV infection for these independent factors are shown in Table 4.4.

Table 4.4: Best Predictors for Baseline HPV infection

BASELINE HPV	Multivariate logistic regression		
	aOR	95% CI	p-value
Baseline CD4	0.41	0.18 – 0.93	0.033
Transactional sex	0.54	0.23 – 1.24	0.146
Baseline HSV-2	1.54	0.91 – 2.58	0.104
Baseline HIV	1.91	0.91 – 4.02	0.087
Religion	1.31	1.10 – 1.56	0.002
Circumcision	2.01	1.08 – 3.74	0.027
Marital status	0.49	0.26 – 0.90	0.021
Wash genitals before sex	0.46	0.21 – 1.01	0.053

Baseline HPV infections stratified by baseline HIV status

Of the 149 fishermen infected with HPV at baseline, 48 (32%) had HIV infection. Majority, 75% (36/48) of those infected with HIV had multiple HPV genotypes compared to 53 (52%) who had multiple HPV genotypes among 101 men that were negative for HIV. Therefore, HIV positive men were almost three times more likely to be infected with multiple HPV genotypes compared to HIV negative men (OR=2.72; 95% CI: 1.20 – 6.26). Figure 4.6 shows that the burden of multiple HPV infections was consistently and significantly higher among HIV positive men compared to HIV negative men (with P–values <0.01). Among HIV positive fishermen, those circumcised were less likely to have HPV infection at baseline compared to the uncircumcised (OR=0.19; 95% CI: 0.04 – 0.89). Lack of circumcision was significantly associated with a 5-fold increased odds of being detected with HPV among HIV positive men ($p<0.05$).

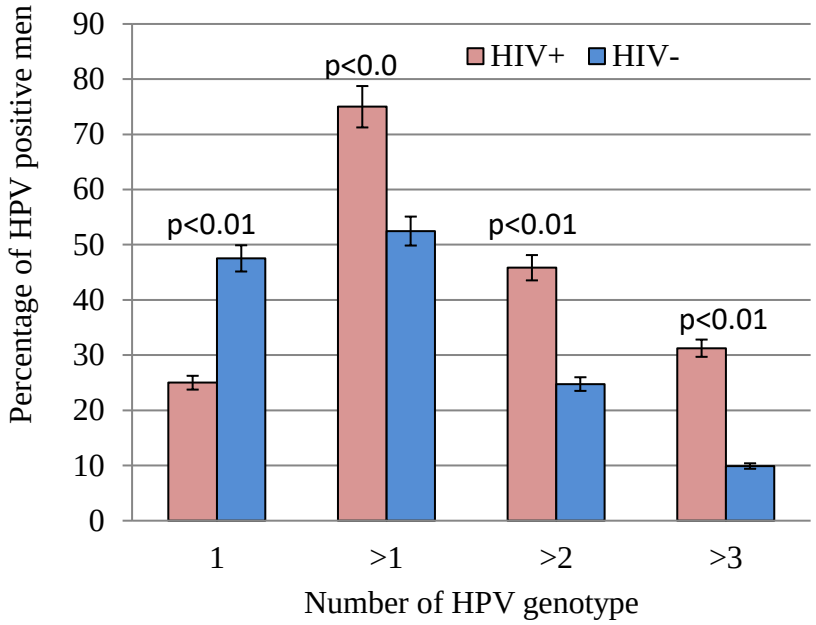


Figure 4.6: Multiple genotypic HPV infection by HIV status

Similarly, low CD4 (≤500 cells/µl) and a history of STI were both independently associated with a 3-fold increased odds of HPV infection (Table 4.5). However, these specific associations were not significant among HIV negative men (Table 4.5). Conversely, among men without HIV, being married and rare condom use (frequency of <25% of the time) with new sexual partner were significantly associated with almost a 2-fold and a 4-fold increased odds of baseline HPV infection respectively (Table 4.5).

Fishermen infected with both HIV and HSV-2 at baseline were three times more likely to be detected with HPV infection at baseline compared to men negative for both HIV and HSV-2 infections at baseline; OR=3.37 (1.60 – 7.13). Only 38.6% (44/114) and 64.3% (36/53) were detected with HPV at baseline among those negative for both HIV/HSV-2 and those positive for both HIV/HSV-2infections respectively.

Table 4.5: Bivariate logistic regression of factors associated with baseline HPV infection among Fishermen stratified by HIV status

Characteristic	HIV Positive men (n=70)			HIV Negative men (n=230)		
	OR	95% CI	p-value	OR	95% CI	p-value
Age (≤ 28 yrs.)	0.65	0.21-2.01	0.401	1.12	0.61-2.05	0.706
Circumcision	0.19	0.04-0.89	0.012	0.78	0.41-1.47	0.402
Marital status (married)	1.91	0.37-9.61	0.368	1.94	1.01-3.74	0.033
Wash genitals before sex	0.67	0.08-6.27	0.668	1.81	0.78-4.20	0.131
Wash genitals after sex	1.34	0.33-5.84	0.654	1.13	0.61-2.10	0.667
Baseline HSV-2	0.88	0.23-3.32	0.837	1.54	0.88-2.69	0.107
Baseline CD4: CD4 ≤ 500	3.43	1.01-11.89	0.024	1.10	0.32-3.80	0.867
Relation with recent partner						
Regular partner	1.00	Reference		1.00	Reference	
Casual partner	1.15	0.36-3.70	0.797	1.47	0.79-2.73	0.189
History of STI	3.27	1.01-10.97	0.026	1.26	0.61-2.61	0.506
Condom use with recent partner	0.01	0.01-8.04	0.137	0.53	0.24-1.15	0.082
Condom use with regular partner						
Most of the time (>75%)	1.00	Reference		1.00	Reference	
Some of the time (25-75%)	1.07	0.17-6.69	0.936	1.78	0.51-6.36	0.313
Rarely (<25%)	1.08	0.24-5.17	0.908	1.58	0.52-5.00	0.382
Condom use with new partner						
Most of the time (>75%)	1.00	Reference		1.00	Reference	
Some of the time (25-75%)	1.14	0.17-7.85	0.873	1.80	0.86-3.79	0.090
Rarely (<25%)	2.40	0.44-13.19	0.238	3.97	1.99-7.99	0.000

4.2.1.2 Incidence of HPV among Fishermen in Kisumu, Kenya

Of the 185 participants who met the criteria for HPV incidence evaluation, 99 had at least one new HPV genotype infection during study follow-up visit. This provided a rather conservative but cumulative annual HPV incidence of 53.5%. The distribution of the number of incident HPV genotypes was as shown in Figure 4.7.

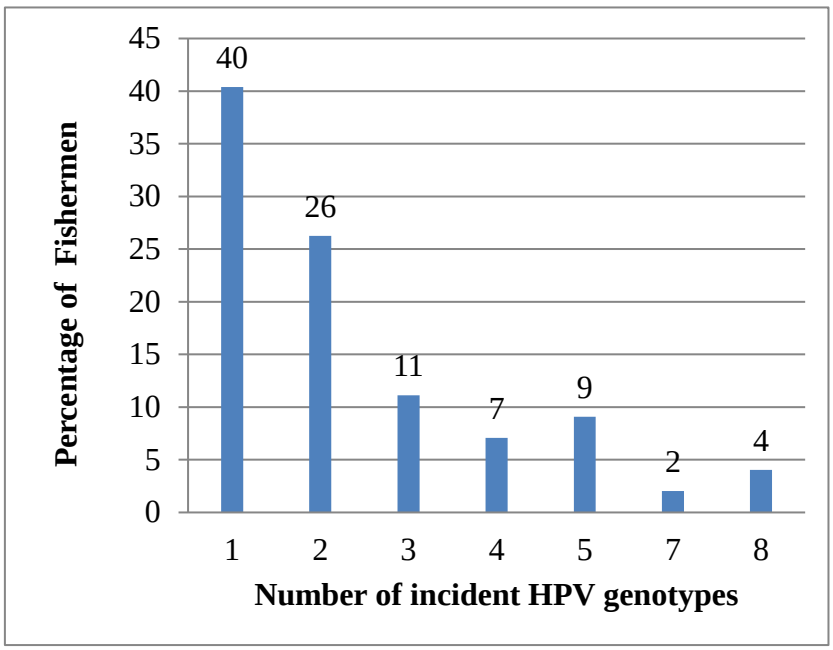


Figure 4.7: Distribution of number of incident HPV types among fishermen

Of the 99 men with incident HPV infection, 77 (78%) acquired new HPV genotypes that contained at least one HR HPV genotype while 22 (22%) had only LR incident HPV genotypes (Figure 4.8). A total of 243 new HPV genotype infections were experienced among the 99 men that had incident HPV infection. Sixty percent (59/99) of these men had multiple (2-8 types) incident HPV genotypes of which 95% (55/59) had at least one HR HPV genotype. Men who acquired multiple incident HPV genotype infections were 11-times more likely to be infected with at least one HR HPV genotype compared to men who acquired a single type of new HPV infection; OR=11.25 95% CI: 3.09–44.77 ($p<0.001$).

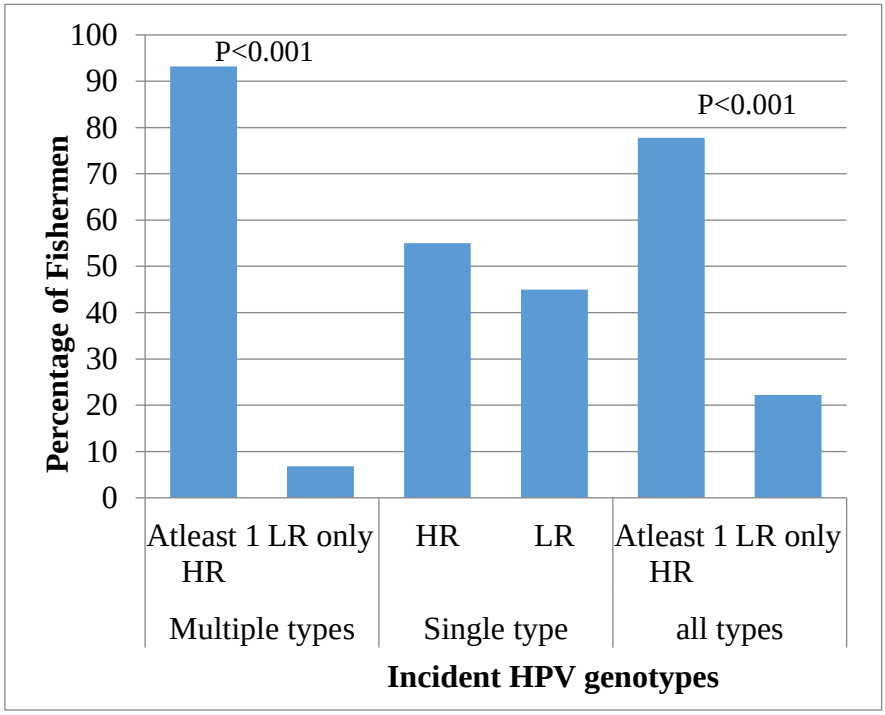


Figure 4.8: Proportion of fishermen infected with HR and LR incident HPV genotypes

Baseline age, marital status, HIV status and circumcision status were not associated with incident HPV infections (Table 4.6). Men who reported multiple sexual partners in the past one year at baseline were at increased risk of acquiring new HPV infection during follow-up compared to those who reported only one sexual partner in the same period (aRR = 14.5; 95% CI: 1.70–31.58). In bivariate analysis, men detected with HPV infection at baseline were four times more likely to have incident HPV infection during follow up compared to HPV negative men at baseline (OR=4.03; (95% CI: 2.01–8.09). The odds did not change significantly for fishermen infected with multiple HPV genotypes at baseline (OR=3.6, 5.8 and 4.5 for baseline number of genotypes >1, >2, and >3 respectively).

Table 4.6: Factors associated with HPV incidence among fishermen in the study

Characteristic	Bivariate logistic regression			Multivariate logistic regression		
	IRR	95% CI	p-value	aIRR	95% CI	p-value
Age	0.98	0.93-1.02	0.282		Not included	
Marital status						
Single	1.30	0.64-2.62	0.465		Not included	
Ever married	0.78	0.39-1.56	0.465			
Circumcision						
Yes	0.78	0.38-1.61	0.508		Not included	
No	1.28	0.62-2.63	0.508			
Sexual partners in 6 months						
< 2 partners	0.76	0.43-1.36	0.362		Not included	
≥ 2 partners	1.56	0.58-4.15	0.362			
Sexual partners in 12 months						
< 2 partners	0.52	0.28-0.76	0.022		Reference	
≥ 2 partners	6.12	1.29-13.13	0.022	14.50	1.70-31.58	0.014
Sexual partners in lifetime						
< 6 partners	0.57	0.26-1.25	0.098		Reference	
≥ 6 partners	1.64	0.91-2.93	0.098	1.34	0.35-5.14	0.666
Transactional sex						
Yes	0.75	0.26-2.20	0.600		Reference	
No	1.33	0.45-3.91	0.600	4.16	0.84-20.74	0.082
Wash genitals before sex						
Yes	1.34	0.52-3.46	0.539		Not included	
No	0.74	0.29-1.91	0.539			
Wash genitals after sex						
Yes	1.64	0.84-3.22	0.149		Not included	
No	0.61	0.31-1.19	0.149			
Baseline HSV						
No	0.79	0.44-1.43	0.434		Not included	
Yes	1.27	0.70-2.28	0.434			
Baseline CD4						
Yes	1.14	0.58-2.23	0.708		Not included	
No	0.88	0.45-1.72	0.708			
Baseline HPV						
No	0.25	0.12-0.50	<0.001		Reference	
Yes	4.03	2.01-8.09	<0.001	9.35	3.03-28.90	<0.001
Baseline HIV						
No	0.88	0.46-1.68	0.701		Not included	
Yes	1.13	0.60-2.16	0.701			
Used condom with recent partner						
No	1.67	0.59-4.71	0.336		Reference	
Yes	0.60	0.21-1.70	0.336	0.08	0.01-0.47	0.005
Condom use with regular partner						
Most of the time (≥50%)	0.14	0.02-1.15	0.067		Reference	
Some of the time (<50%)	1.25	0.53-2.98	0.067	1.84	0.27-12.36	0.530
Condom use with new partner						
Most of the time (≥50%)	0.52	0.23-0.86	0.030		Reference	
Some of the time (<50%)	2.43	1.09-5.43	0.030	3.85	0.74-20.15	0.110
Ever had STI						
Yes	1.16	0.61-2.19	0.649		Not included	
No	0.86	0.46-1.63	0.649			

The highest risk for HPV incidence was among men infected with at least one HPV genotype at baseline (Figure 4.9). There was no significant additional risk of incident HPV infection for any extra genotypes among men that had multiple HPV genotypes at baseline. Multivariate logistics regression strengthened this relationship significantly, where baseline HPV infection was significantly associated with a 9-fold increased risk of incident HPV infections ($p<0.001$). Conversely, men who reported using condom with the most recent sexual partner were significantly at reduced risk of new HPV acquisition (aRR = 0.08; 95% CI: 0.01 – 0.47).

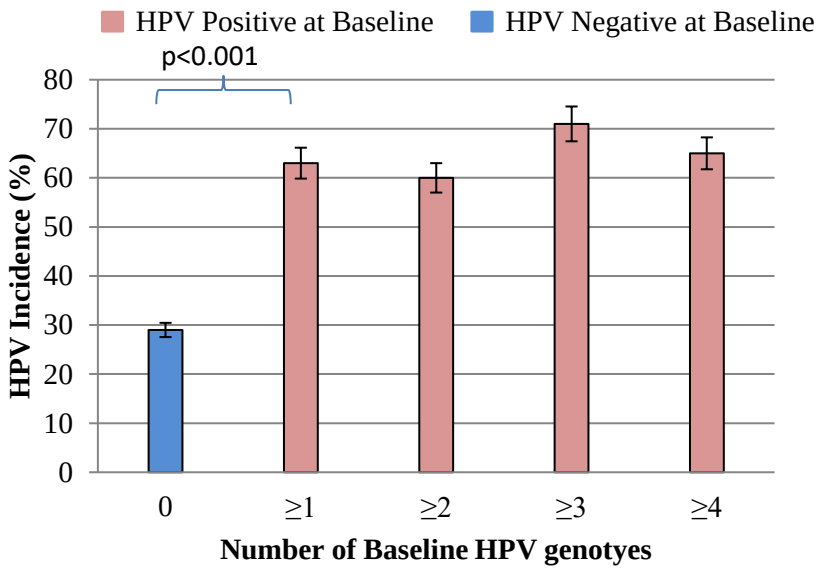


Figure 4.9: HPV incidences by number of baseline HPV genotypes

Incident HPV infections stratified by baseline HIV status

Of the 99 fishermen with incident HPV infection, 29 (29.3%) had HIV infection at baseline. Whereas among 86 fishermen who never acquired new HPV infection, 23 (26.7%) were infected with HIV at baseline. Stratifying by HIV status, 29 (56%) of the 52 men infected with HIV at baseline were detected with incident HPV infection. Conversely 70 (53%) of the 133 men without HIV at baseline were detected with incident HPV infection during follow-up. Table 4.7 shows HPV incidence analysis stratified by HIV status. Age, marital status and circumcision status were not associated with incident HPV infection irrespective of HIV status. Among HIV negative men, those who were HPV positive at baseline were significantly at increased risk of acquiring new HPV infections compared to those negative for HPV at baseline (RR=4.68; 95% CI: 2.12–10.36).

However, baseline HPV status was not a risk factor for incidence HPV infection among HIV positive fishermen. Number of sexual partners was not associated with incident HPV infection among fishermen with or without baseline HIV infection; except for number of sexual partners one year prior to the study among HIV negative men that was associated with increased risk of incident HPV infection (RR=2.70; 95% CI: 1.2–6.12). Interestingly, genital washing immediately after sex was associated with an increased risk of incident HPV infection. Among HIV positive men (RR=11.58; 95% CI: 1.36–18.94) but not among HIV uninfected fishermen.

Immediate genital washing before sex was not associated with increased risk of incident HPV infection irrespective of baseline HIV status. Additionally, among HIV negative fishermen, genital washing immediately after sex was not associated with any risk of incident HPV infection. However, HIV positive men who reported washing genitals immediately after sex had a significant 12-fold increased odds ($p<0.05$) of being detected with a new HPV infection compared to HIV positive men who reported never washing their genitals immediately after sex (OR=11.58; 95% CI: 1.36–98.94).

Table 4.7: Characteristics associated with incident HPV infection stratified by HIV status

Characteristics	HIV Positive (+) Participants (n = 52)			HIV Negative (-) Participants (n = 133)		
	OR	95% CI	p-value	OR	95% CI	p-value
Age	0.98	0.91-1.05	0.559	0.96	0.90-1.03	0.258
Marital status						
Single	1.07	0.21-5.33	0.937	1.40	0.64-3.08	0.403
Married	0.72	0.15-3.39	0.678	0.78	0.36-1.70	0.534
Circumcision						
Yes	0.55	0.11-2.74	0.464	0.87	0.39-1.97	0.743
No	1.82	0.36-9.12	0.464	1.15	0.51-2.59	0.743
Sexual partners in 6 months						
At most 1 partner	0.98	0.33-2.94	0.974	0.69	0.35-1.36	0.280
2 - 3 partners	0.95	0.31-2.93	0.930	1.20	0.60-2.38	0.606
More than 3 partners	1.21	0.18-7.93	0.841	1.71	0.54-5.41	0.360
Sexual partners in 12 months						
At most 1 partner	0.67	0.22-2.02	0.474	0.45	0.21-0.96	0.038
2 - 3 partners	1.20	0.37-3.89	0.757	1.28	0.64-2.55	0.483
More than 3 partners	1.37	0.38-4.94	0.629	1.76	0.80-3.89	0.161
Sexual partners in lifetime						
At most 3 partners	0.38	0.03-4.42	0.436	0.61	0.26-1.41	0.246
4 - 8 partners	0.56	0.18-1.70	0.306	0.94	0.43-2.03	0.868
More than 8 partners	2.20	0.72-6.73	0.166	1.47	0.74-2.91	0.273
Wash genitals just before sex						
Yes	0.79	0.05-13.28	0.867	1.49	0.54-4.12	0.440
No	1.27	0.08-21.51	0.867	0.67	0.24-1.85	0.440
Wash genitals immediately after sex						
Yes	11.58	1.36-18.94	0.025	1.08	0.51-2.32	0.838
No	0.09	0.01-0.74	0.025	0.92	0.43-1.98	0.838
Baseline HSV						
No	0.60	0.17-2.11	0.422	0.87	0.44-1.73	0.700
Yes	1.68	0.47-5.93	0.422	1.14	0.58-2.26	0.700
Baseline CD4						
Yes	1.62	0.49-5.36	0.433	0.61	0.18-2.04	0.427
No	0.62	0.19-2.05	0.433	1.63	0.49-5.42	0.427
Baseline HPV						
No	0.42	0.09-1.96	0.267	0.21	0.10-0.47	0.000
Yes	2.41	0.51-11.37	0.267	4.68	2.12-10.36	0.000
Condom use with new partner						
Most of the time (>75%)	0.85	0.25-26.18	0.434	0.40	0.16-0.98	0.036
Some of the time (25 – 75%)	0.77	0.14-4.22	0.763	0.89	0.27-2.92	0.848
Rarely (<25%)	2.54	0.25-26.18	0.434	2.77	1.07-7.17	0.036
Ever had STI						
Yes	0.60	0.20-1.82	0.367	1.67	0.70-3.98	0.249
No	1.67	0.55-5.06	0.367	0.60	0.25-1.43	0.249

The genital washing practice was significantly associated with a 2-fold increased risk for acquiring new HPV infection (RR=1.96; 95% CI: 1.34 – 2.86) among the 52 HIV infected men of which only seven (13%) were circumcised. Condom use was not associated with incident HPV infection among fishermen positive for HIV at baseline.

However, among those negative for HIV at baseline, men who reported consistent condom use with a frequency of >75% were significantly less likely to be detected with incident HPV infection (OR=0.4; 95% CI: 0.16 – 0.98).

4.2.1.3 Persistent HPV infections among Fishermen in Kisumu, Kenya

Of the 300 fishermen enrolled into the study, 187 (62.3%) met the clear definition for evaluation of HPV persistence and clearance. Over 86% (161/187) had persistent non-type specific HPV infection during the one year prospective follow up. LR-HPV genotypes persisted among 118 (73.3%) of the 161 men with persistent HPV, whereas HR-HPV genotypes persisted among 117 (72.7%) of these men. Over 45% (74/161) of the men were detected with both HR-HPV and LR-HPV persistent genotypes.

Factors associated with persistent infection of any HPV genotype among fishermen

In bivariate logistic regression, a unit decrease in WBC count significantly increased the odds of HPV persistence of any HPV genotype ($p<0.05$). Number of sexual partners was not associated with persistence of any HPV genotype (Table 4.8). However, being married was associated with a 2–fold increased odds of HPV persistence compared to single (never married) fishermen (OR=2.71; 95% CI: 1.14 – 6.42). Conversely, fishermen who reported the most recent sexual act with a woman other than their wife were significantly protected from persistent HPV infection if the sexual partners was married compared to when the partner was not married (OR=0.44; 95% CI: 0.22 – 0.86). Most recent sexual act with a casual partner was associated with a 2-fold increased risk for HPV persistence compared to regular sexual partner (OR=2.06; 95% CI: 1.02 – 4.14). Men who used condom at least 50% of the time with a new sexual partner were protected from persistent HPV infections compared to men who never or used condom <50% of the time with a new sexual partner; OR=0.50 (95% CI: 0.26 – 0.96). On the other hand, men with HIV infection at baseline had a 3-fold increased risk of HPV persistence compared to HIV negative men; OR=3.52 (95% CI: 1.60 – 7.72). Additional factors which were not significantly associated with HPV persistence are as shown in Table 4.8.

Table 4.8: Bivariate logistic regression of factors associated with HPV persistence among Fishermen

	Bivar logistic regression: any (all) HPV type			Bivar logistic regression: high-risk HPV			Bivar logistic regression: low-risk HPV		
Characteristic	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Age	0.98	0.93-1.02	0.298	0.98	0.93-1.03	0.453	0.99	0.94-1.04	0.648
Age first sex	1.06	0.97-1.16	0.176	1.02	0.93-1.12	0.630	1.13	1.01-1.27	0.029
No. of sexual partners past 1 yr.	1.05	0.93-1.18	0.459	1.06	0.91-1.23	0.447	1.02	0.96-1.08	0.516
No. of sexual partners lifetime	1.01	0.99-1.04	0.224	1.02	0.99-1.04	0.177	1.00	0.98-1.02	0.974
WBC	1.27	1.01-1.59	0.043	1.29	1.00-1.68	0.051	1.10	0.87-1.41	0.424
Married	2.71	1.14-6.42	0.024	3.44	1.25-9.41	0.016	3.26	1.24-8.53	0.016
Circumcision	1.22	0.57-2.64	0.605	0.98	0.42-2.27	0.962	1.27	0.51-3.19	0.609
Condom recent sex	NA	0.00	1.000	0.98	0.19-5.07	0.984	9.72	1.19-79.49	0.034
Partner married	0.44	0.22-0.86	0.016	0.46	0.21-0.99	0.048	0.83	0.36-1.93	0.664
Condom use with Wife >50 of the time	0.85	0.32-2.28	0.751	1.31	0.46-3.76	0.617	1.72	0.47-6.22	0.411
Condom use with new partner >50 of the time	0.50	0.26-0.96	0.036	0.79	0.38-1.64	0.534	0.46	0.20-1.04	0.062
History of STI	1.50	0.77-2.93	0.238	1.13	0.54-2.36	0.753	1.56	0.71-3.41	0.267
Wash Genital	0.64	0.26-1.57	0.325	1.15	0.43-3.06	0.775	0.31	0.09-1.02	0.054
Wash genitals After Sex	0.43	0.17-1.07	0.069	0.65	0.23-1.83	0.416	0.54	0.17-1.78	0.313
HIV	3.52	1.60-7.72	0.002	2.47	1.12-5.43	0.025	4.03	1.67-9.71	0.002
HSV-2	1.65	0.87-3.11	0.122	1.26	0.612.59-	0.536	1.40	0.66-3.00	0.384
CD4 >350	0.89	0.20-4.07	0.884	0.27	0.05-1.50	0.136	1.13	0.22-5.67	0.886
Sex worker/Casual partner	2.33	0.93-5.83	0.070	2.41	0.87-6.67	0.089	1.57	0.49-5.02	0.446
Casual partner	2.06	1.02-4.14	0.043	1.24	0.55-2.75	0.605	1.03	0.45-2.39	0.939
Baseline HPV	2.27	1.39-3.70	<0.001	1.85	1.13-3.05	0.009	2.01	1.22-3.32	0.003
Baseline multiple-type HPV	2.25	1.30-3.92	0.002	1.73	1.02-2.95	0.032	1.70	0.990-2.89	0.039

In multivariate logistic regression (Table 4.9), marital status (aOR=3.00; 95% CI: 1.15 – 7.83), HIV status (aOR=4.30; 95% CI: 1.83 – 10.11) and recent sexual intercourse with casual partner (aOR=2.33; 95% CI: 1.08 – 5.04) were positively associated with persistence of any HPV genotype. However, marital status (married) of the most recent sexual partner (aOR=0.45; 95% CI: 0.21 – 0.97) was negatively associated with persistence of any HPV genotype. Baseline HPV infection including infection with multiple HPV genotypes independently increased the odds of HPV persistence by 3-fold (Table 4.9).

Table 4.9: Multivariate logistic regression of factors associated with persistent HPV infection among Fishermen

Characteristic	Any (all) HPV type			High-risk HPV			Low-risk HPV		
	aOR	95% CI	P-value	aOR	95% CI	P-value	aOR	95% CI	P- value
WBC	1.23	0.94-1.60	0.131	1.19	0.90-1.57	0.219		Excluded	
Married	3.00	1.15-7.83	0.025	3.42	1.19-9.87	0.023	4.54	1.55-13.27	0.006
Partner married	0.45	0.21-0.97	0.043	0.44	0.19-1.03	0.059		Excluded	
Age at sexual debut		Excluded			Excluded		1.13	0.94-1.36	0.192
Age of partner		Excluded			Excluded		0.87	0.69-1.11	0.267
Condom use recent sex		Excluded			Excluded		12.75	3.43-23.8	0.023
Condom use with new partner >50 of the time	0.78	0.35-1.74	0.551		Excluded		0.80	0.29-2.20	0.669
Wash Genitals		Excluded		0.23	0.05-1.15	0.074	0.32	0.08-1.32	0.117
Always wash after sex	0.45	0.17-1.20	0.111		Excluded			Excluded	
HIV	4.30	1.83-10.11	0.001	3.29	1.40-7.74	0.006	6.41	2.45-16.76	<0.001
Sex worker/Casual partner	2.33	1.08-5.04	0.031	2.10	0.70-6.31	0.187		Excluded	
Baseline HPV	3.19	1.21-5.63	0.001	2.86	1.22-3.32	0.003	1.41	1.11-4.36	0.020
Baseline multiple type HPV	4.98	1.29-8.46	0.001	5.13	1.52-9.28	0.012	1.59	0.69-3.67	0.087

Factors associated with persistent HR-HPV infections among Fishermen

In bivariate logistic regression, men who reported being married at baseline were more likely to experience persistent HR- HPV infection compared to single (never married) fishermen (OR=3.44; 95% CI: 1.25 – 9.41). However, among fishermen who reported the most recent sexual act with a woman other than their wife were significantly protected from persistent HR-HPV infection if the female sexual partner was reported to be married compared to when the partner was not married (OR=0.46; 95% CI: 0.21 – 0.99). Conversely, men detected with HIV infection at baseline were 2 times more likely to have persistence HR-HPV infection compared to men negative for HIV at baseline; OR=2.47 (95% CI: 1.12 – 5.43). Table 4.9 shows additional factors that were not significantly associated with persistent HR-HPV infection. In multivariate logistic regression, marital status (aOR=3.42; 95% CI: 1.19 – 9.87), baseline HIV status (aOR=3.29; 95% CI: 1.40 – 7.74) and Baseline HPV infection (aOR=2.86; 95% CI: 1.22 – 3.32) remained positively associated with persistence HR-HPV infections. Infection with multiple HPV types at baseline was independently associated with persistence of HR-HPV (aOR=5.13; 95% CI: 1.52 – 9.28).

Factors associated with persistent LR-HPV infections among Fishermen

In bivariate logistic regression, a unit increase in age of sexual debut significantly increased the odds of LR-HPV persistence ($p<0.05$). Additionally, married men at baseline were more likely to experience persistent LR- HPV infections compared to single (never married) fishermen (OR=3.26; 95% CI: 1.24 – 8.53). Fishermen who reported baseline condom use during the most recent sexual act were 9 times more likely to experience persistent LR-HPV infections compared to those who reported no condom use (OR=9.72; 95% CI: 1.19 – 79.49). Like for any HPV and HR-HPV persistence, men who tested positive for HIV at baseline were more likely to have persistence LR-HPV infection compared to men that tested negative for HIV infection at baseline; (OR=4.03; 95% CI: 1.67 – 9.71). Other risk factors for persistent LR-HPV infection explored in this analysis are shown in Table 4.9.

In multivariate logistic regression, marital status (aOR=4.54; 95% CI: 1.55 – 13.27), baseline HIV status (aOR=6.41; 95% CI: 2.45 – 16.76) and baseline HPV

infection (aOR=1.41; 95% CI: 1.11 – 4.36) were positively associated with persistent LR-HPV infections. The association between condom use during the most recent sexual act and persistence of LR-HPV infection was even stronger in multivariate analysis (aOR=12.75; 95% CI: 3.43 – 23.98), (Table 4.9). Other risk factors were found to be insignificantly associated with persistent LR-HP infection (Table 4.9).

4.2.2 HIV Infections among Fishermen

4.2.2.1 Baseline HIV Infection Among Fishermen in Kisumu, Kenya

Of the 300 fishermen studied, 70 were confirmed to be HIV positive at baseline estimating a 23.3% (95% CI: 18.5 – 28.1) HIV prevalence, of whom 59 (84.3%) were uncircumcised at baseline. HIV prevalence among fishermen in this study, increased with age (Figure 4.10). HIV negative men were significantly younger ($p<0.001$), reported no history of STI ($p<0.001$) and reported higher condom use with a new partner ($p<0.001$) compared to HIV positive men (Table 4.10).

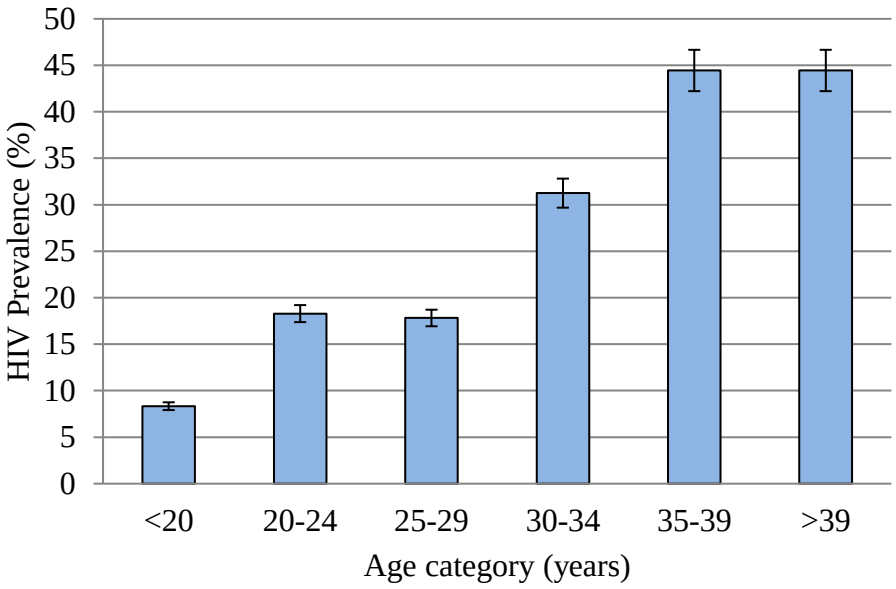


Figure 4.10: Trend of baseline HIV prevalence among different age categories

Table 4.10: Characteristics of Fishermen by HIV status at enrollment into the study

Characteristic	HIV Positive (N=70)		HIV Negative (N=230)		Total (N=300)	
	n (%)	P – Value	n (%)	P – Value	n (%)	P – Value/ (95% CI)
Age		0.396		<0.001		<0.001
18-28 years	33 (47.1)		161 (70)		194 (64.7)	(59.2–70.1)
29-47 years	37 (52.9)		69 (30)		106 (35.3)	(29.9–40.8)
Marital status		<0.001		<0.001		<0.001
single	9 (12.9)		55 (23.9)		64 (21.3)	(16.7–26.0)
Ever married	61 (87.1)		175 (76.1)		236 (78.7)	(74.0–83.3)
Circumcised		<0.001		<0.001		<0.001
Yes	11 (15.7)		61 (26.5)		72 (24.0)	(19.1–28.9)
No	59 (84.3)		169 (73.5)		228 (76.0)	(71.1–80.7)
Sexual partners in 6 months		0.090		0.777		0.777
≤ 1 partner	39 (55.7)		113 (49.1)		152 (50.7)	(45.0–56.4)
> 1 partners	31 (44.3)		117 (50.9)		148 (49.3)	(43.6–55.0)
Sexual partners in 12 months		0.090		<0.001		<0.001
< 2 partner	31 (44.3)		81 (35.2)		112 (37.3)	(31.8–42.8)
≥ 2 partners	39 (55.7)		149 (64.8)		188 (62.7)	(57.2–68.2)
Sexual partners in lifetime		<0.001		<0.001		<0.001
≤ 5 partners	10 (14.3)		48 (20.9)		108 (36.0)	(30.5–41.5)
> 5 partners	60 (85.7)		182 (79.1)		192 (64.0)	(58.5–69.5)
Transactional sex		<0.001		<0.001		<0.001
No	1 (1.4)		33 (14.3)		34 (11.3)	(7.7–14.9)
Yes	69 (98.6)		197 (85.7)		266 (88.7)	85.1–92.3)
Most recent sexual partner						
Wife	4 (5.7)	Reference	37 (16.1)	Reference	41 (13.7)	(9.8–17.6)
Girl friend	39 (55.7)	<0.001	128 (55.7)	<0.001	167 (55.7)	(50.0–61.3)
Casual partner/Sex worker	27 (38.6)	<0.001	65 (28.3)	<0.001	92 (30.7)	(25.4–35.9)
Condom use on last sexual act		<0.001		<0.001		<0.001
No	69 (98.6)		189 (82.2)		258 (86.0)	(82.1–90.0)
Yes	1 (1.4)		41 (17.8)		42 (14.0)	(10.1–18.0)
Condom use with new partner						
Rarely (<25%)	44 (62.9)	Reference	63 (27.4)	Reference	107 (35.7)	Ref
Some of the time (25 – 75%)	17 (24.3)	0.011	48 (20.9)	0.221	65 (21.7)	0.003
Most of the time (>75%)	9 (12.9)	<0.001	119 (51.7)	<0.001	128 (42.7)	0.265
Condom Use		<0.001		<0.001		<0.001
Ever used condom	48 (68.6)		195 (84.8)		243 (81.0)	(76.5–85.5)
Never used condom	22 (31.4)		35 (15.2)		57 (19.0)	
History of STI		0.777		<0.001		<0.001
No	34 (48.6)		189 (82.2)		223 (74.3)	(69.3–79.2)
Yes	36 (51.4)		41 (17.8)		77 (25.7)	(20.8–30.7)
Baseline HSV-2		<0.001		1.000		0.090
Negative	17 (24.3)		114 (49.6)		131 (43.7)	(38.0–49.3)
Positive	53 (75.7)		116 (50.4)		169 (56.3)	(50.7–62.0)
Baseline HPV		<0.001		0.090		1.000
Negative	22 (31.4)		129 (56.1)		151 (50.3)	(44.6–56.0)
Positive	48 (68.6)		101 (43.9)		149 (49.7)	(44.0–55.4)

Conversely, proportions of baseline HSV-2 (76% vs 50%) and HPV (69% vs 44%) infection were significantly higher among HIV positive men compared to HIV negative men ($p<0.001$). In bivariate logistic regression (Table 4.11**)**, baseline history of STI, HPV and HSV-2 infection were significantly associated with baseline HIV infection ($p<0.001$). A unit increase in age was significantly associated with a one-fold increase in odds of being HIV infected at baseline; OR=1.08 (95% CI: 1.04–1.13). Older men (>28 year old) were 2.6 times more likely to test positive for HIV at baseline compared to younger men (≤28 years old); OR=2.62 (95% CI: 1.46–4.69) (Table 4.11**)**. Men who reported ever being married were 2-times more likely to be HIV positive at baseline in bivariate analysis (OR=2.13; 95% CI: 1.02–4.93).

Men in this study reported very low condom use: (7%) at sexual debut, 10% with new sexual partner (at a frequency >75% of the time), 14% at most recent sexual act and 43% with new sexual partner (at frequency >75% of the time). However, those who reported ever using a condom (OR=0.39; 95% CI: 0.20–0.76), condom use with a frequency greater than 75% of the time with a new sexual partner (OR=0.18; 95% CI: 0.06–0.51) or used condom on the most recent sexual act (OR=0.07; 95% CI: 0.01–0.50), were less likely to test HIV positive at baseline. Circumcision status and/ or the number of sexual partners at enrolment were not associated with baseline HIV status (Table 4.11).

Married men who reported having their most recent sexual act with a girlfriend OR=3.52 (95% CI: 1.08–12.69) or sex worker /casual partner OR=4.44 (95% CI: 1.30–16.59) were more likely to be HIV positive at baseline compared to those who reported having the most recent sexual act with their wife. In stratified analysis, men infected with both HPV and HSV-2 at baseline were 6 times at increased risk of being detected with HIV infection at baseline compared to men negative for both HPV and HSV-2 infections at baseline; OR=6.19 (2.55–15.03). Only 6.7% (5/75) and 38.7% (36/93) were detected with HIV at baseline among those negative for both HPV/HSV-2 and those positive for both HPV/HSV-2 infections respectively.

Table 4.11: Bivariate logistic regression of factors associated with baseline HIV infection among Fishermen

Characteristic	OR	95% CI	p-value
Age (continuous)	1.08	1.04 – 1.13	<0.001
Age: 18-28 years		Reference	
29-47 years	2.62	1.46 – 4.69	<0.001
Marital status: Single		Reference	
Ever married	2.13	1.02 – 4.93	0.048
Circumcision: Yes		Reference	
No	1.94	0.95 – 3.93	0.067
Sexual partners in 6 months: < 2 partner		Reference	
≥ 2 partners	1.19	0.68 – 2.08	0.523
Sexual partners in 12 months: ≤ 2 partner		Reference	
> 2 partners	1.04	0.58 – 1.85	0.897
Sexual partners in lifetime: ≤ 5 partners		Reference	
> 5 partners	4.45	2.17 – 9.14	<0.001
Transactional sex: No		Reference	
Yes	11.56	1.65 – 23.12	0.017
Baseline HSV-2: Negative		Reference	
Positive	3.06	1.67 – 5.61	<0.001
Baseline HPV: Negative		Reference	
Positive	2.79	1.58 – 4.92	<0.001
Relation with recent partner			
All men: Girlfriend	1.00	0.59 – 1.72	0.993
Casual partner / Sex worker	2.23	0.36 – 13.59	0.386
Wife	0.32	0.11 – 0.92	0.035
Single men: Girlfriend		Reference	
Casual partner / Sex worker	0.80	0.15 – 4.50	0.761
Married men: Wife		Reference	
Girlfriend	3.52	1.08 – 12.69	0.020
Casual partner / Sex worker	4.44	1.30 – 16.59	0.007
Ever use of Condom: Yes	0.39	0.20 – 0.76	0.002
No	2.55	1.31 – 4.96	0.002
Condom use on recent sexual act:			
No	14.97	2.15 – 29.82	0.005
Yes	0.07	0.01 – 0.50	0.005
Condom use with new partner:			
Most of the time (>75%)	0.18	0.06 – 0.51	0.001
Some of the time (>25%)	1.71	0.66 – 4.43	0.266
Rarely (<25%)	4.14	2.35 – 7.29	0.001
Ever had STI: No		Reference	
Yes	4.86	2.72 – 8.65	<0.001

In multivariate logistic regression analysis, a unit increase in age was significantly associated with a one-fold increase in odds of being HIV infected at baseline (aOR=1.05; 95% CI: 1.02–1.11), as shown in Table 4.12. Older fishermen (>28 year old) were twice more likely to test positive for HIV at baseline compared to younger men (≤28 years old); aOR=2.13 (95% CI: 1.25–5.07). Although fishermen who reported ever being married were 2-times more likely to be HIV positive at baseline in bivariate analysis, marital status was not significantly associated with baseline HIV infection in multivariate analysis (p=0.409).

Table 4.12: Multivariate logistic regression of factors associated with baseline HIV infection

Characteristic	aOR	95% CI	p-value
Age: 18-28 years		Reference	
29-47 years	2.13	1.25 – 5.07	0.001
Marital status: Single		Reference	
Ever married	1.57	0.15 – 2.17	0.409
Circumcision: Yes		Reference	
No	1.82	0.55 – 5.98	0.325
Sexual partners in lifetime: ≤ 5 partners		Reference	
> 5 partners	5.76	2.41 – 11.57	<0.001
Transactional sex: No		Reference	
Yes	10.98	1.86 – 19.34	0.021
Baseline HSV: Negative		Reference	
Positive	1.54	0.67 – 3.57	0.312
Baseline HPV: Negative		Reference	
Positive	2.13	1.05 – 4.77	0.047
Ever use of Condom: Ever used condom		Reference	
Never used condom	2.67	1.19 –5.99	0.017
Condom use on recent sexual act: No		Reference	
Yes	0.09	0.03 – 0.61	0.009
Condom use with new partner:			
Rarely (<25%)		Reference	
Most of the time (>75%)	0.21	0.08 – 0.55	0.002
Ever had STI: No		Reference	
Yes	4.21	2.07 – 9.34	<0.001

Men with a higher number (>5) of lifetime sexual partners were 5-times more likely to have HIV infection at baseline (aOR=5.76; 95% CI: 2.41 – 11.57). Most recent sexual act with a sex worker increased the odds of being detected with HIV at baseline by 2-fold in bivariate analysis (Table 4.11), however, this was not statistically significant. Nonetheless, men who reported engaging in transactional sex were significantly more likely to be HIV positive at baseline (aOR=10.98; 95% CI: 1.86 – 19.34) as shown in Table 4.12. History of an STI was associated with 4–fold increased odds of HIV infection at baseline (aOR=4.21; 95% CI: 2.07 – 9.34). Men who used condom consistently over 75% of the time were less likely to test HIV positive at baseline, compared to those who rarely used (<25% of the time) condoms with regular (aOR=2.6; 95% CI: 1.17–7.77); new (aOR=4.02; 95% CI: 2.14–8.07); or most recent (aOR=14.18; 95% CI: 2.31–28.39) sexual partners (Table 4.12).

4.2.2.2 Incident HIV Infection Among Fishermen in Kisumu, Kenya

Of the 300 fishermen enrolled, 230 were HIV negative at baseline of which 191 (83%) returned for the scheduled study exit follow-up visit after 12 months. This represented a total of 191 person–years (pyr) of follow–up. During the one year follow-up period, eight of the 191 (4.2%) fishermen who were HIV negative at baseline got infected with HIV, indicating an HIV incidence of 4.2 (95% CI: 1.3–7.1) per 100 person-years. The men who seroconverted were significantly younger ($p<0.001$) with mean (SD) age 22 (2.19) years compared to those men who tested HIV negative at study exit that had a mean (SD) age 27.0 (6.05) years (Figure 4.11). Seven (87.5%) of these men were below 25 years old with a range of 18 – 24 years. All men with incident HIV infection were not circumcised, belonged to the Luo ethnic group and five (62.5%) were single (never married). In the exit questionnaire, they all reported having had more than two sexual partners during the one year of study follow-up period, >5 sexual partners in a lifetime and engaging in transactional sex (exchange of fish, money or other valuables for sex). Seventy five percent (6/8) of men with incident HIV infection were HSV-2 positive at exit. However, only three (37.5%) of these men had HSV-2 at baseline. Of the men with new HIV infection 63% (5/8) had HPV infection at baseline. Moreover, 88% (7/8) of fishermen with new HIV infection were detected with at least one HPV genotype (of the 33 detectable genotypes indicated in

Table 3.1) in the course of study follow-up and 71% (5/7) of these men had multiple HPV genotypes. These suggested that the risk factors for new acquisition of HIV, HPV and HSV-2 could be similar.

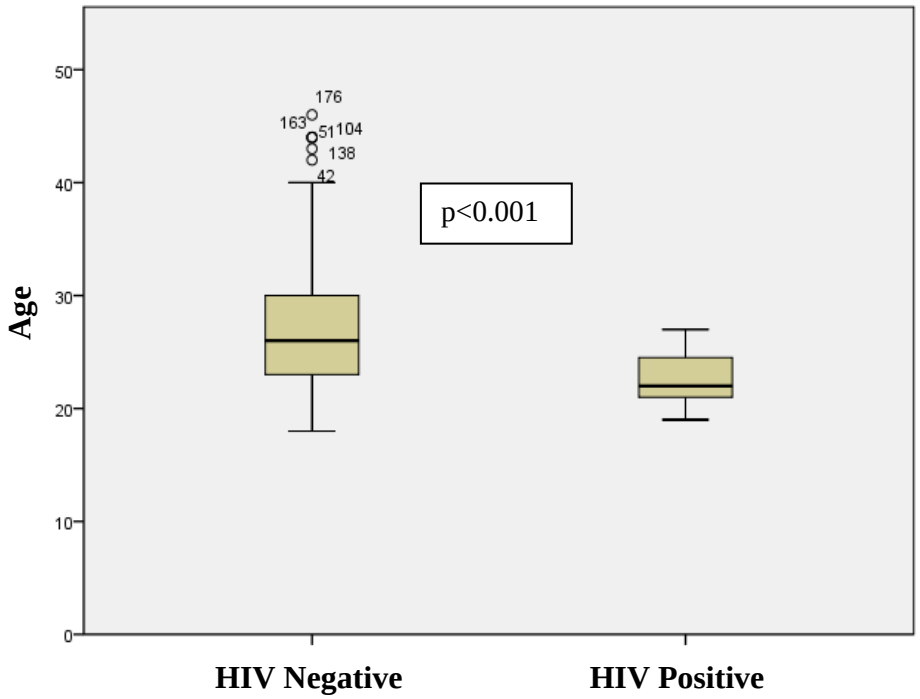


Figure 4.11: Age distributions of men with or without incident HIV infection

Fishermen enrolled in this study had a baseline CD4>700 (mean=852, SD=162.5) ranging from 729 to 1091 cells/ml of blood before getting HIV infection, but 63% (5/8) had their CD4 significantly ($p<0.001$) dropped to a mean (SD) CD4 of 516 (277.5) ranging from 205 to 845 cells/ml of blood, by the time of study exit. Five of eight (62.5%) already had their CD4 dropped to <500 cells/µl of blood ranging from 205 to 477 cells/ml of blood. Five (62.5%) had high-risk HPV infection (with multiple types including low-risk HPV genotypes) at baseline that persisted for the entire study period. Additionally all the eight fishermen acquired a new HPV type during follow up. Condom use was rare among these men, seven out of eight reported using condom less than 25% of the time with a new sexual partner. The most recent sexual partner

for all these men was a sex worker/casual partner and majority 87.5% (7/8) did not use condom during this most recent sexual act.

In bivariate analysis (Table 4.13), younger men (≤25 years of age) were at increased risk for acquiring new HIV infection compared to older men (>25 years of age), incidence risk ration (IRR) of 8.19 (95% CI: 1.03–65.32). Similarly, the risk of incident HIV infections among single men (never married) was significantly higher compared to men who reported ever being married at baseline (IRR=5.41; 95% CI: 1.34–21.75). However, condom use ≥50% of the time with new sexual partner compared to <50% of the time (IRR=8.19; 95% CI: 1.03–65.32) and most recent sexual act with wife/ regular partner (IRR=8.19; 95% CI: 1.03 – 65.32) compared to casual partner/ sex worker, significantly reduced the risk of new HIV acquisition. In stratified analysis, men infected with both HPV and HSV-2 at baseline were 6 times at increased risk of being detected with HIV infection at baseline compared to men negative for both HPV and HSV-2 infections at baseline (IRR=6.19; 95% CI: 2.55–15.03). Only 6.7% (5/75) and 38.7% (36/93) were detected with HIV at baseline among those negative for both HPV/HSV-2 and those positive for both HPV/HSV-2 infections respectively.

Multivariate logistic regression strengthened the association between marital status and HIV incidence (Table 4.13), where single men were independently at a greater risk for HIV acquisition compared to married men, (aIRR=8.32; 95% CI: 1.27 – 54.67). Young age had a borderline association (p=0.066) with HIV acquisition (aIRR=5.57; 95% CI: 0.89 – 34.64). Men who had most recent sexual act with their wife/regular partner (aIRR=0.03; 95% CI: 0.01 – 0.35) and those who used condom (≥50% of the time) with new sexual partners (aIRR=0.11; 95% CI: 0.01 – 0.89) were significantly protected from acquiring new HIV infection, compared to men whose most recent sexual act was with a sex worker/ casual partner and those who inconsistently used condom (<50% of the time) with new sexual partners.

Table 4.13: Factors associated with incident HIV infection among Fishermen

Factors	n	Incidence (Rate %)	IRR (95% CI)	P - Value	aIRR (95% CI)	P- Value
Age						
18-25 years	88	7 (8.0)	8.19 (1.03–65.32)	0.025	5.57 (0.89 – 34.64)	0.066
26-47 years	103	1 (1.0)	Reference			
Marital status						
Single	45	5 (11.1)	5.41 (1.34–21.75)	0.019	8.32 (1.27 – 54.67)	0.027
Ever married	146	3 (2.1)	Reference			
Circumcised						
Yes	51	1 (2.0)	0.39 (0.05 – 3.11)	0.092	0.99 (0.09 – 10.68)	0.991
No	140	7 (5.0)	Reference			
Sexual partners in 6 months						
≤ 1 partner	94	4 (4.3)	0.95 (0.24–3.68)	1.00	Not included	---
> 1 partners	93	4 (4.3)	Reference			
Sexual partners in 12 months						
≤ 1 partner	70	1 (1.4)	0.25 (0.03–1.97)	0.102	0.87 (0.07 – 10.92)	0.913
> 1partners	121	7 (5.8)	Reference			
Sexual partners in lifetime						
≤ 5 partners	78	1 (1.3)	0.21 (0.03–1.65)	0.101	0.65 (0.05 – 8.93)	0.747
> 5 partners	113	7 (6.2)	Reference			
Transactional sex						
No	25	0 (0.0)	0.00 (0.00 – 4.58)	0.600	Not included	---
Yes	166	8 (4.8)	Reference			
Most recent sexual partner						
Wife / regular girlfriend	139	1 (0.7)	0.05 (0.00 – 0.39)	0.001	0.03 (0.01 -0.35)	0.004
Casual partner/Sex worker	53	7 (13.2)	Reference			
Condom use on last sexual act						
No	162	7 (4.3)	1.25 (0.16 – 9.81)	1.000	Not included	---
Yes	29	1 (3.4)	Reference			
Condom use with regular partner						
≥ 50% of the time	14	0 (0.0)	0.00 (0.00 – 9.17)	1.000	Not included	---
< 50% of the time	177	8 (4.5)	Reference			
Condom use with new partner						
≥ 50% of the time	108	1 (0.9)	0.10 (0.00 – 0.85)	0.022	0.08 (0.01 – 0.89)	0.039
< 50% of the time	83	7 (8.4)	Reference			
Condom use						
Ever used condom	164	8 (4.9)	Reference			
Never used condom	27	0 (0.0)	0.00 (0.00 – 4.17)	0.603	Not included	---
History of STI						
No	157	5 (3.2)	Reference			
Yes	33	3 (9.1)	3.04 (0.54 –15.74)	0.104	2.10 (0.28 – 15.84)	0.470
Baseline HSV-2						
Negative	94	5 (5.3)	0.57 (0.10 – 2.83)	0.493	Not included	---
Positive	97	3 (3.1)	Reference			
Baseline HPV						
Negative	106	3 (2.8)	Reference			
Positive	85	5 (5.9)	2.15 (0.43 –11.73)	0.470	Not included	---

Men who reported most recent sexual partnership with a casual partner/sex worker had a five-fold increased risk of HIV infection during follow up (aIRR=5.29; 95% CI: 2.05–15.12). Reported condom use with a new sexual partner (over 75% of the time) was most protective against incident HIV infection; (aIRR= 0.21; 95% CI: 0.09–0.62) compared to rare use of condom (<25% of the time) with a new sexual partners that conferred a five-fold increased risk for HIV acquisition during follow up (aIRR=5.63; 95% CI: (1.15–22.47). Interestingly, incident HIV infection was not associated with baseline HSV-2 infection (p=0.443), circumcision (p=0.991) or number of lifetime sexual partners (p=0.747).

4.2.3 HSV-2 Infections among Fishermen

4.2.3.1 Prevalence of HSV-2 Infection Among Fishermen in Kisumu, Kenya

Of the 300 men tested for HSV-2 at baseline, 169 were positive resulting in a baseline HSV-2 prevalence of 56.3% (95% CI: 50.7 – 62.0) . Of the 131 men who were negative for HSV-2 at baseline, 106 (81%) returned for their exit visit and were significantly younger mean (SD) age 25.8 (5.84) years compared to men infected with HSV-2 at baseline (mean age 29.3, SD ±6.37), (Table 4.14). Persons with prevalent HSV-2 infection were more likely to be older (>28 years old) (70% vs. 49%), married (62% vs. 34%), HIV-1 positive (76% vs. 50%), ever had an STI (71% vs. 51%) and co-infected with HPV (62% vs. 50%), (Table 4.14**)**. Among men with baseline HSV-2 infection, 93 (55%) were positive for HPV DNA, of which 70 (75%) were infected with at least one HR HPV genotype. Similarly, 75% (42/56) of HPV positive men had at least one HR HPV genotype among HSV-2 negative men.

Table 4.14: Characteristics of Fishermen with or without HSV-2 infection at baseline

Characteristic	All (N=300)	HSV-2 Positive (n=169)	HSV-2 Negative (n=131)
Age: Mean (SD)	27.7 (6.40)	29.3(6.37)	25.8(5.84)
18-28 years	194	95(49.0)	99(75.6)
29-47 years	106	74(68.9)	32(24.4)
Marital status:			
Single	64	22(34.4)	42(32.1)
Ever married	236	147(62.3)	89(67.9)
Circumcised:			
Yes	72	36(50.0)	36(27.5)
No	228	133(58.0)	95(72.5)
Sexual partners in last 12 months:			
< 2 partner	112	65(58.0)	47(35.9)
≥ 2 partners	188	104(55.3)	84(64.1)
Sexual partners lifetime:			
≤ 5 partners	108	59(54.6)	49(37.4)
> 5 partners	192	110(57.3)	82(62.6)
Engaged in transactional sex:			
Yes	34	14(41.1)	20(15.3)
No	266	155(58.3)	111(84.7)
Baseline HIV status:			
Negative	230	116(50.4)	114(87.0)
Positive	70	53(75.7)	17(13.0)
Baseline HPV status:			
Negative	151	76(50.1)	75(57.3)
Positive	149	93(62.4)	56(42.7)
Type of partner for recent sexual act:			
Single men:	(N=69)	(n=24)	(N=45)
Girlfriend (regular partner)	51	19(37.3)	32(71.1)
Casual partner/sex worker	18	5(27.8)	13(28.9)
Married men:	(N=231)	(n=145)	(n=86)
Wife	41	17(41.5)	24(27.9)
Girlfriend	116	75(64.7)	41(47.7)
Casual partner/sex worker	74	53(71.6)	21(24.4)
Used condom in recent sexual act:			
Yes	42	18(42.9)	24(18.3)
No	258	151(58.5)	107(81.7)
Condom use with new partner:			
Most of the time (>75%)	107	65(60.7)	42(32.1)
Some of the time (25 – 75%)	65	49(75.4)	16(12.2)
Rarely (<25%)	128	55(43.0)	73(55.7)
Ever had STI:			
No	223	114(51.1)	109(83.2)
Yes	77	55(71.4)	22(16.8)

HSV-2 prevalence increased across different age categories from the youngest category to the oldest (Figure 4.12), with a significant sharp increase observed at ages ≤29 years and gradually levels off with advancing age beyond 29 years (p<0.001). In bivariate analysis, every unit increase in age among the studied fishermen resulted in a one-fold increase in odds of having HSV-2 at baseline (OR=1.11; 95% CI, 1.06–1.16). Older age of >28 years (OR=2.41; 95% CI, 1.42–4.11), ever married (OR=2.13; 95% CI, 1.02–4.93), HIV infection (OR=3.06; 95% CI, 1.67–5.61), HPV infection (OR=1.64; 95% CI, 1.03–2.60), history of STI (OR=2.41; 95% CI, 1.38–4.22) or inconsistent condom use <25% of the time (OR=5.31; 95% CI, 2.29–12.33) were significantly associated with HSV-2 prevalence.

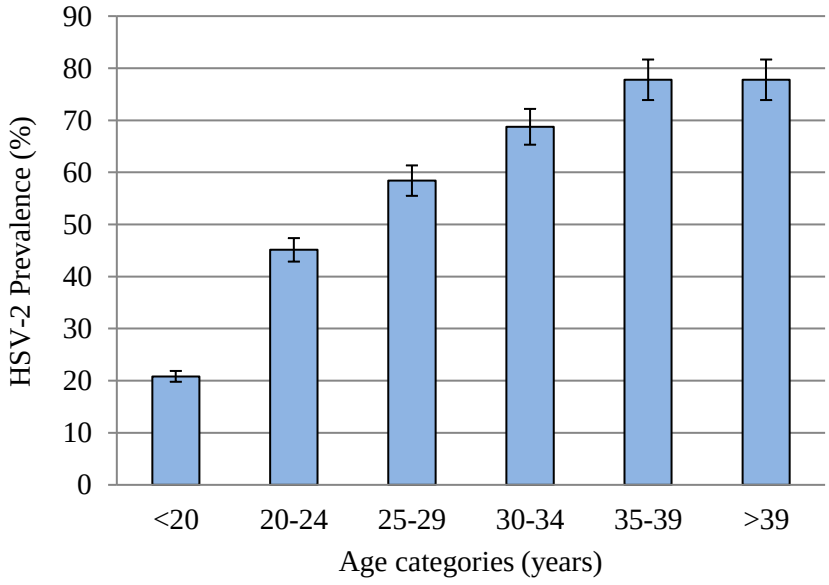


Figure 4.12: Trend of baseline HSV-2 prevalence among different age categories

Men who reported being married at baseline were 3-times more likely to have HSV-2 at baseline compared to single (never married) men (OR=3.16; 95% CI: 1.80 – 5.55). Additionally, among married fishermen, most recent sexual act with partner other than the wife was associated with increased odds of HSV-2 infection; girlfriend (OR=2.58; 95% CI, 1.17–5.71) or casual partner/sex worker (OR=3.56; 95% CI, 1.49–8.62). However, number of sexual partners from the last one month to lifetime was not associated with baseline HSV-2 infection (Table 4.15).

On the multivariate logistic regression model, inconsistent condom use <25% of the time (aOR=6.34; 95% CI, 2.24–13.04), HIV positive status (aOR=2.22; 95% CI, 1.17–4.22), and a history of STI (aOR=2.12; 95% CI, 1.19–3.91) were independently associated with increased odds of baseline HSV-2 prevalence (Table 4.15). Conversely, young age (≤28 years) was independently associated with reduced likelihood of baseline HSV-2 infection (aOR=0.49; 95% CI: 0.27 – 0.86) compared to older age (>28 years), that had a 2-fold increased odds of being detected with HSV-2 infection at baseline. Men who reported ever having an STI were more likely to test positive for HSV-2 at baseline compared to those who reported no history of STI (aOR=2.05; 95% CI: 1.22 – 5.01).

Any level of condom use with recent or regular sexual partner was not associated with HSV-2 infection at baseline (Table 4.15). However, consistent condom use (over 75% of the time) compared to (less than 25% of the time) with new sexual partner was significantly associated with reduced likelihood of testing positive for HSV-2 at baseline (aOR=0.25; 95% CI: 0.09 – 0.51). Baseline HIV infection was associated with a 2-fold increased odds of being detected with HSV-2 at baseline (aOR=2.22; 95% CI: 1.17 – 4.22). Baseline CD4 and HPV infection were significantly associated with baseline HSV-2 infection in bivariate analysis ($p<0.01$ and $p<0.05$) respectively. However, in multivariate analysis, this association was not statistically significant. In a final multiple logistic regression model, the strongest predictors for baseline HSV-2 infection were age and HIV status ($p<0.01$ and $p<0.05$) respectively.

Table 4.15: Factors associated with baseline HSV-2 infection among Fishermen

Characteristic	OR(95% CI)	p-value	aOR (95% CI)	p-value
Age (continuous)	1.11(1.06 – 1.16)	<0.001	1.09(1.04 – 1.15)	<0.001
Age (stratified):				
18-28 years	Reference		Reference	
29-47 years	2.41(1.42 – 4.11)	<0.001	1.96(1.16 – 2.85)	0.003
Marital status :				
Single	Reference		Reference	
Ever married	2.13(1.02 – 4.93)	<0.001	3.80(1.42 – 11.90)	0.001
Circumcised:				
Yes	Reference		Not included	
No	1.40(0.82 – 2.38)	0.215		
Sexual partners in last 12 months:				
< 2 partner	Reference		Not included	
≥ 2 partners	0.90(0.54 – 1.48)	0.646		
Lifetime Sexual partners:				
≤ 5 partners	Reference		Not included	
> 5 partners	1.11(0.67 – 1.84)	0.655		
Engaged in transactional sex:				
Yes	Reference		Reference	
No	0.50(0.24 – 1.04)	0.062	0.56(0.28 – 1.33)	0.189
Baseline HIV status:				
Negative	Reference		Reference	
Positive	3.06(1.67 – 5.61)	<0.001	2.22(1.17 – 4.22)	0.015
Baseline HPV status:				
Negative	Reference		Reference	
Positive	1.64(1.03 – 2.60)	0.035	1.36(0.78 – 2.37)	0.283
Partner in recent sexual act:				
Single men:				
Girlfriend (regular)	Reference		Not included	
Casual partner/sex worker	1.54(0.42 – 5.92)	0.468		
Married men:				
Wife	Reference		Not included	
Girlfriend	2.58(1.17 – 5.71)	0.009		
Casual partner/sex worker	3.56(1.49 – 8.62)	0.001		
Condom use in recent sexual act:				
Yes	Reference		Reference	
No	1.88(0.97 – 3.64)	0.06	1.06(0.48– 2.34)	0.885
Condom use with new partner:				
Most of the time (>75%)	0.45(0.26 – 0.78)	0.005	Reference	
Rarely (<25%)	5.31(2.29 – 12.33)	<0.001	6.34(2.24–13.04)	0.001
Some of the time (25 – 75%)	2.03(0.76 – 5.38)	0.155		
Ever had STI:				
No	Reference		Reference	
Yes	2.41(1.38 – 4.22)	0.002	2.12(1.19 –3.91)	0.014

In stratified analysis, men infected with both HIV and HPV at baseline were 3-times more likely to test positive for HSV-2 infection at baseline compared to men who were negative for both HIV and HPV infections at baseline (OR=3.56; 95% CI: 1.61 – 7.99). Only 45.7% (59/129) and 75.0% (36/48) fishermen were detected with HSV-2 at baseline among those negative for both HIV/HPV and those positive for both HIV/HPV infections respectively.

4.2.3.2 Incidence of HSV-2 Infections Among Fishermen in Kisumu, Kenya

Among the 131 fishermen negative for HSV-2 at baseline, 106 had exit data and were included in the HSV-2 incidence analysis. During the one year follow-up period, 25 of the 106 (23.6%) fishermen who were HSV-2 negative at baseline got infected with HSV-2, resulting in an HSV-2 incidence of 23.6 per 100 person-years. Age distribution was similar ($p<0.555$) between men with incident HSV-2 infection who had a mean age of 25.8 (5.01 SD) years and those without whose mean age was 26.5 (5.97 SD) years as presented in Figure 4.13.

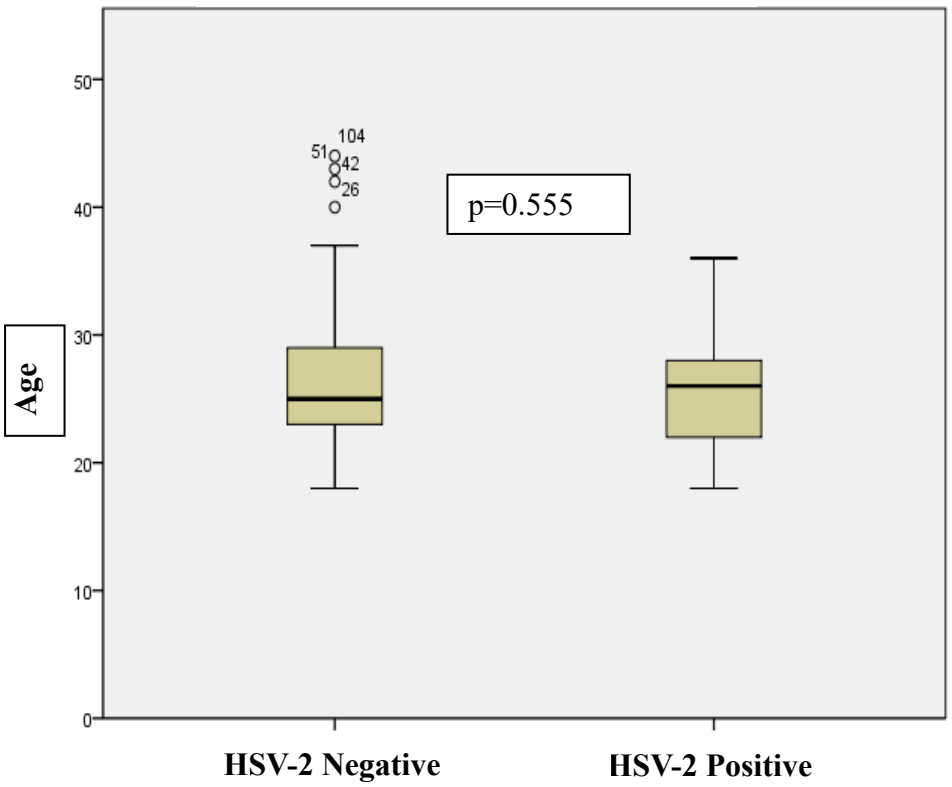


Figure 4.13: The distribution of age by HSV-2 incident status

Incidence rates were higher among men with a history of STI (25.0 vs 23.3/100 pyr), positive for HIV (28.6 vs 22.8/100 pyr), HPV infection (31.9 vs 16.9/100 pyr), those whose most recent sexual act was with a casual partner/sex worker (44.8 vs 15.6/100 pyr)and those with multiple sexual partners in the last six months (31.3 vs. 17.2/100 pyr), 12 months, (33.3 vs. 9.3/100 pyr), and >5 sexual partners lifetime (31.3 vs. 10.3/100 pyr). The incidence rate was much lower among men not engaging in transactional sex (3.8 vs 30.0/100 pyr) and those who reported condom use on the last sexual act (7.1 vs 33.3/100 pyr) or with new sexual partner (14.5 vs 36.4/100 pyr) as shown in Table 4.16.

Table 4.16: Factors associated with incident HSV-2 infection among Fishermen

Characteristic	n	Incidence (%)	IRR (95% CI)	P - Value	aIRR (95% CI)	P- Value
Age:						
26-47 years	50	11 (22.0)	Reference			
18-25 years	56	14 (25.0)	1.14 (0.57–2.27)	0.716	Not included	
Marital status:						
Ever married	77	18 (23.4)	Reference			
Single	29	7 (27.4)	1.03 (0.48–2.21)	0.934	Not included	
Circumcised:						
No	77	19 (24.7)	Reference			
Yes	29	6 (20.7)	0.84 (0.37–1.89)	0.667	Not included	
Sexual partners in 12 months:						
< 2 partner	43	4 (9.3)	0.28 (0.10–0.76)	0.004	Reference	
≥ 2partners	63	21 (33.3)	3.58 (1.32–9.71)	0.004	4.77 (1.12–11.28)	0.009
Sexual partners in lifetime:						
≤ 5 partners	39	4 (10.3)	Reference			
> 5 partners	67	21 (31.3)	3.06 (1.13–8.26)	0.014	3.76 (0.86 – 9.15)	0.089
Transactional sex:						
Yes	80	24 (30.0)	Reference		Reference	
No	26	1 (3.8)	0.09 (0.00–0.71)	0.006	0.15 (0.08 – 0.95)	0.047
Most recent sexual partner:						
Wife/regular girlfriend	77	12 (15.6)	0.05 (0.00–0.39)	0.002	Reference	
Casual partner/Sex worker	29	13 (44.8)	4.40(1.53–12.82)	0.002	3.03 (1.17 – 8.58)	0.012
Condom use with new partner:						
≥ 50% of the time	62	9 (14.5)	0.30 (0.10 – 0.83)	0.009	Reference	
< 50% of the time	44	16 (36.4)	3.37 (1.21 –9.56)	0.009	2.53 (1.12 – 7.38)	0.021
Condom use:						
Ever used condom	84	17 (20.2)	Reference			
Never used condom	22	8 (36.4)	0.44 (0.14 –1.38)	0.113	Not included	
History of STI:						
No	90	21 (23.3)	Reference			
Yes	16	4 (25.0)	1.10 (0.26 –4.23)	0.885	Not included	
Baseline HIV:						
Negative	92	21 (22.8)	Reference			
Positive	14	4 (28.6)	1.35 (0.32 – 5.40)	0.637	Not included	
Persistent all HPV:						
Negative	51	8 (15.6)	Reference		Reference	
Positive	59	17 (28.8)	2.4 (0.85 –6.92)	0.065	1.12 (0.37 – 3.41)	0.844
Persistent HR HPV:						
Negative	67	10 (14.9)	Reference		Reference	
Positive	39	15 (38.5)	3.56 (1.28–10.06)	0.006	3.35 (1.21– 11.37)	0.009
Persistent LR HPV:						
Negative	63	13 (20.6)	Reference			
Positive	43	12 (27.9)	1.49 (0.55–4.03)	0.387	Not included	

Of the 25 men with incident HSV-2, 15 (60%) had HPV infection at baseline that persisted in 73.3% (11/15) during follow up. Among the 106 men who were evaluated for HSV-2 incidence, 14 (13.2%) were HIV positive at baseline, of which only four (28.6%) acquired HSV-2 infection during follow-up. Bivariate analysis revealed that the number of sexual partners in the last 12 months (IRR=3.58; 95% CI, 1.32–9.71), number of lifetime sexual partners (IRR=3.06; 95% CI, 1.13–8.26) and most recent sexual act with casual partner/sex worker (IRR=4.40; 95% CI, 1.53–12.82) were significantly associated with increased risk for HSV-2 incidence. Condom use with new sexual partner (IRR=0.30; 95% CI, 0.10–0.83) and lack of transactional sex (IRR=0.09; 95% CI, 0.01–0.71) were protective against incident HSV-2 infection. However, age, marital status, circumcision status and baseline HIV were not associated with acquisition of new HSV-2 infection.

Despite lack of association between HSV-2 incidence and overall baseline HPV infection, of the 25 cases with incident HSV-2 infection, 15 (60.0%) were infected with persistent HR HPV genotypes compared to 29.6% (24/81) among men who remained HSV-2 negative at study exit. Therefore men with persistent HR HPV infection were 3.6 times more likely to acquire new HSV-2 infection compared to those without (IRR=3.56; 95% CI, 1.28–10.06). Persistence of all HPV types combined increased the risk for new HSV-2 acquisition by 2-fold (IRR=2.40; 95% CI, 0.85–6.92) but this association was on the borderline (p=0.065). However, exclusive infection with persistent LR HPV genotypes was not associated with HSV-2 incidence (Table 4.16).

In multivariate logistic regression (Table 4.16), fishermen with less than two sexual partners one year prior to study participation were significantly protected from acquiring HSV-2 (aIRR=0.16; 95% CI: 0.05–0.50) compared to those who had a higher number of (≥2) sexual partners in the same period. Multiple sexual partnerships in the last 12 months prior to study participation was independently associated with a 4-fold increased risk for new HSV-2 acquisition (aIRR=4.77; 95% CI: 1.12–11.38).

However, incident HSV-2 infection was not significantly associated with baseline HIV infection (p=0.637) or baseline CD4 (p=0.543) level. Fishermen who reported

the most recent sexual act with a casual partner or sex worker at baseline were at increased risk of incident HSV-2 infection (aIRR=3.03; 95% CI: 1.17–8.58) compared to those reporting this sexual act with the wife/ regular sexual partner. Infrequent (<50 % of the time) condom use during sexual intercourse with a new partner independently increased the risk for HSV-2 incident infection significantly compared to condom use ≥50% of the time with new sexual partners (aIRR=2.53; 95% CI: 1.12–7.38). Men who reported not engaging in transactional sex were significantly protected from new HSV-2 infections compared to those who engaged in type of sexual partnerships (aIRR=0.15; 95% CI: 0.08–0.95). Persistent HR HPV infection remained an independent risk factor for incident HSV-2 infection in multivariate analysis (aIRR=3.35; 95% CI: 1.21–11.37).

In stratified analysis (Table 4.17); among 92 fishermen eligible for incidence evaluation for both HIV and HSV-2, three of five men with new HIV infection had incident HSV-2 infection compared to 18 of 87 men without new HIV infection. Therefore, men with new HIV infection were 5-times more likely to acquire new HSV-2 infection (IRR=5.07; 95% CI, 0.91–28.37) compared to those without. However, the association between incident HSV-2 infection and HIV acquisition was on the borderline (p=0.076).

Similarly, among men who completed the study that were negative for HIV and HSV-2 at baseline, 22.8% (21/92) acquired new HSV-2 infection during follow up, of which 52% (11/21) reported sex worker/casual as the most recent sexual partner at baseline. Whereas 77.2% (71/92) that remained negative for HSV-2 at study exit, only 14.1% (10/71) reported similar sexual partnership at baseline. Therefore, in bivariate analysis, most recent sexual partnership with a sex worker/casual partner increased the risk for acquiring new HSV-2 infection by 6-fold (RR=6.71; 95% CI: 2.00–23.12). Frequent condom use (≥50% of the time) compared to (<50% of the time), RR=0.20; 95% CI: 0.06–0.62; and number of sexual partners (<2) compared to (≥2), RR=0.09; 95% CI: 0.02–0.39; were significantly associated with decreased risk for new HSV-2 acquisition among fishermen negative for HIV infection at baseline. These factors: In multivariate analysis; inconsistent condom use, most recent sexual intercourse with a

casual partner/sex worker and multiple sexual partnerships 12 months prior to study participation, remained important factors significantly associated with increased risk for new HSV-2 acquisition (Table 4.17).

Table 4.17: Factors associated with HSV-2 incidence among the 92 Fishermen negative for HIV at baseline

Characteristics	n/N (%)	Bivariate analysis			Multivariate analysis		
		OR	95% CI	p-value	aOR	95% CI	p-value
Age		0.98	0.90 – 1.06	0.629		Not included	
Marital status							
Single (never married)	7/26 (27)	1.77	0.68 – 4.60	0.241		Not included	
Ever married	14/66 (21)	0.65	0.25 – 1.68	0.377			
Circumcision							
Yes	6/29 (21)	0.69	0.24 – 1.95	0.480		Not included	
No	15/64 (23)	1.46	0.51 – 4.15	0.480			
Sexual partners in 6 months							
< 2 partners	8/50 (16)	0.42	0.14 – 1.28	0.089		Reference	
≥ 2 partners	13/42 (31)	2.35	0.28 – 7.21	0.089	1.16	0.11 – 9.73	0.321
Sexual partners in 12 months							
< 2 partners	2/36 (6)	0.09	0.02 – 0.39	0.002		Reference	
≥ 2 partners	19/56 (34)	8.73	1.75 – 18.73	0.002	4.09	1.12 – 22.61	0.012
Sexual partners in lifetime							
< 4 partners	3/22 (14)	0.37	0.10 – 1.38	0.139		Not included	
≥ 4 partners	18/70 (26)	1.50	0.57 – 3.96	0.413			
Wash genitals before sex							
Yes	3/15 (20)	0.56	0.15 – 2.16	0.401		Not included	
No	18/77 (23)	1.78	0.46 – 6.84	0.401			
Wash genitals after sex							
Yes	5/28 (18)	0.56	0.20 – 1.59	0.277		Not included	
No	16/64 (25)	1.78	0.63 – 5.02	0.277			
Baseline HPV							
No	10/56 (18)	0.43	0.17 – 1.07	0.071		Reference	
Yes	11/36 (31)	2.33	0.93 – 5.85	0.071	1.87	0.62 – 7.24	0.539
Relation with most recent sex partner							
Regular partner (Girlfriend/wife)	10/71 (14)	0.15	0.04 – 0.50	<0.001		Reference	
Casual partner / Sex worker	11/21 (52)	6.71	2.00 – 23.12	<0.001	7.78	2.74 – 22.04	<0.001
Condom use with recent partner							
Yes	1/14 (7)	0.36	0.08 – 1.71	0.199		Not included	
No	20/78 (26)	2.79	0.58 – 13.30	0.199			
Condom use with new partner							
≥50%	7/58 (12)	0.20	0.06 – 0.62	0.001		Reference	
<50%	14/34 (41)	5.10	1.61 – 16.61	0.001	3.09	1.32 – 12.37	0.009
Ever had STI							
Yes	3/11 (27)	1.82	0.53 – 6.16	0.339		Not included	
No	18/81 (22)	0.55	0.16 – 1.87	0.339			

4.2.4 HPV, HIV and HSV-2 co-infections among Fishermen in Kisumu, Kenya

In the course of the one-year study, 269 (90%) of the 300 fishermen were infected with any of the three viruses. These included: 228 (76%) fishermen detected with at least one HPV genotype, 78 (26%) fishermen infected with HIV, and HSV-2 detected among 194 (65%) fishermen. Among the 269 infected fishermen, majority 173 (64%) of these viral infections occurred as co-infections rather than single 96 (36%) infection. In HPV infected fishermen, 168 (74%) were co-infected with HIV, HSV-2 or both (Figure 4.14). For men testing positive for HIV, 76 (97%) were co-infected with HPV, HSV-2 or both. While fishermen detected with HSV-2, 160 (82%) were co-infected with HPV, HIV or both.

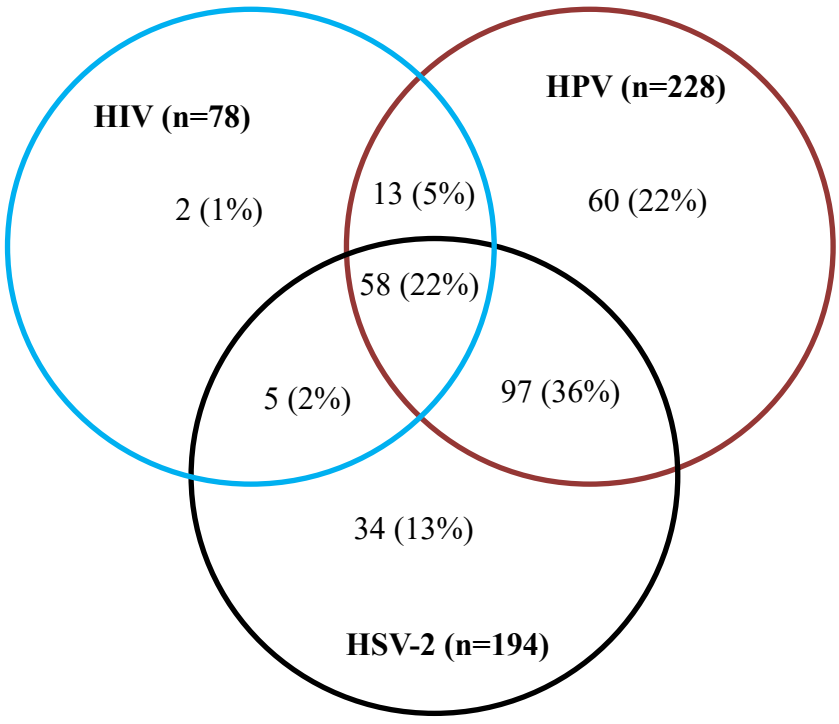


Figure 4.14: Vane diagram showing the number of men with HPV, HIV and HSV-2 co-infections among fishermen in one year of study participation

4.3 Molecular Characterization of HPV Infections among Fishermen

To characterize genotypic HPV infections among fishermen in relevance to HPV vaccination, all the 228 men infected with at least one HPV genotype at any time in the course of the one year study participation were included in this analysis (Table 4.18).

Table 4.18: Vaccine-relevant HPV genotype distribution among Fishermen within one year of study participation

HPV infection	n (%)	95% CI
HPV (+)	228 (76.0)	70.7 – 80.6
HPV (-)	72 (24.0)	19.4 – 29.3
Total	**300 (100)**	
Total single infections	**52 (22.8)**	**17.6 – 28.9**
Single infections HPV-6 genotype	2 (0.9)	0.2 – 3.1
Single infections HPV-11 genotype	0	
Single infections HPV-16 genotype	5 (2.2)	0.8 – 5.3
Single infections HPV-18 genotype	0	
Single infections HPV-31 genotype	2 (0.9)	0.2 – 3.1
Single infections HPV-33 genotype	0	
Single infections HPV-45 genotype	0	
Single infections HPV-52 genotype	0	
Single infections HPV-58 genotype	3 (1.3)	0.3 – 4.2
Total single infections HPV-6/11/16/18/31/33/45/52/58 genotypes	12 (5.3)	2.9 – 9.2
Total single infections HPV-16/18/31/33/45/52/58 genotypes	10 (4.4)	2.3 – 8.2
Total Single infections others non-vaccine HPV genotype	40 (17.5)	13.0 – 23.2
Total multiple infections	**176 (77.2)**	**71.1 – 82.4**
Multiple infections 6/11/16/18 excluding any other HPV genotypes	0	
Multiple infections HPV-6/11/16/18 with or without any other HPV genotypes	83 (36.4)	30.2 – 43.1
Multiple infections vaccine genotypes HPV-6/11/16/18/31/33/45/52/58 excluding any other non-vaccine HPV genotypes	2 (0.9)	0.2 – 3.1
Multiple infections HPV-6/11/16/18/31/33/45/52/58 with or without another non-vaccine HPV genotype	125 (54.8)	48.1 – 61.4
Multiple infections HPV-16/18/31/33/45/52/58 with or without another non-vaccine HPV genotype	105 (46.1)	39.5 – 52.8
Multiple infections other HPV genotypes excluding Vaccine genotypes HPV-6/11/16/18/31/33/45/52/58	51 (22.4)	17.3 – 28.5

Of the 228 fishermen, 137 (60.1% [95% CI: 53.4 – 66.4]) were infected with at least one nonavalent HPV vaccine genotype (Table 4.18). Multiple genotypic infections were detected in 176 (77.2% [95% CI: 71.1 – 82.4]) men, while 52 (22.8% [95% CI: 17.6 – 28.9]) had single HPV genotypes. Among 176 men infected with multiple HPV genotypes, 125 (71.0% [95% CI: 63.6–77.5]) had at least one vaccine genotype while only 12 (23.1% [95% CI: 13.0–37.2]) of 52 men with single HPV genotype infections had a vaccine genotype. Men infected with multiple HPV genotypes were significantly ($p<0.00001$) at increased odds of being detected with a vaccine relevant HPV genotype compared to men with single HPV genotype infection (OR=8.17; 95% CI: 3.77 – 18.01).

Considering HPV genotypes with higher oncogenic risk, 172 (75.4% [95% CI: 69.5 – 80.6]) of the 228 fishermen were infected with high-risk HPV genotypes. Of these men, 34 (19.8% [95% CI: 14.5 – 26.4]) had high-risk HPV genotypes exclusively covered in the nonavalent HPV vaccine, 57 (33.1% [95% CI: 26.5 – 40.5]) were exclusively infected with high-risk HPV genotypes not covered by the nonavalent HPV vaccine and 81 (47.1% [95% CI: 39.8 – 54.5]) were co-infected with both vaccine and non-vaccine high-risk HPV genotypes. Therefore, 80.2% [95% CI: 73.6 – 85.8]) of men detected with high-risk HPV genotypes were infected with at least one high-risk HPV genotype not included in the nonavalent vaccine. Overall, among the 228 fishermen detected with any HPV, 138 men (60.5% [95% CI: 54.1 – 66.7]) were infected with high-risk HPV genotypes not covered by nonavalent HPV vaccine.

HPV-68b (16.7%), HPV-16 (15.8%) and HPV-58 (14.5%) were the top three most prevalent high-risk HPV genotype infections during the course of study, whereas HPV-40 (24.6%), HPV-6 (18.9%) and HPV-62 (18.4%) were the most prevalent low-risk genotypes (Figure 4.15). This trend for low-risk HPV genotypes was consistently observed at the three study visits: baseline (Figure 4.16), month six visit (Figure 4.17) and month-12 study exit visit (Figure 4.18). For high-risk HPV genotypes, the trend was similar for all study visits except month-12 exit visit where HPV-58 (12.4%), HPV-35 (10.7%) and HPV-52 (10.4%) were the three most prevalent genotypes.

except month-12 exit visit where HPV-58 (12.4%), HPV-35 (10.7%) and HPV-52 (10.4%) were the three most prevalent genotypes.

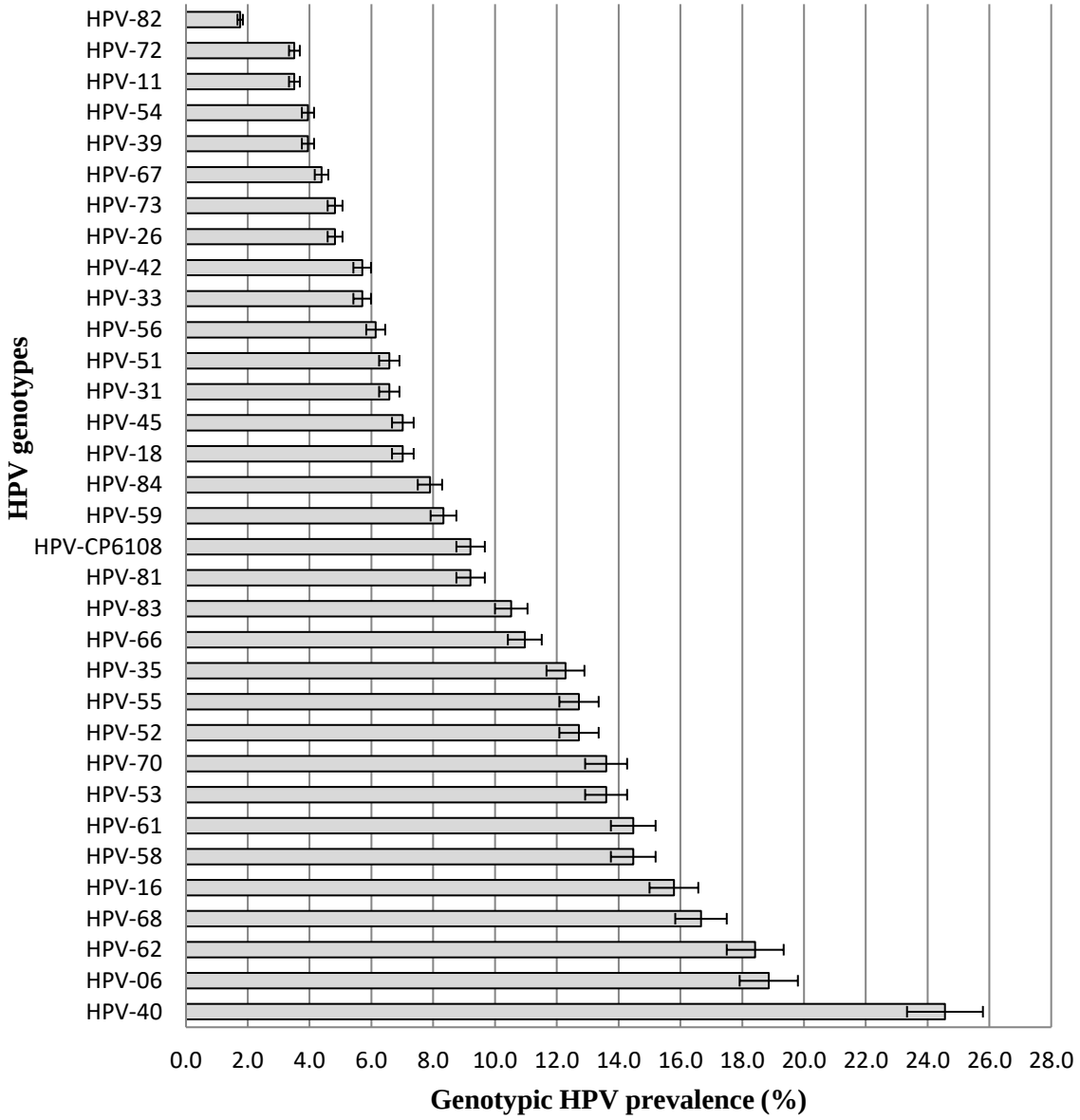


Figure 4.15: Overall distribution of HPV genotypes among studied fishermen during one year of study participation

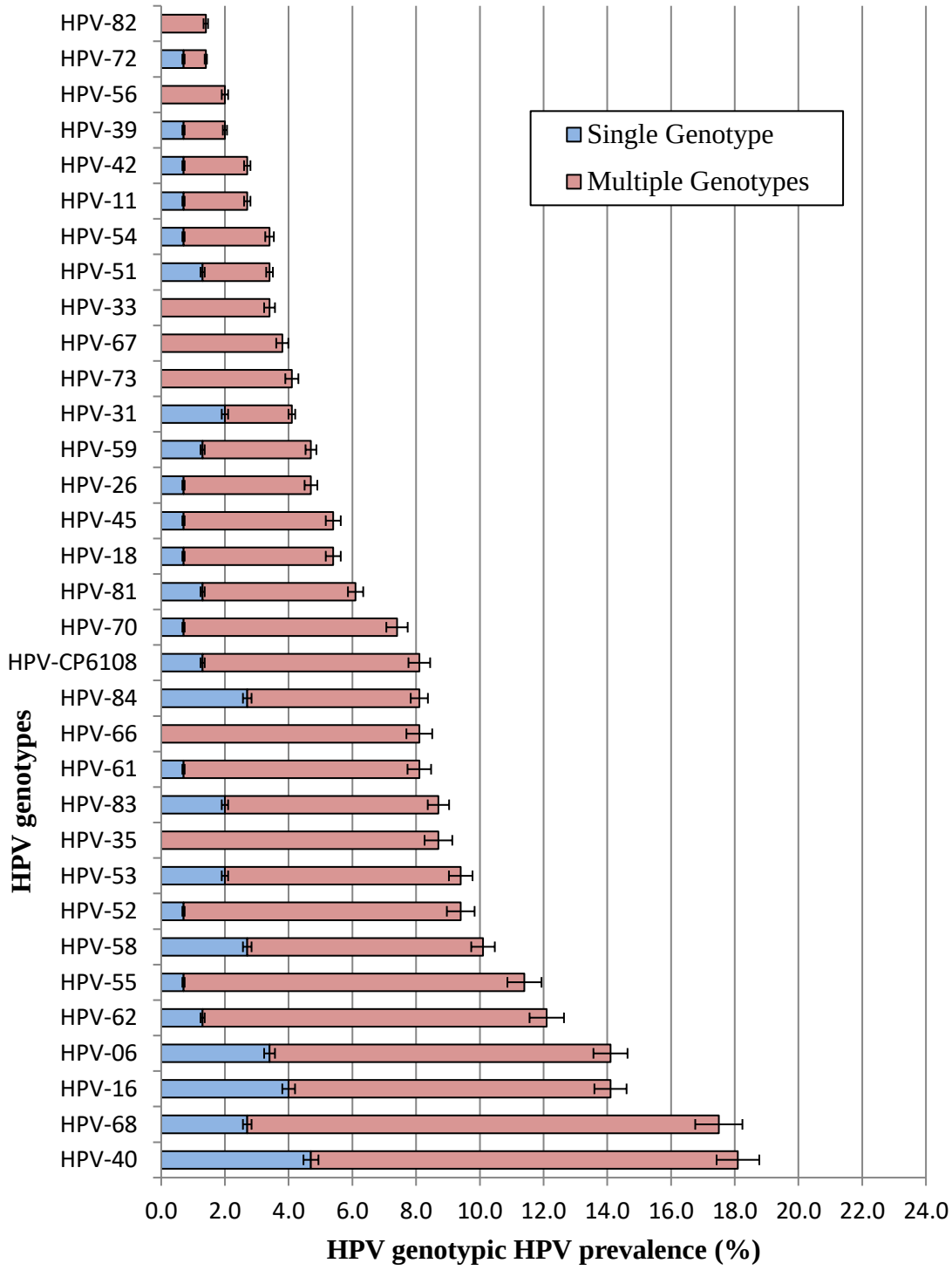


Figure 4.16: Baseline distribution of single and multiple HPV genotypes among studied fishermen

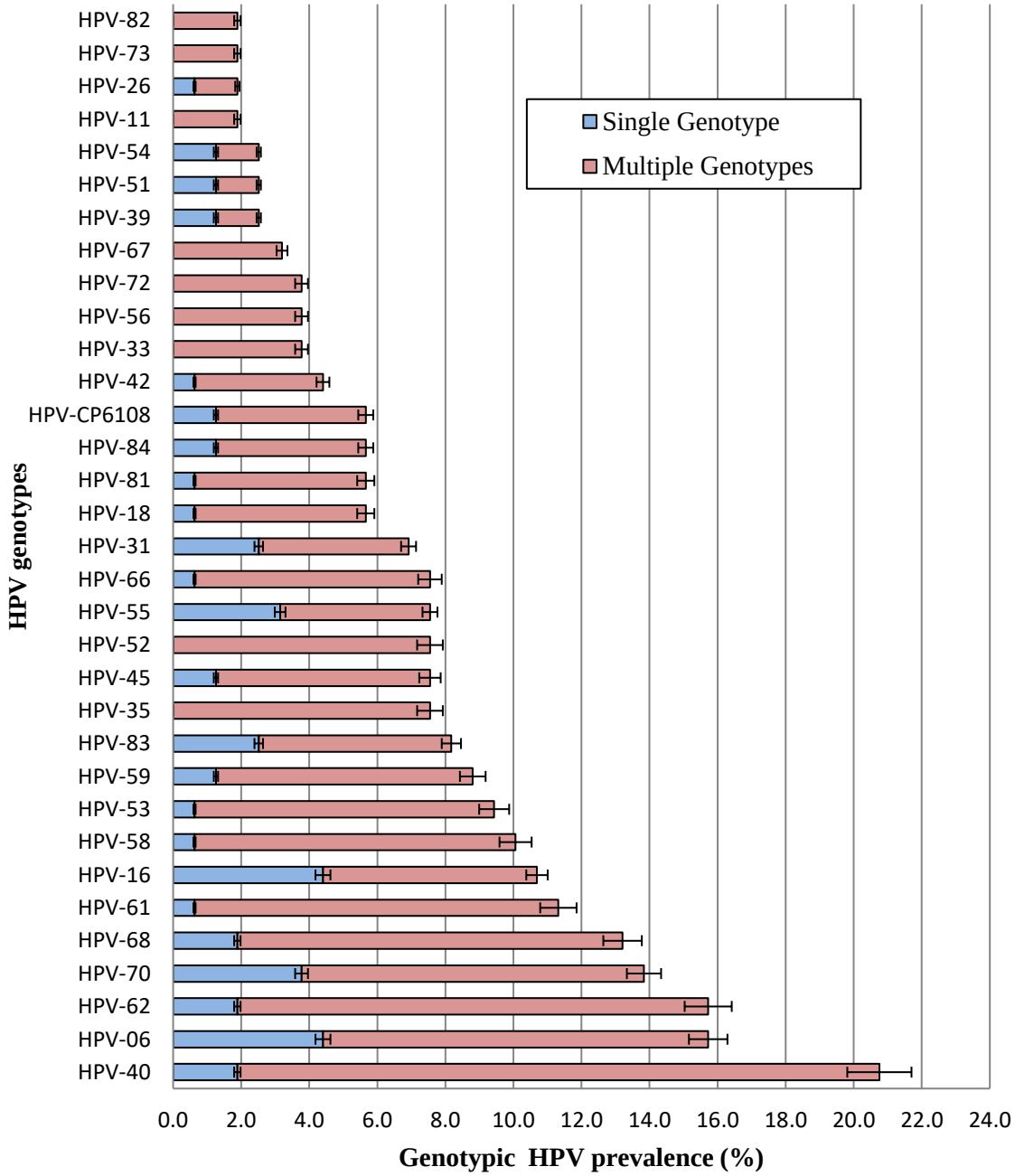


Figure 4.17: Distribution of single and multiple HPV genotype infections among fishermen at month-6 follow up visit

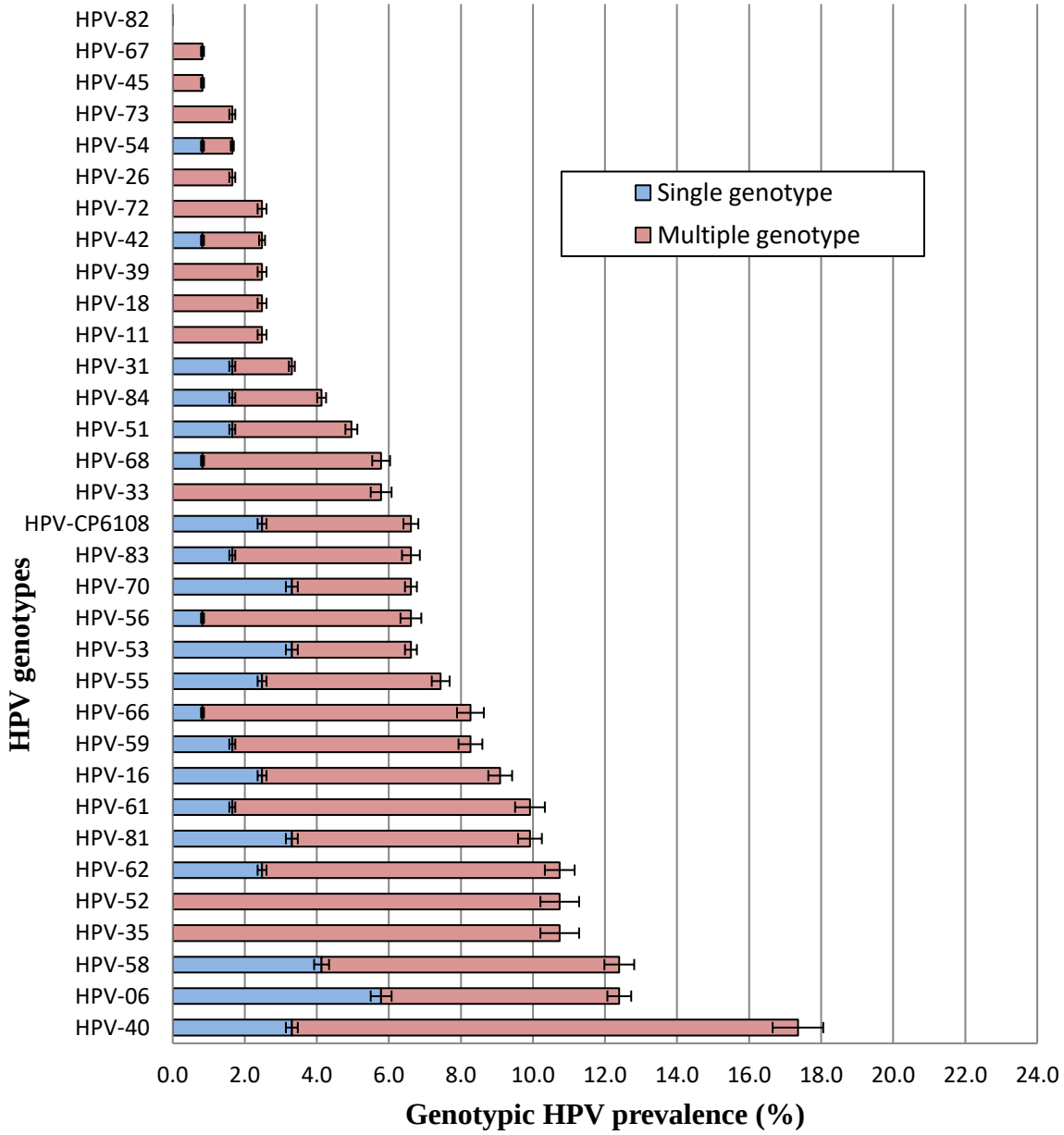


Figure 4.18: Distribution of single and multiple HPV genotype infections among fishermen at month-12 follow up visit

Among HIV positive fishermen, HPV-68 (29.7%), HPV-53 (17.2%) and HPV-66 (15.6%) were the top three HR-HPV genotypes. In this subpopulation, HPV-58 (15.6%) and HPV-16 (12.5%) were ranked 5th and 7th respectively. However, among HIV negative men, HPV-16 (15.9%), HPV-68 (14.6%) and HPV-58 (13.4%) were the top three most prevalent HR-HPV genotypes, with HPV-53 (12.2%) and HPV-66 (9.1%) ranking 4th and 8th respectively. Although HPV-40 (34.4%) and HPV-62 (29.7%) were significantly ($p<0.05$ and $p<0.01$ respectively) more prevalent among HIV positive men compared to those without HIV infection {HPV-40 (20.1%) and HPV-62 (14.0%)}, the top three most prevalent LR-HPV genotypes remained the same when stratified by HIV status.

In this study, there were no exclusive genotype-specific HPV infections observed. All the 33 detectable HPV genotypes had a chance to exist in presence of any of the other evaluated HPV genotypes. HPV-6 was highest, co-existing with 31 genotypes of the other 32 genotypes detected, while HPV-72 had the lowest (Table 4.19).

Table 4.19: HPV genotypic co-existence among Fishermen infected with multiple HPV

Secondary genotypic infection with any of the 33 detectable HPV genotypes

Primary HPV genotypic infection

Genotypes	06	11	16	18	26	31	33	35	39	40	42	45	51	52	53	54	55	56	58	59	61	62	66	67	68	70	72	73	81	82	83	84	CP	**Total**
HPV-06		+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**31**
HPV-11	+		+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	+	+	-	-	+	+	+	-	-	+	+	-	+	+	-	-	**20**
HPV-16	+	+		+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	**26**
HPV-18	+	+	+		+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-	+	+	-	+	+	+	-	-	+	**22**
HPV-26	-	-	+	+		+	-	+	-	+	+	-	+	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	+	-	+	**22**
HPV-31	+	+	+	+	+		-	+	-	+	-	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	**19**
HPV-33	+	+	-	-	-	-		-	+	+	-	+	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	**18**
HPV-35	+	-	+	+	+	+	-		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	**26**
HPV-39	+	+	+	+	-	-	+	-		+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	**13**
HPV-40	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	**30**
HPV-42	+	-	+	+	+	-	-	+	-	+		+	+	+	+	-	+	-	-	+	+	+	+	-	+	+	-	+	+	+	-	-	-	**20**
HPV-45	+	+	+	+	-	+	+	+	+	+	+		+	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	**26**
HPV-51	+	+	-	+	+	+	-	+	+	+	+	+		+	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+	+	-	-	+	**23**
HPV-52	+	-	+	+	+	+	-	+	-	+	+	+	+		+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	**25**
HPV-53	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	**30**
HPV-54	+	-	-	-	+	-	-	+	-	+	-	-	-	+	+		-	-	+	+	+	+	-	+	-	-	-	-	-	-	+	+	+	**14**
HPV-55	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-		+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	**28**
HPV-56	+	+	-	-	+	+	-	+	-	+	-	-	-	+	+	-	+		+	+	+	+	+	+	+	-	+	-	+	-	+	-	-	**19**
HPV-58	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+		+	+	+	+	+	+	-	+	+	+	+	+	+	+	**27**
HPV-59	+	-	+	+	-	+	-	+	-	+	+	+	+	+	-	+	+	+	+		+	+	+	-	+	+	+	+	+	-	+	+	+	**25**
HPV-61	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	-	+	+	+	+	**29**
HPV-62	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	-	+	+	-	+	+	+	+	+	+	**27**
HPV-66	+	+	+	-	-	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+		-	+	+	+	+	+	-	+	+	+	**24**
HPV-67	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-	+	-	+	+	-	+	-	-		-	-	+	-	-	-	-	+	+	**13**
HPV-68	+	-	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-		+	-	+	+	+	+	+	+	**25**
HPV-70	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	+	-	+		-	+	+	+	+	+	+	**24**
HPV-72	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	-	-		-	-	-	-	-	-	**11**
HPV-73	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	+	+	+	-	+	+	-		+	-	-	-	+	**21**
HPV-81	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+	-	+		-	+	+	+	**24**
HPV-82	+	+	-	+	+	+	+	-	-	-	+	+	+	-	+	-	+	-	+	-	+	+	-	-	+	+	-	-	-		-	-	-	**16**
HPV-83	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	-		+	-	**23**
HPV-84	+	-	+	-	-	-	-	+	-	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+		-	**19**
CP6108	+	-	+	+	+	-	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	-	-		**22**

To characterize genotypic interactions of HPV infections among fishermen in relation to vaccine relevant genotypes, dendrograms demonstrating relative probability of genotypic co-infections with any of the nine genotypes included in nonavalent HPV vaccine were plotted (Figure 4.19 and Figure 4.20). Among men infected with HPV-6, genotypes with the highest probabilities of co-infections with vaccine genotype HPV-6 included HPV-53 for high-risk genotypes and HPV-40 for low-risk genotypes (Figure 4.19A). Low-risk HPV-6 and high-risk HPV-33/HPV-55 had the highest chance of co-infections with vaccine genotype HPV-11, in men detected with HPV-11 (Figure 4.19B). Low-risk HPV-61 had the highest co-infection probability in men detected with HPV-18 and HPV-45, while High-risk HPV-26 and HPV-58 had the highest chance of co-infection among men infected with HPV-18 and HPV-45 respectively (Figure 4.19C–D).

HPV-61 had the highest probability of low-risk HPV co-infection among men infected with vaccine genotypes HPV-16 and HPV-31, whereas high-risk HPV-68b and HPV-16 genotypes respectively had the highest probability of co-infection among these men (Figure 4.20A–B). In men detected with HPV-33, high-risk HPV-58/HPV-53 and low-risk HPV-40 genotypes had the highest probability of co-infection with vaccine genotype HPV-33 (Figure 4.20C). High-risk HPV-35 and low-risk HPV-62 genotypes had the highest chance of co-infection with vaccine genotype HPV-52 (Figure 4.20D). HPV-66 and HPV-6/HPV-40 genotypes had the highest co-infection probability with vaccine genotype HPV-58 (Figure 4.20E).

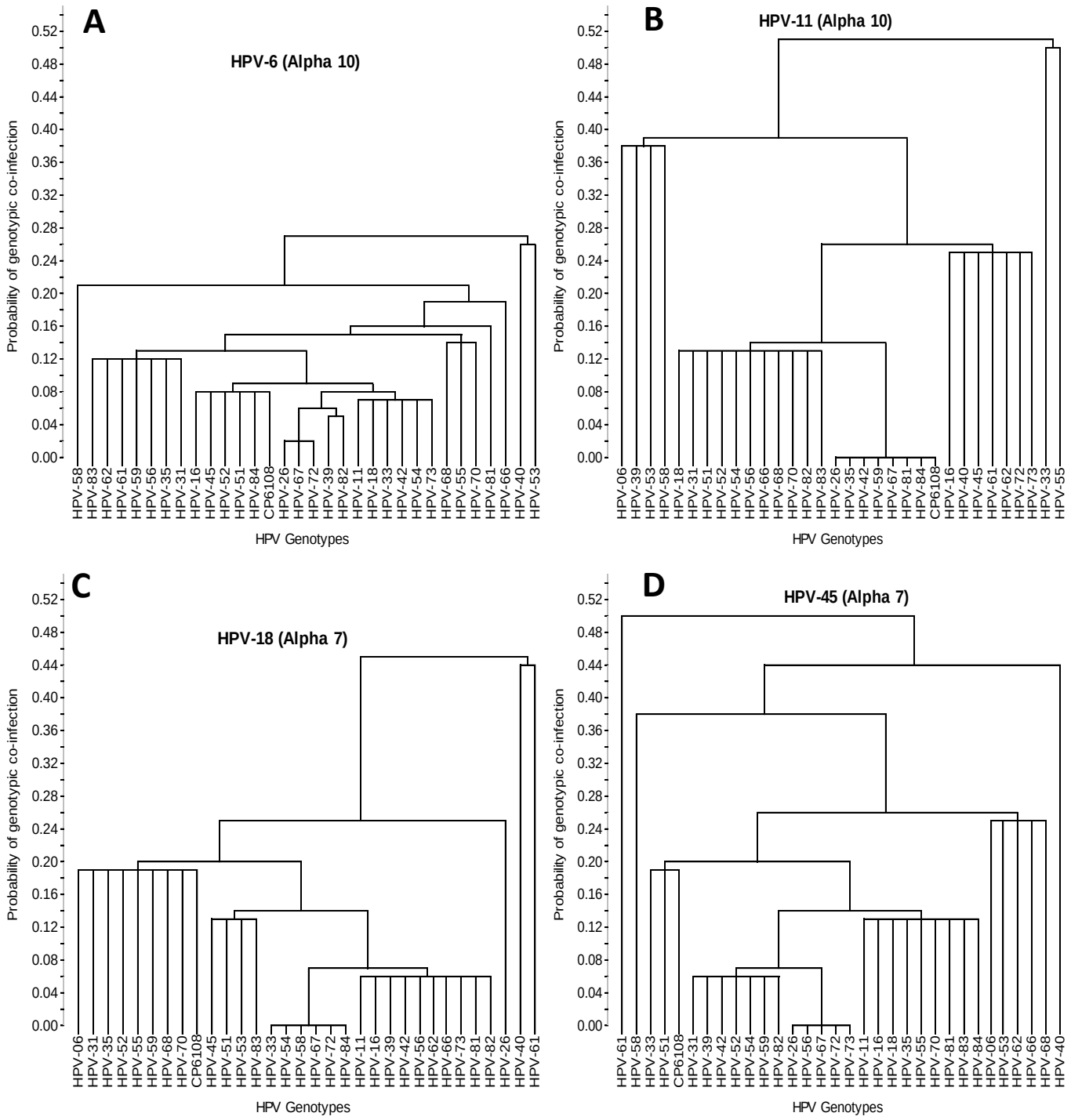


Figure 4.19: Dendrograms showing probability of genotypic co-infections among men infected with Alpha 7 and 10 HPV vaccine genotypes

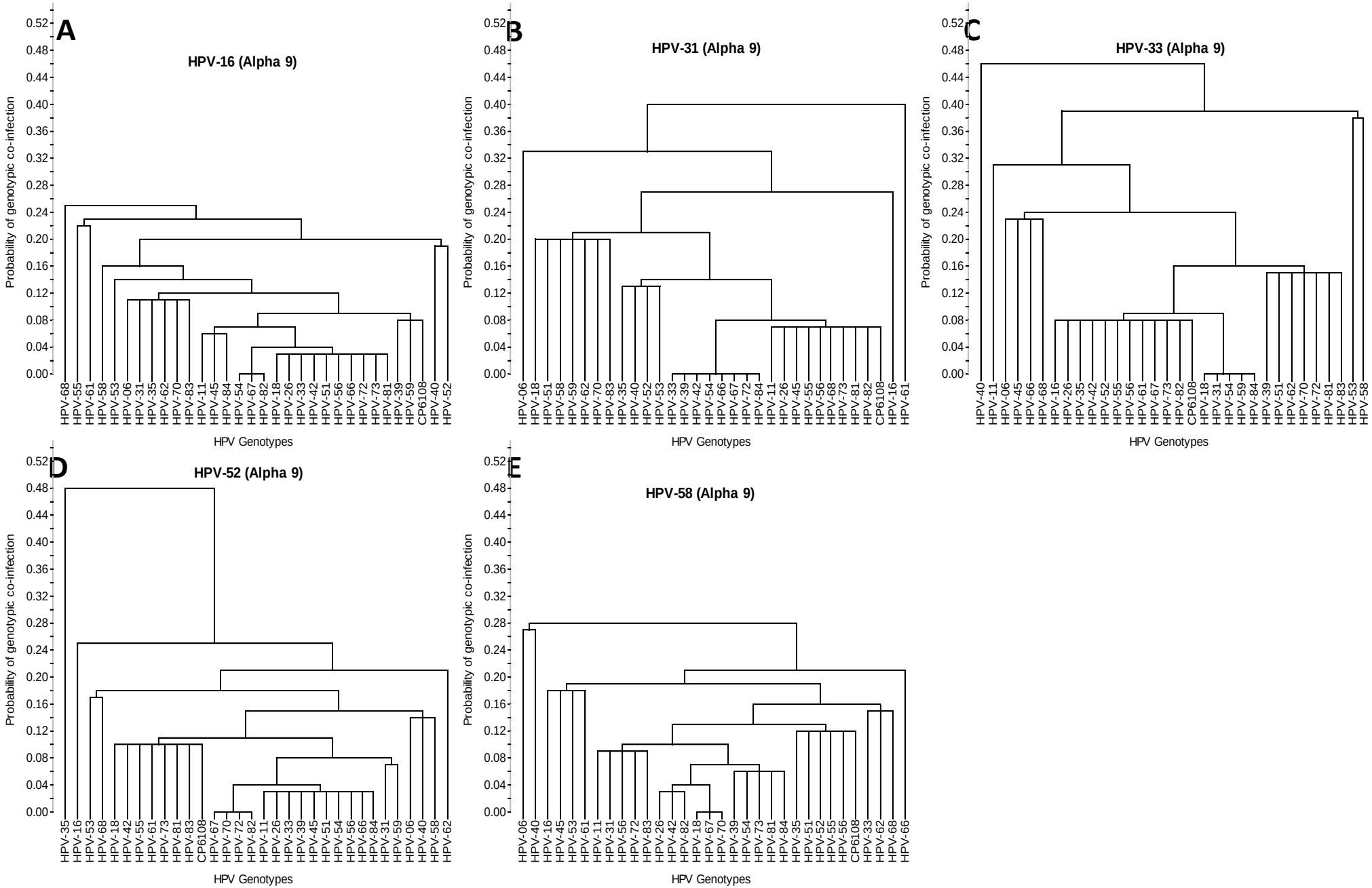


Figure 4.20: Dendrograms showing probability of genotypic co-infections among men infected with Alpha 9 HPV vaccine genotypes

Analysis of potential genotype replacement among these men in the event of 9-valent HPV vaccination did not demonstrate a clear pattern of genotypic differences in associations between the nine vaccine preventable HPV types (HPV-6, 11, 16, 18, 31, 33, 45, 52 and 58) and the other 24 HPV genotypes (Table 4.20). A total of 19 genotypic associations were observed, of which two were negative. Of the 19 genotypic associations, 14 were between vaccine genotypes and non-vaccine genotypes. HPV-33 was positively associated with HPV-45 (aOR=7.52 [1.66 – 32.63]), HPV-58 (aOR=4.17 [1.19 – 15.47]) and non-vaccine genotype HPV-53 (aOR=4.54 [1.18 – 16.94]), while HPV-45 was also associated with HPV-58 (aOR=4.11 [1.21 – 13.65]). HPV-18 was positively associated with 2 non-vaccine genotypes: HPV-26 (aOR=9.76 [2.05 – 45.15]) and HPV-61(aOR=5.56 [1.69 – 18.20]). Additionally, HPV-61 was associated with HPV-31 (aOR=4.59 [1.32 – 15.64]) and HPV-45 (aOR=7.48 [2.30 – 24.51]). HPV-52 was associated with HPV-35 (aOR=12.33 [4.55 – 33.91]), and HPV-58 associated with HPV-66 (aOR=5.22 [1.91 – 14.19]).

For vaccine-preventable low-risk types (HPV-6 and 11), two non-vaccine HPV genotypes (HPV-53 and HPV-66) were positively associated ($p<0.01$) with HPV-6, while three non-vaccine HPV genotypes (HPV-39, HPV-55 and HPV-72) and two vaccine HPV genotypes (HPV-33 and HPV-58) were positively associated with HPV-11 (aORs: 2.84 – 7.80). Only HPV-70 was negatively associated with HPV-52 ($p=0.018$) and HPV-58 ($p=0.011$) among the nine vaccine preventable HPV genotypes (Table 4.20). Together, these results characterizing genotypic HPV infections among fishermen suggest that, infection with one HPV genotype does not preclude concurrent or future infection with other HPV genotypes.

Table 4.20: Genotypic association Between the 9-HPV vaccine genotypes and all other detected HPV genotypes among fishermen during the one year study follow-up

HPV Genotype	HPV-6 aOR (95% CI)	HPV-11 aOR (95% CI)	HPV-16 aOR (95% CI)	HPV-18 aOR (95% CI)	HPV-31 aOR (95% CI)	HPV-33 aOR (95% CI)	HPV-45 aOR (95% CI)	HPV-52 aOR (95% CI)	HPV-58 aOR (95% CI)
HPV-6	**Reference**	2.70 (0.49– 13.71)	0.49 (0.14 -1.57)	0.99 (0.21– 3.98)	2.30 (0.64– 7.91)	1.31 (0.27– 5.51)	2.22 (0.53– 8.70)	0.66 (0.18– 2.15)	1.78 (0.69– 4.46)
HPV-11	2.70 (0.49– 13.71)	**Reference**	1.82 (0.24– 10.64)	1.95 (0.27– 12.23)	2.10 (0.29– 13.16)	**5.44 (2.05– 23.47)**	4.90 (0.62– 31.17)	0.98 (0.15– 6.35)	**6.59 (1.29– 33.69)**
HPV-16	0.49 (0.14 -1.57)	1.82 (0.24– 10.64)	**Reference**	0.34 (0.02– 2.57)	2.06 (0.51– 7.61)	0.43 (0.02– 3.35)	0.75 (0.11– 3.70)	1.87 (0.66– 5.15)	1.22 (0.41– 3.46)
HPV-18	0.99 (0.21– 3.98)	1.95 (0.27– 12.23)	0.34 (0.02– 2.57)	**Reference**	3.85 (0.75– 17.48)	0.01 (0.01– 5.42)	2.02 (0.01– 10.84)	1.65 (0.35– 6.82)	0.01 (0.01– 1.81)
HPV-26	0.42 (0.02-3.31)	0.01 (0.01– 14.83)	0.52 (0.02– 4.17)	**9.76 (2.05– 45.15)**	1.45 (0.19– 10.37)	1.71 (0.23– 11.95)	0.01 (0.01– 6.58)	0.68 (0.03– 5.48)	0.58 (0.03– 4.66)
HPV-31	2.30 (0.64– 7.91)	2.10 (0.29– 13.16)	2.06 (0.51– 7.61)	3.85 (0.75– 17.48)	**Reference**	0.01 (0.01– 5.84)	0.94 (0.13– 6.69)	1.06 (0.28– 5.37)	1.52 (0.32– 6.31)
HPV-33	1.31 (0.27– 5.51)	**5.44 (2.05– 23.47)**	0.43 (0.02– 3.35)	0.01 (0.01– 5.42)	0.01 (0.01 -5.84)	**Reference**	**7.52 (1.66– 32.63)**	0.56 (0.03– 4.41)	**4.17 (1.19– 15.47)**
HPV-35	0.93 (0.29– 2.80)	0.01 (0.01– 4.93)	0.88 (0.24– 2.91)	1.73 (0.36– 7.16)	1.11 (0.29– 5.62)	0.58 (0.03– 4.60)	1.02 (0.27– 5.14)	**12.33 (4.55– 33.91)**	0.98 (0.27– 3.29)
HPV-39	1.24 (0.01– 6.88)	**3.14 (1.40– 14.79)**	2.82 (0.53– 13.59)	1.70 (0.22– 12.43)	0.01 (0.01– 8.96)	5.40 (0.68– 34.29)	1.70 (0.22– 12.43)	0.85 (0.11– 6.61)	1.73 (0.24– 9.76)
HPV-40	1.07 (0.46– 2.43)	1.02 (0.14– 5.85)	0.70 (0.26– 1.82)	2.59 (0.82– 8.10)	0.45 (0.07– 2.21)	2.83 (0.80– 9.93)	2.59 (0.82– 8.10)	0.45 (0.13– 1.46)	1.18 (0.47– 2.90)
HPV-42	1.31 (0.27– 5.51)	0.01 (0.01– 12.18)	0.43 (0.02– 3.35)	1.11 (0.15– 7.96)	0.01 (0.01 -5.84)	1.41 (0.19– 9.80)	1.11 (0.15– 7.96)	2.18 (0.44– 9.43)	0.48 (0.02– 3.74)
HPV-45	2.22 (0.53– 8.70)	4.90 (0.62– 31.17)	0.75 (0.11– 3.70)	2.02 (0.01– 10.84)	0.94 (0.13– 6.69)	**7.52 (1.66– 32.63)**	**Reference**	0.44 (0.02– 3.38)	**4.11 (1.21– 13.65)**
HPV-51	1.62 (0.41– 5.92)	2.10 (0.29– 13.16)	0.36 (0.02– 2.79)	2.19 (0.01– 11.86)	4.19 (0.81– 19.29)	2.83 (0.39– 15.90)	3.85 (0.75– 17.48)	0.47 (0.02– 3.67)	2.31 (0.57– 8.60)
HPV-52	0.66 (0.18– 2.15)	0.98 (0.15– 6.35)	1.87 (0.66– 5.15)	1.65 (0.35– 6.82)	1.06 (0.28– 5.37)	0.56 (0.03– 4.41)	0.44 (0.02– 3.38)	**Reference**	0.94 (0.26– 3.13)
HPV-53	**2.84 (1.14– 6.97)**	4.11 (0.73– 21.35)	1.03 (0.32– 3.11)	0.90 (0.02– 4.50)	0.98 (0.26– 4.92)	**4.54 (1.18– 16.94)**	2.28 (0.57– 8.43)	1.39 (0.42– 4.29)	1.51 (0.50– 4.35)
HPV-54	2.24 (0.42– 10.67)	3.79 (0.49– 24.30)	0.01 (0.01– 3.16)	0.01 (0.01– 8.31)	0.01 (0.01– 8.96)	0.01 (0.01– 10.60)	1.70 (0.22– 12.43)	0.85 (0.11– 6.61)	1.73 (0.24– 9.76)
HPV-55	1.14 (0.39– 3.23)	**7.80 (1.52– 40.33)**	2.33 (0.85– 6.23)	1.65 (0.35– 6.82)	0.47 (0.02– 3.67)	0.56 (0.03– 4.41)	0.98 (0.26– 4.91)	0.77 (0.17– 2.93)	0.94 (0.26– 3.13)
HPV-56	2.57 (0.70– 9.05)	2.27 (0.31– 14.25)	0.39 (0.02– 3.05)	1.02 (0.14– 7.30)	1.10 (0.15– 7.80)	1.29 (0.18– 8.99)	0.01 (0.01– 4.97)	0.51 (0.02– 4.01)	2.55 (0.63– 9.69)
HPV-58	1.78 (0.69– 4.46)	**6.59 (1.29– 33.69)**	1.22 (0.41– 3.46)	0.01 (0.01– 1.81)	1.52 (0.32– 6.31)	**4.17 (1.19– 15.47)**	**4.11 (1.21– 13.65)**	0.94 (0.26– 3.13)	**Reference**
HPV-59	1.61 (0.47– 5.18)	0.01 (0.01– 7.80)	1.00 (0.22– 3.94)	2.83 (0.57– 12.31)	3.08 (0.62– 13.57)	0.01 (0.01– 4.44)	0.72 (0.10– 5.17)	0.79 (0.12– 3.89)	1.12 (0.24– 4.44)
HPV-61	0.74 (0.23– 2.19)	2.03 (0.27– 11.92)	1.91 (0.71– 5.01)	**5.56 (1.69– 18.20)**	**4.59 (1.32– 15.64)**	0.48 (0.02– 3.74)	**7.48 (2.30– 24.51)**	0.65 (0.15– 2.46)	1.38 (0.46– 3.95)
HPV-62	0.53 (0.17– 1.53)	1.50 (0.20– 8.67)	0.51 (0.14– 1.63)	0.28 (0.01– 2.10)	1.12 (0.24– 4.54)	0.80 (0.12– 4.03)	1.53 (0.39 -5.49)	1.18 (0.40– 3.35)	0.76 (0.24– 2.26)
HPV-66	**3.34 (1.30– 9.01)**	1.17 (0.18– 7.45)	0.20 (0.01– 1.47)	0.52 (0.02– 4.07)	0.01 (0.01– 2.72)	2.63 (0.53– 11.56)	3.03 (0.75– 11.46)	0.26 (0.01– 1.93)	**5.22 (1.91– 14.19)**
HPV-67	3.06 (0.69– 13.05)	0.01 (0.01– 16.60)	1.35 (0.01– 7.35)	0.01 (0.01– 7.35)	0.01 (0.01– 7.92)	1.91 (0.25– 13.43)	0.01 (0.01– 7.35)	0.01 (0.01– 3.64)	4.34 (0.96– 18.89)
HPV-68b	0.78 (0.27– 2.13)	0.71 (0.03– 6.01)	1.87 (0.73– 4.71)	1.17 (0.25– 4.72)	0.34 (0.02– 2.60)	1.54 (0.32– 6.53)	1.75 (0.44– 6.33)	1.05 (0.32– 3.18)	0.88 (0.27– 2.62)
HPV-70	1.04 (0.35– 2.91)	0.90 (0.14– 5.91)	0.76 (0.21– 2.51)	1.52 (0.32– 6.23)	1.65 (0.35– 6.87)	1.17 (0.30– 6.03)	0.90 (0.24– 4.50)	**0.02 (0.01– 0.97)**	**0.02 (0.01– 0.83)**
HPV-72	0.61 (0.03– 5.12)	**2.89 (1.34– 9.92)**	0.76 (0.10– 6.01)	0.01 (0.01– 9.54)	0.01 (0.01– 10.29)	6.33 (0.78– 41.86)	0.01 (0.01– 9.54)	0.01 (0.01– 4.72)	3.80 (0.68– 19.62)
HPV-73	1.66 (0.33– 7.32)	7.81 (0.94– 53.78)	0.52 (0.02– 4.17)	1.35 (0.18– 9.71)	1.45 (0.19– 10.37)	1.71 (0.23– 11.95)	0.01 (0.01– 6.58)	2.75 (0.54– 12.50)	1.33 (0.30– 7.11)
HPV-81	2.38 (0.80– 6.88)	0.01 (0.01– 6.94)	0.25 (0.01– 1.83)	0.64 (0.03– 5.06)	0.69 (0.03– 5.49)	1.88 (0.45– 10.06)	1.45 (0.36– 7.51)	1.16 (0.25– 4.59)	0.60 (0.09– 2.87)
HPV-82	4.46 (0.43– 45.99)	1.33 (0.01– 14.29)	0.01 (0.01– 8.41)	4.42 (0.49– 40.08)	5.00 (0.52– 42.80)	5.89 (0.62 -49.40)	4.64 (0.49– 40.08)	0.01 (0.01– 10.95)	2.00 (0.21– 18.37)
HPV-83	1.15 (0.35– 3.54)	1.22 (0.18– 7.79)	1.08 (0.29– 3.64)	1.23 (0.31– 6.30)	2.29 (0.47– 9.76)	1.60 (0.40– 8.43)	1.23 (0.31– 6.30)	0.98 (0.22– 3.81)	0.83 (0.18– 3.19)
HPV-84	1.25 (0.33– 4.39)	0.01 (0.01– 8.31)	0.65 (0.10– 3.15)	0.01 (0.01– 3.72)	0.01 (0.01– 4.00)	0.01 (0.01– 4.72)	1.75 (0.42– 9.23)	0.38 (0.02– 2.91)	0.72 (0.11– 3.53)
CP6108	1.01 (0.27– 3.45)	0.01 (0.01– 6.94)	0.88 (0.19– 3.42)	2.49 (0.51– 10.67)	0.69 (0.03– 5.49)	0.81 (0.12– 5.29)	2.49 (0.51 -10.67)	1.16 (0.25– 4.59)	1.44 (0.38– 5.02)

aORs for pairwise associations were adjusted for marital status, circumcision status, history of STI, HIV status and consistent condom use.

4.4 Cellular immune responses to HPV Infections among Fishermen

4.4.1 IFN–γ immune responses to HPV-16 Infections among Fishermen

To explore cell-mediated immune responses among men, a preliminary assessment of induced IFN-γ using ELISPOT immune assay was performed. Figure 4.21 shows a snapshot of ELISPOTs resulting from secreted IFN–γ immune response following stimulation of PBMCs collected from three HPV-16 positive participants at study exit using HPV-16 E6 peptide pool.

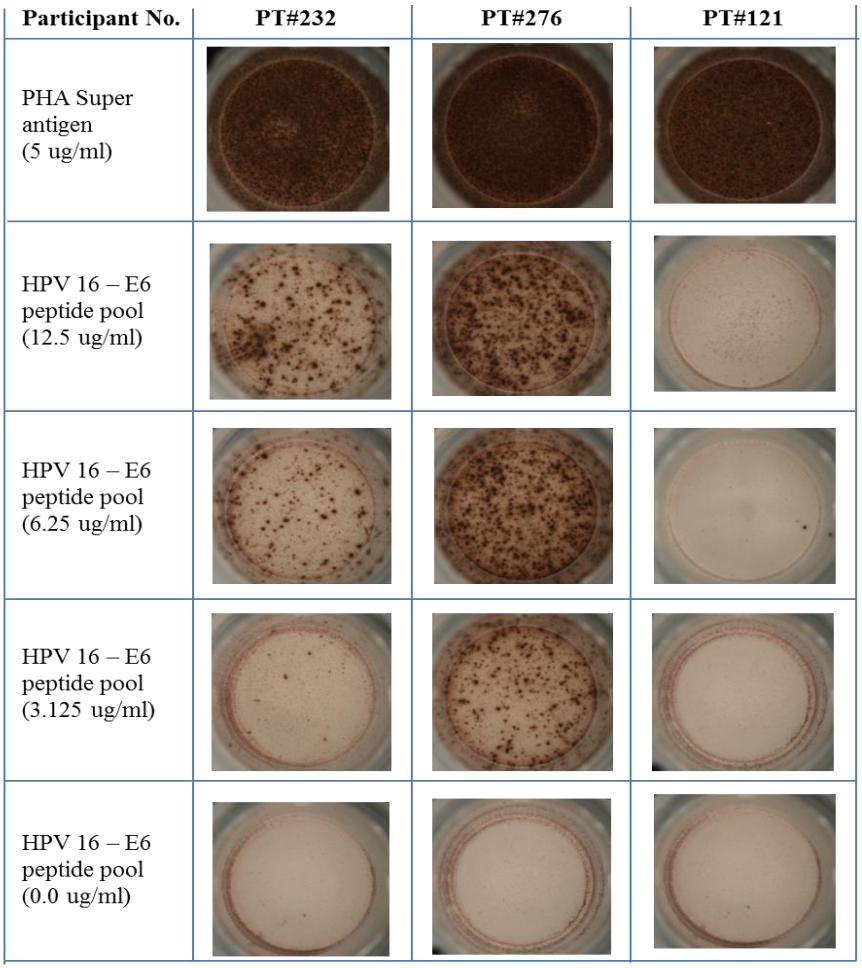


Figure 4.21: IFN–γ ELISPOT for three participants with weak strong and no response to HPV-16 peptide pool

PBMCs demonstrated a robust and strong IFN–γ immune response when stimulated by PHA super antigen (positive control) as shown in first row of Figure 4.21, for the three different participants. Each spot represent IFN–γ released from an activated

viable PBMC after successful stimulation by the supper antigen. The saturation of spots in the supper antigen row (first row) indicates that the PBMCs were viable enabling response to antigenic stimulation.

On stimulation with different concentrations of HPV type 16 E6 peptide pool as antigen, PBMCs from participant number (PT#) 121 had no IFN–γ immune response, indicating absence of memory on cell-mediated HPV-16 immune priming. However, PT#232 had moderate IFN–γ immune response, while PT#276 had a strong IFN–γ immune response (Figure 4.21) suggesting presence of memory on successful HPV-16 immune priming. None of the three participants had a reactive IFN–γ immune response in absence of antigenic (peptide) stimulation, indicating that the IFN–γ secretion was highly specific to the HPV-16 peptide pool (last row of Figure 4.21).

IFN–γ Immune response among fishermen with persistent HPV-16 infections

Among all the 149 participants positive for HPV at study enrollment, 21 (14.1%) of fishermen were detected with HPV-16. Of these, seven (33.3%) persistently tested positive for HPV16 at month-6 follow up visit, while three (14.3%) persistently tested positive for HPV-16 at both month-6 and month-12 (exit) follow up visits. Among participants negative for HPV-16 at baseline, ten men were newly detected with HPV-16 at month six, of whom two men persistently tested positive for HPV-16 at month-12 visit. Additionally, five fishermen who were negative for HPV-16 at baseline and at month six were detected with HPV-16 at study exit (month-12 visit). This provided a total of 36 fishermen infected with HPV-16 at any of the three HPV testing points, of whom nine were infected with persistent HPV-16 that potentially lasted for a period of over 6–12 months. HPV-16 clearance was well characterized only among eight men (positive at baseline) of the 36 participants detected with HPV-16 over the study period.

Using IFN–γ ELISPOT assay, evidence of HPV-16 genotype-specific immune priming and activation against HPV infection was evaluated among fishermen who had persistent HPV-16 infection compared to those who cleared HPV-16 (Table 4.21). Eight out of nine fishermen with persistent HPV-16 and all the eight who cleared HPV-16 infection had adequate PBMC viability for analysis. Two fishermen who

tested negative for any HPV types at baseline and throughout the study were included in the immune response assay.

Table 4.21: Immune response among Fishermen by HPV-16 status duration study

Description	n	M0	M3	M6	M9	M12	Classification	Duration	INFγ + response
Baseline	8	+	NT	+	NT	+/ -	Persistent	>6-12 months	7/8
Baseline	8	+	NT	-	NT	-	Cleared	by month 6	8/8
New infection at months 6	7	-	-	+	-	-	Cleared	by month 9	1/7
New infection at months 12	5	-	NT	-	-	+	New infection	<3 months	1/5
No HPV infection	2	-	NT	-	NT	-	Negative	---	0/2

Key:
NT -Not Tested; + positive for HPV 16; – negative for HPV16; +/ – either positive or negative for HPV 16

Seven (87.5%) of eight participants with persistent HPV-16 infection tested positive for HPV-16 specific INF–γ immune response. Among HPV-16 clearers, 100% (8/8) had a positive INF–γ response on the ELISPOT assay. However, among the seven men who were newly detected with HPV-16 at month six visit and cleared infection by month-9 visit, only 14.3% (1/7) tested positive for the cell mediated immune response. Of the five men who were newly detected with HPV-16 at study exit visit and not at any other study visit, only one (20%) reacted positively for IFN-γ. None of the two men that were never detected with any HPV genotype during study participation demonstrated evidence of immune memory resulting from priming of immune system by natural HPV-16 infection.

4.4.2 Induced cytokine immune responses to HPV infection among Fishermen

With this evidence of natural HPV-16 immune priming from preliminary ELISPOT experiments, a total of 135 fishermen were examined for a panel of eight cytokines using a quantitative multiplex Luminex assay to enable characterization of cell-mediated immune response against nine HPV genotypes among men. These included HPV uninfected men (n=50), men with HPV clearance (n=44) and men with HPV persistence (n=41). The mean (SD) ages for these groups were: 26.4 (4.81), 27.6 (6.49) and 28.9 (6.79) years respectively (Table 4.22). From baseline data, men in HPV persistence group that had significantly higher mean CD8+ T cell count

(p=0.001), mean absolute lymphocyte count (p=0.018), and mean platelet count (p=0.019) compared to HPV uninfected group (Table 4.22).

Table 4.22: Baseline immunologic characteristics of men included in the evaluation of natural cytokine immune response among Fishermen

Characteristic	Mean (SD) or n (%)	OR (95% CI)[a]	P value[b]	P value[c]
Age: [Mean(SD)] years				
HPV uninfected group	26.4 (4.81)	-	Reference	-
HPV CLR group	27.6 (6.49)		0.333	Reference
HPV PST group	28.9 (6.79)		0.045	0.348
White Blood Cells: [Mean (SD)] x 10^3/µL				
HPV uninfected group	5.5 (1.35)	-	Reference	-
HPV CLR group	5.6 (1.67)		0.759	Reference
HPV PST group	6.1 (1.42)		0.052	0.156
Absolute Lymphocytes: [Mean (SD)] x 10^3/µL				
HPV uninfected group	2.32 (0.647)	-	Reference	-
HPV CLR group	2.38 (0.870)		0.726	Reference
HPV PST group	2.68 (0.741)		0.018	0.094
CD4: [Mean (SD)] cells/µL				
HPV uninfected group	927 (355.8)	-	Reference	-
HPV CLR group	793 (301.8)		0.062	Reference
HPV PST group	762 (381.0)		0.043	0.688
CD8: [Mean (SD)] cells/µL				
HPV uninfected group	602 (272.9)	-	Reference	-
HPV CLR group	685 (388.3)		0.249	Reference
HPV PST group	884 (446)		0.001	0.033
Absolute Neutrophils: [Mean (SD)] x 10^3/µL				
HPV uninfected group	2.19 (0.802)	-	Reference	-
HPV CLR group	2.13 (0.846)		0.736	Reference
HPV PST group	2.24 (1.034)		0.810	0.609
Platelets: [Mean (SD)] x 10^3/µL				
HPV uninfected group	199 (71.6)	-	Reference	-
HPV CLR group	194 (64.6)		0.788	Reference
HPV PST group	236 (74.5)		0.019	0.008
HIV Infection: n (%)				
HPV uninfected group	6 (12)	Reference	Reference	-
HPV CLR group	13 (30)	2.36 (0.98 – 5.68)	0.044	Reference
HPV PST group	17 (41)	3.34 (1.44 – 7.62)	<0.002	0.2508
HSV-2 Infection: n (%)				
HPV uninfected group	27 (54)	Reference	Reference	-
HPV CLR group	26 (59)	1.09 (0.77 – 1.56))	0.619	Reference
HPV PST group	25 (61)	1.13 (0.79 – 1.61)	0.503	0.859
Cytokine Response at study exit: n (%)				
HPV uninfected group	11 (22)	Reference	Reference	-
HPV CLR group	28 (64)	6.20 (2.29 – 17.19)	<0.0001	Reference
HPV PST group	21 (51)	3.72 (1.37 – 10.25)	0.0037	0.247

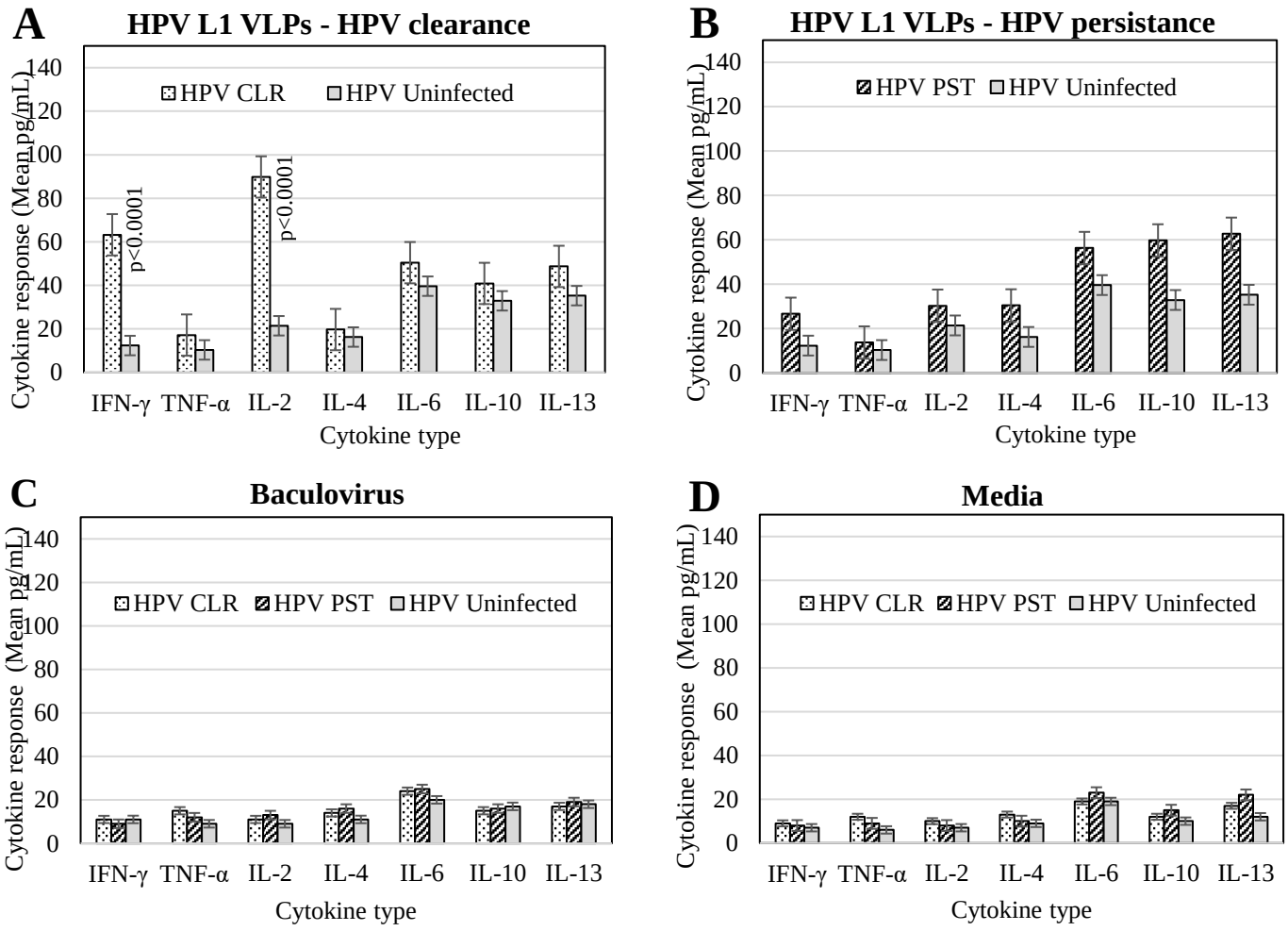
a Odds Ratio (OR) computed for binary variables only
b P-values computed for HPV clearance (HPV CLR) group (n=44) and HPV persistence (HPV PST) group (n=41) in comparison to HPV uninfected group (n=50)
c P-values computed for HPV PST group in comparison to HPV CLR group

Moreover, mean platelet count and mean CD8+ T cell count were significantly higher among men with HPV persistence compared to those with HPV clearance. The rest of baseline immunological characteristics were not significantly different between the three groups. Compare to HPV uninfected men, men with HPV persistence were more likely to have HIV infection; OR=3.34 (95% CI: 1.44 – 7.62). Therefore, analysis stratified by HIV status showed that compared to men without HIV infection (665.9 cells/mL), the mean CD8+ T cell count was significantly ($p<0.0001$) higher among men with persistent HPV, co-infected with HIV (1213.3 cells/mL). In this analysis, Mean platelet counts were not significantly different ($p=0.803$) between HIV negative (238.5×10^6 cells/mL), compared to HIV positive (232.5×10^6 cells/mL) men with persistent HPV infection.

Of the 135 men studied, positive cytokine immune response induced by L1 VLP stimulation was detected in 60 men (44%; 95% CI: 36.3 – 52.9). The proportion of immune response among HPV uninfected men was 22% (95% CI: 12.8 – 35.2), in HPV clearance group was 64% (95% CI: 48.9 – 76.2) and in HPV persistence group was 51% (95% CI: 36.5 – 65.8) (Table 4.22). Compared to men without HPV infection, men with HPV clearance were six times (OR=6.20; 95% CI: 2.29 – 17.19) more likely to mount induced cytokine immune responses to L1 VLPs. Additionally, men with HPV persistence had increased odds (OR=3.72; 95% CI: 1.37 – 10.25) of inducing cytokine responses compared to HPV uninfected men.

Of the eight cytokines evaluated in supernatants harvested from L1 VLP treated PBMC cultures, levels of IL-5 were below detection limit in all analyzed specimens excluding it from further analysis. Of the remaining seven cytokines, two were significantly ($p<0.0001$) upregulated among men with HPV clearance compared to HPV uninfected men (Figure 4.22A). These included: IFN-γ (5.1-fold) and IL-2 (4.2-fold) cytokines (mean n-fold increases). Among men with HPV clearance compared to those with persistent HPV infection, IFN-γ (2.4–fold) and IL-2 (3.0–fold) were the only cytokines significantly ($p<0.0001$) upregulated. IL-4 (1.5-fold, $p=0.021$) and IL-10 (1.5–fold, $p=0.011$) were upregulated among men with persistent HPV infection compared to men in the HPV clearance group, but never reached statistical

significance after correcting for multiple measurements. Between the three groups of men, there were no significant changes in cytokine levels between antigenic control (Figure 4.22C) and media from unstimulated (Figure 4.22D) cell cultures, indicating that observed changes in immune response were HPV-specific.



The key represents: HPV clearance (HPV CLR) group, n=28; HPV persistence (HPV PST) group, n=21; and HPV uninfected group, n=11. Bars represent mean ± standard error of the mean (SEM). Independent t-test was used to determine statistical significance. Panel A: HPV CLR group compared to HPV uninfected men; Panel B: HPV PST group compared to HPV uninfected men; Panel C: Baculovirus stimulated PBMCs for HPV CLR, PST and uninfected groups of men (as antigenic control); and Panel D: Unstimulated PBMCs for HPV CLR, PST and uninfected groups (negative control).

Figure 4.22: Cytokine response among Fishermen with HPV CLR or PST compared to unifected men

In the HPV clearance group, TNF-α (1.7-fold, p=0.009) and IL-13 (1.4-fold, p=0.032) were upregulated, but these changes in cytokine levels did not reach statistical significance after correction for multiple measurements (Table 4.23). Changes in cytokine levels for IL-4, IL-6, and IL-10 were not statistically different.

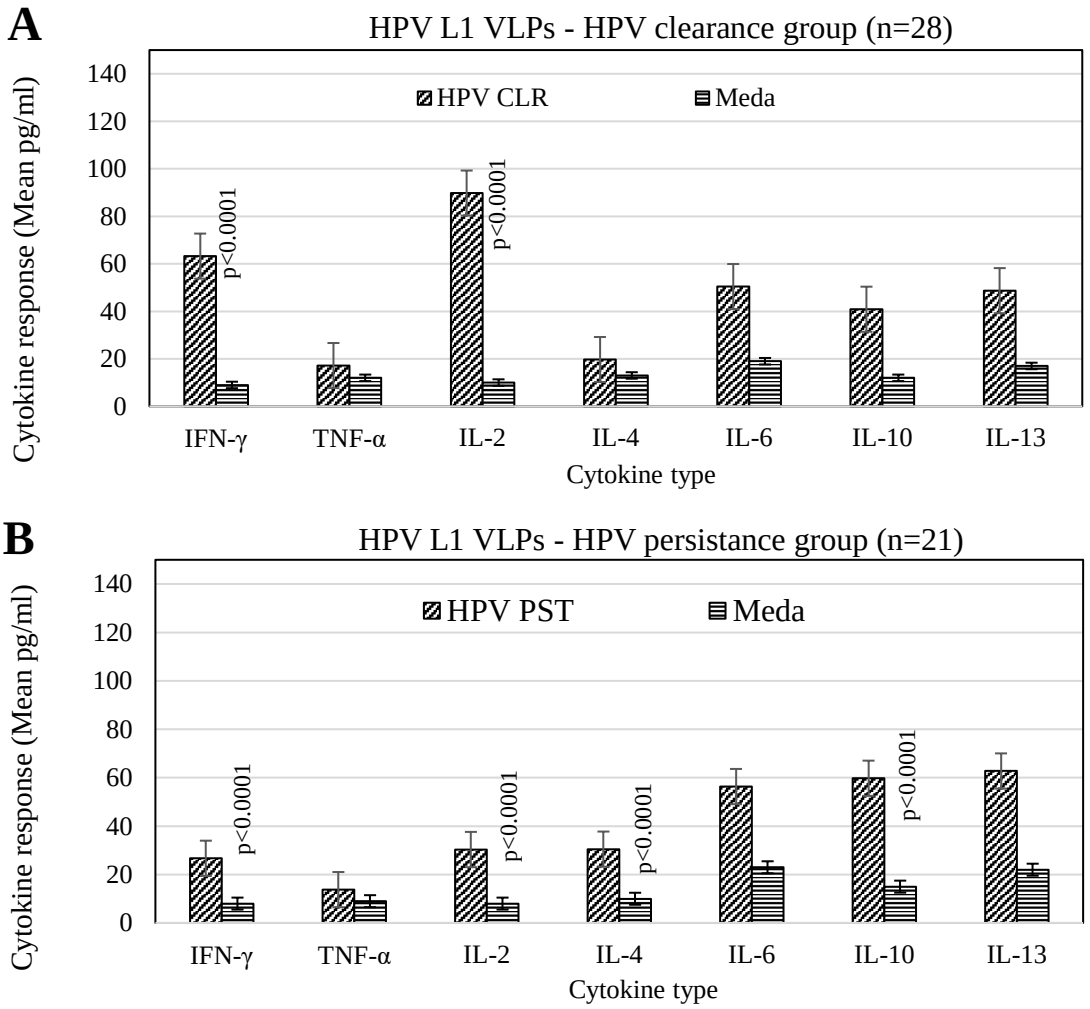
Table 4.23: Comparison of mean cytokine levels detected among Fishermen:

Cytokine	Mean (95% CI) pg/mL	± SD	p-value[(a)]	p-value[(b)]
IFN-γ				
HPV Uninfected	12.4 (8.34-16.39)	5.99	Ref	----
HPV CLR	63.3 (55.17-71.33)	20.83	<0.0001	<0.0001
HPV PST	26.7 (21.17-32.26)	12.19	0.008	Ref
TNF-α				
HPV Uninfected	10.4 (7.71-13.02)	3.96	Ref	----
HPV CLR	17.2 (14.15-20.21)	7.81	0.009	0.1141
HPV PST	13.8 (10.72-16.80)	6.68	0.133	Ref
IL-2				
HPV Uninfected	21.5 (16.62-26.29)	7.20	Ref	----
HPV CLR	89.8 (76.92-102.65)	33.17	<0.0001	<0.0001
HPV PST	30.3 (23.59-37.07)	14.81	0.072	Ref
IL-4				
HPV Uninfected	16.3 (13.05-19.50)	4.80	Ref	----
HPV CLR	19.7 (15.25-24.18)	11.52	0.347	Ref
HPV PST	30.5 (21.50-39.45)	19.91	0.026	0.021
IL-6				
HPV Uninfected	39.6 (30.23-49.04)	14.00	Ref	----
HPV CLR	50.4 (41.37-59.49)	23.36	0.162	Ref
HPV PST	56.3 (43.42-69.24)	28.36	0.077	0.428
IL-10				
HPV Uninfected	32.9 (26.95-38.87)	8.87	Ref	----
HPV CLR	40.9 (34.04-47.75)	17.68	0.164	Ref
HPV PST	59.8 (45.20-74.33)	32.00	0.011	0.011
IL-13				
HPV Uninfected	35.3 (27.25-43.30)	11.94	Ref	----
HPV CLR	48.7 (41.57-55.85)	18.41	0.032	Ref
HPV PST	62.8 (49.10-76.43)	30.02	0.007	0.049

(a) p-values for independent t-test of mean cytokine levels between HPV CLR (n=28) or HPV PST (n=21) groups and HPV uninfected (n=11) group

(b) p-values for independent t-test of mean cytokine levels between HPV CLR and HPV PST group

Compared to culture media from unstimulated cells, IFN-γ (7.0–fold) and IL-2 (9.0–fold) were the only cytokines significantly ($p<0.0001$) upregulated among men who cleared HPV infections after correcting for multiple measurements (Figure 4.23A). IFN-γ (3.3–fold), IL-2 (3.8–fold), IL-4 (3.0–fold) and IL-10 (4.0–fold) were significantly ($p<0.0001$) upregulated among men with persistent HPV infection compared to media (Figure 4.23B).



The key represents: HPV clearance (HPV CLR) group and HPV persistence (HPV PST) group, and culture media from unstimulated cells. Bars represent mean ± standard error of the mean (SEM). Independent t-test was used to determine statistical significance. Panel A: HPV CLR group compared to media; Panel B: HPV PST group compared to media.

Figure 4.23: Cytokine response among Fishermen with HPV CLR or PST compared to media

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussions of Study Findings

5.1.1 Epidemiology of HPV, HIV and HSV-2 infection among Fishermen

5.1.1.1 HPV infections among Fishermen in Kisumu, Kenya

5.1.1.1.1 Prevalence, Persistence and Incidence of HPV infection among Fishermen

The baseline HPV prevalence of 50% found in this study was consistent with those found among men in Kisumu Kenya: 58% among uncircumcised fishermen (Ng'ayo *et al.,* 2008a), 51% among young uncircumcised men (Smith *et al.,* 2010a), and 54% among young men (Smith *et al.,* 2007). The high proportion of men (60%) infected with multiple HPV genotypes that was observed in this study has similarly been reported among men in several other studies; 73% (Ng'ayo *et al.,* 2008a), 51% (Lajous *et al.,* 2005) and 42% (Nielson *et al.,* 2007). Strikingly, the proportion of fishermen (75%) infected with HR-HPV demonstrated in this study was very similar to 74.1% found among HIV positive heterosexual men in Uganda (Tobian *et al.,* 2013).

The HPV incidence of 53.3 per 100 person-years observed in this study was consistent with those observed elsewhere. Few studies have evaluated the incidence and persistence of HPV infections among men, and found that 13.8% to 22.7% of men who tested negative for HPV at baseline, acquired new HPV infection (i.e., tested positive) during follow-up (Van Doornum *et al.,* 1994; Wikstrom *et al.,* 2000; Kjaer *et al.,* 2005; Lajous *et al.,* 2005). The definition of persistence varied by study and persistence occurred in 6%–11% of men in the study or in 50%–57.5% of men with HPV infection at the baseline visit (Van Doornum *et al.,* 1994; Wikstrom *et al.,* 2000; Kjaer *et al.,* 2005; Lajous *et al.,* 2005).

Van Doornum *et al.* (1994), in their prospective HPV study of heterosexual men and women found an incidence density of 50.5 per 100 person-years among military men

in Mexico (Van Doornum *et al.,* 1994). In the same study HPV was found to have intermediate persistence in 6% of HPV positive men. In a recent longitudinal study, Lajous *et al.* (2005), detected same HPV type in 58 (11%) of 336 men one year after the initial detection (Lajous *et al.*, 2005), translating to 29% of 126 men who had HPV infection at baseline. They further demonstrated an incidence of 17.9/1000 men-months during the one year follow-up period – with HPV detection being done at baseline and one year later. However, the work done by Lajous *et al.* (2005), did not consider the fact that some HPV incident infections are cleared within just a few months. Therefore, there is a possibility that Lajous *et al.* (2005), could have missed out some new HPV infections in this study leading to an underestimation of HPV incidence among these men. Wikstrom and coauthors reported HPV persistence of at least one HPV type in seven of 14 HPV positive men (Wikstrom *et al.,* 2000). However, the follow-up time duration was not defined in this study. In yet another longitudinal study of men followed up for a period of 6-8 months, Kjaer *et al.* (2005), found that HPV persisted in 42 (58%) of 72 men testing positive for a known HPV type at baseline (Kjaer *et al.*, 2005).

Natural history studies of the cervical HPV infections have clearly demonstrated that HPV persistence is a preliminary step toward advanced neoplastic changes, thereby persistence in men is thought to have implications for their own cancer risk as well as becoming an important HPV reservoir for women (Moscicki *et al.,* 2006). Only a few studies have evaluated persistence of HPV infections among men, recording a persistence of 29% to 67% after 6–12 months of follow-up (Van Doornum *et al.,* 1994; Wikstrom *et al.,* 2000; Kjaer *et al.,* 2005; Lajous *et al.,* 2005; Silva *et al.,* 2011; Giuliano *et al.*, 2011b). However, the question whether adaptive immunity is involved in preventing future HPV infections among men has not been explored.

5.1.1.1.2 Risk factors for HPV Prevalence, Incidence and Persistence among Fishermen

Factors associated with HPV prevalence

In this study, marital status (married), HIV infection, low baseline CD4 count and history of STI were independently associated with increased odds of baseline prevalent HPV infection. The finding that married men were more likely to have additional sexual partners apart from their wives could explain why being married was found to a major risk factor for prevalent HPV infection. Circumcision and consistent condom use was found to be associated with reduced odds of HPV infection at baseline. Measures of sexual behavior have been associated with HPV infection in men. Several cross-sectional studies found risk factors related to sexual behavior, including young age at first sexual intercourse (Smith *et al.,* 2007; Castellsague *et al.,* 1997); a greater number of regular, lifetime, and recent sex partners (Svare *et al.,* 2002; Smith *et al.,* 2007; Smith *et al.,* 2010a); and a greater number of sex partners before and during marriage (Castellsague *et al.,* 1997; Castellsague *et al.,* 2002). In addition, female sex partners' lifetime number of sex partners (Castellsague *et al.,* 2002) and a high frequency of sexual intercourse (Baldwin *et al.,* 2004) have been shown to be associated with HPV detection. However, only two of these studies included a multivariate analysis that adjusted for confounding (Koutsky *et al.,* 1997; Jamal *et al.,* 2003). Longitudinal studies found that anal intercourse with men (Lajous *et al.,* 2005) and having had ≥2 sex partners were independently associated with HPV acquisition (Kjaer *et al.,* 2005) and HPV infection (Smith *et al.,* 2007).

This study found high risk sexual activity including unprotected sex as the primary risk factor for HPV infection. Although condoms may not prevent HPV infections to uncovered genital anatomical sites (Bleeker *et al.,* 2005), this study demonstrated that consistent use of condom (>75% of the time) was significantly protective ($p<0.0001$) against HPV infections. In agreement with results from this study, Baldwin *et al* (2004) found a significant reduction in the risk of HPV infection in men who used condoms consistently (Baldwin *et al.,* 2004). Consistent condom use during the preceding three months was associated with a decreased risk of both any and HR-HPV infection; condom use during the last occurrence of anal sex in the same study

was associated with a decreased risk of low-risk HPV infection (Baldwin *et al.*, 2004). Another study demonstrated that condom use significantly reduced the risk of HPV in circumcised men but not in uncircumcised men (Castellsague *et al.*, 2002). However, these results were not replicated in a few cross-sectional studies exploring the effect of condom use on protection against HPV among men (Franceschi *et al.*, 2002; van der Snoek *et al.*, 2003; Smith *et al.*, 2007).

Nonetheless, condoms have been shown to offer some protection against HPV associated diseases. A recent prospective randomized clinical trial studying the effect of condom use on the regression of CIN lesions found a 2-year cumulative CIN regression rate of 53% in the "condom" group and 35%in the "non-condom" group; and 2-year cumulative HPV clearance rate of 23% in the condom group and 4% in the non-condom group (Hogewoning *et al.*, 2003).

The effect of circumcision was evaluated in 5 cross-sectional studies (Castellsague *et al.*, 2002; Svare *et al.*, 2002; Xi *et al.*, 2002; Baldwin *et al.*, 2004; Hernandez *et al.*, 2008b), four of which found, after adjusting for confounding factors, that circumcision was associated with a statistically significant lower risk of HPV infection and or persistence (Castellsague *et al.*, 2002; Svare *et al.*, 2002; Baldwin *et al.*, 2004; Hernandez *et al.*, 2008b). The statistically significant reduction in risk ranged from 20% to 48%. Two longitudinal studies found that self-reported circumcision was independently associated with a reduced risk of HPV persistence (Kjaer *et al.*, 2005; Lajous *et al.*, 2005).

Few cross-sectional studies have evaluated factors associated with the detection of high-risk versus low-risk HPV types (Svare *et al.*, 2002; Baldwin *et al.*, 2004); only one study adjusted for confounding factors (Baldwin *et al.*, 2004). Factors associated with high-risk HPV types were young age (Svare *et al.*, 2002), a higher lifetime number of sex partners (Svare *et al.*, 2002), and higher frequency of sexual intercourse (Baldwin *et al.*, 2004). High-risk HPV types were less likely to be detected in circumcised men or in those who had used condoms consistently during the preceding 3 months (Baldwin *et al.*, 2004).

Factors associated with the detection of low-risk HPV types were young age (Svare *et al.,* 2002), a history of genital warts (Svare *et al.,* 2002), the presence of genital warts (Baldwin *et al.,* 2004), and the number of sex partners during the preceding year (Svare *et al.,* 2002). Low-risk HPV types were less likely to be detected in circumcised men and in those who had used condoms during their last occurrence of anal sex (Baldwin *et al.,* 2004).

Factors associated with HPV incidence

This study observed that baseline HPV infection and multiple sexual partners within the year preceding the study were independent risk factors for acquisition of new HPV infection, while condom use was protective. These findings were consistent with results from previous studies (that had varied duration of follow-up) which prospectively evaluated HPV infection among men (Van Doornum *et al.,* 1994; Wikstrom *et al.,* 2000; Kjaer *et al.,* 2005; Lajous *et al.,* 2005). Independent predictors of HPV acquisition in multivariate analysis were found to be anal sex with other men (Lajous *et al.,* 2005) and having had ≥3 sex partners since the last clinic visit (Kjaer *et al.,* 2005). Factors independently associated with a reduced risk of HPV acquisition were consistent or occasional condom use (Kjaer *et al.,* 2005) and high socioeconomic status (Lajous *et al.,* 2005). Therefore, the risk factors for incident HPV infection among men apart from baseline HPV were factors related to high-risk sexual behavior during study follow-up: number of recent sexual partners and condom use with recent sexual partners. However, the protective effect of condom against HPV infection in both men and women still remain controversial. Results from this study are in agreement with some (Hippela¨inen *et al.,* 1993; Baldwin *et al.,* 2004), but in contrast with others (Franceschi *et al.,* 2002). Notably, the multifocal nature of genital HPV infections in both men and women to sites otherwise covered by condom during foreplay or sexual intercourse may explain these controversial findings. The finding in this study that infection with multiple HPV types at enrollment was an important risk factor for subsequent acquisition of a new HPV type and even a greater risk of acquiring multiple new HPV types was also observed elsewhere among men (Kjaer *et al.,* 2005) and women (Franco *et al.,* 1999; Thomas *et al.,* 2000). This suggested

that risk factors for the initial HPV infection were likely not to change over time predisposing these men to infection with additional new HPV genotypes.

This is the first study to evaluate penile hygiene and acquisition of new HPV infection among men. Hypothetically, routine male genital hygiene before and after sexual intercourse is generally believed to confer reduced risk for acquisition of STIs. The finding that genital washing immediately after sex significantly increased the risk of HPV acquisition among men infected with HIV in this study was unexpected and should be interpreted with caution. However, a few studies have found that genital washing immediately after sexual intercourse significantly increased the risk of HIV acquisition among uncircumcised men (Tobian *et al.,* 2009b; Makumbi *et al.,* 2016). Makumbi *et al*. (2016) documented HIV incidence of 1.34/100 pyr among uncircumcised men who reported washing their penis after intercourse compared to 0.62/100 pyr among uncircumcised men who reported not washing their penis after sex (Makumbi *et al.,* 2016). Tobian *et al*. (2009) also demonstrated a 1.4 infections per 100 pyr among uncircumcised men who reported washing their genitals immediately after sexual intercourse compared to only 0.38 infections per 100 pyr among uncircumcised men who did not wash their genitals after sex (Tobian *et al.,* 2009b). Notably, majority of men (84%) with baseline HIV infection in this study were not circumcised. Conversely, Meier *et al*. (2006) in their cross-sectional study found that genital washing was negatively associated with HIV infection among circumcised men in Kenya (Meier *et al.,* 2006).

However, studies evaluating female genital hygiene practices found that vaginal washing (douching) was associated with increased risk of HIV acquisition (McClelland *et al.,* 2006; Low *et al.,* 2011). These studies suggested that douching (vaginal washing) has several negative implications: First, imbalance of vaginal pH, microbiota and immune molecules that provide protective environment against invading pathogens, which may facilitate viral survival and possible infectivity; Second, the disruption of the delicate mucosal membrane that provide the primary barrier to infection may contribute to micro-ulceration that facilitates HIV infection; and Third, the process may introduce pathogenic organisms in the vaginal canal whose

infection may increase susceptibility to HIV infection. Synonymously, genital washing among uncircumcised men may disrupt the mucosal membranes under the foreskin, creating microscopic cracks that serve as portal of entry for HPV. Hypothetically, the acidic nature of the vaginal fluid and its constituent immune molecules while on the penis could be potentially protective against HPV among uncircumcised men. Therefore, genital washing immediately might be neutralizing this protective effect.

Factors associated with HPV persistence

This study found marital status (married) an independent risk factor for HPV persistence in addition to underlying baseline HPV and HIV infection detected at enrolment. Moreover, the results indicated that baseline infection with multiple HPV genotypes increased the risk of HPV persistence. These findings were consistent with those from other studies among men (Kjaer *et al.*, 2005; Lajous *et al.*, 2005) and among women (Ho *et al.*, 1998) that demonstrated genotypic multiplicity of baseline HPV infection to be an independent risk factor for HPV persistence.

The finding in this study that infection with multiple HPV types was strongly associated with HIV infection, provide biological plausibility that an underlying immune condition in certain individuals may increase susceptibility to multiple and persistent HPV infections. Indeed, HPV infection was associated with low CD4 count in this study and elsewhere (Rowhani-Rahbar *et al*., 2007; Mbulawa *et al*., 2010), suggesting a weaker immune system among HIV positive compared to HIV negative men that could favor persistence of HPV infection. Put together, the findings of this study suggest that HIV infection could be the most important risk factor for HPV persistence. Although epidemiological data strongly supports the evident relationship between HIV and HPV (Williamson 2015; Poljak *et al*., 2017), the biological mechanism through which these two viruses increase associated infection/disease risk are not well understood. In a recent study, HIV-1 Tat protein secreted by HIV infected lymphocytes was linked to increased expression of HPV E6 protein by human uterine cervical carcinoma cells and reduced levels of p53 oncosuppressor protein. (Barillari

et al., 2016), suggesting a direct role of HIV in increasing the risk of developing HPV-related diseases.

Married fishermen participating in this study had increased odds of HPV infection and persistence compared to fishermen who were single. Although this is the first study to show this association for HPV, several studies have found marital status (being married) significantly associated with HIV infection in high HIV endemic regions in Sub Saharan Africa (de Walque *et al.,* 2012; Mohamed *et al.,* 2013; Mkandawire-Valhmu *et al.,* 2013). Studies from low HIV endemic regions have contradicted this association (Morgan *et al.,* 2012; Kposowa *et al.,* 2013). Nevertheless, HIV transmission among married couples has been found to be higher among HIV discordant couples compared to single individuals (Hugonnet *et al.,* 2002; Dunkle *et al.,* 2008; Wabwire-Mangen *et al.,* 2009). The HIV burden is generally higher among women compared to men. Because HPV is more infectious compared to HIV and thus has higher transmission rates, it implies that HPV burden is similarly much higher among women.

Therefore, the high HPV burden among married fishermen observed in this study, likely suggests even a higher HPV burden among their spouses (female sexual partners), leading to increased HPV persistence due to repeated exposure to the same HPV genotypes. Complicated by the high HIV burden and increased risk for both HIV and HPV infection, HPV is likely to have long term persistence among these men and subsequently their spouses. This eventually provides continuous exposure within the couple, elevating the risk of HPV-related diseases and increasing the risk of transmission to extramarital sexual partners. Unlike HIV, HPV acquisition is almost certain among sexually exposed men and women. The observation in this study that married men had their most recent sexual act with a woman other than their wife, coupled with low condom use during this sexual act, supports the finding that married men are at increased risk for infection with multiple HPV genotypes and HPV persistence.

5.1.1.2 Prevalence, Incidence and risk factors for HIV infection among Fishermen

This study presents data from a one year longitudinal follow up of fishermen on the Kenyan beaches of Lake Victoria in Kisumu County. It is one of the very few studies that have evaluated risk factors for HIV acquisition among fishermen. With a baseline HIV prevalence of 23.3%, fishermen remain a subpopulation highly burdened by HIV infection compared to the general population. This was 4-times the national HIV prevalence of 5.6% (KAIS, 2014). Similar high HIV prevalence (of 20 – 28%) among fishermen have been previously reported in Kenya and Uganda (Kwena *et al.,* 2010; Asiki *et al.,* 2011; Kiwanuka *et al.,* 2013; Opio *et al.,* 2013). In comparison to other high-risk groups such as men who have sex with men (MSM), the HIV prevalence in this study was twice that observed in bisexual MSM in Mombasa, Kenya (Sanders *et al.,* 2007). However, the HIV prevalence in this study was lower than 43% observed among exclusive MSM in Mombasa, Kenya (Sanders *et al.,* 2007) and 37% among women with high-risk sexual behavior in Uganda (Vandepitte *et al.,* 2011). The independent risk factors (older age, a higher number of lifetime sexual partners, transactional sex, HPV infection and history of STI) associated with HIV prevalence in this study were also observed in previous studies (Asiki *et al.,* 2011; Vandepitte *et al.,* 2011; Opio *et al.,* 2013).

The HIV incidence rate of 4.2 per 100 person-years among fishermen in this study was similar to rates found in previous studies among male subpopulations considered at higher risk for HIV infection (Xu *et al.,* 2010; Okuku *et al.,* 2011; Zhonghua *et al.,* 2011; Price *et al.,* 2012; Seeley *et al*., 2012; Sanders *et al.,* 2013; Kiwanuka *et al*., 2014). HIV incidence rates of 4.9/100 person-years (5.2/100 person-years among men) (Seeley *et al*., 2012) and 3.4/100 person-years (3.4/100 person-years among men) (Kiwanuka *et al*., 2014) were documented among fishing communities on the shores of Lake Victoria in Uganda. New HIV infection rate of 3.9 per 100 person-years was observed among men reporting high-risk sexual behavior (Okuku *et al.,* 2011) and 5.8 per 100 person-years among bisexual MSM (Sanders *et al.,* 2013) in Costal Kenya. Similarly, HIV incidence of 6.1 per 100 person-years was found among exclusive MSM in Mombasa, Kenya (Price *et al.,* 2012), while 3.4 per 100 person-

years (Zhonghua *et al.,* 2011) and 5.4 per 100 person-years (Xu *et al.,* 2010) observed among MSM in China. Moreover, HIV acquisition rate of 5.8 per 100 person-years was shown among patients and commercial sex workers attending STI clinics in Pune, India (Reynolds *et al.,* 2003) and 3.66 per 100 person-years observed among female sex workers in Kampala, Uganda (Vandepitte *et al.*, 2013).

HIV incidence in this study was higher compared to 2 per 100 person-years and 1.4 per 100 person-years observed among uncircumcised men drawn from the general population in Kisumu, Kenya (Mehta *et al.,* 2012) and Rakai, Uganda (Tobian *et al.,* 2009b) respectively, 0.9 per 100 person-years among heterosexual men in Mombasa, Kenya. Similar contrasting lower incidence rates were observed among high-risk men in low HIV prevalence countries of: 1.24 per 100 person-years among MSM in Victoria, Australia (Guy *et al.,* 2011) and 1.9 per 100 person-years among MSM in six US cities (Brown *et al.,* 2006). This suggests that the sexual behavior of fishermen in this study is comparable to that of men and women with high-risk sexual behavior living in high HIV prevalence communities.

Independent risk factors for HIV acquisition among fishermen in this study were similar to those of previous studies that have assessed factors associated with incident HIV infection. As in previous studies; young age (Seeley *et al.,* 2012; Vandepitte *et al.,* 2013), being single (Tobian *et al.,* 2009b; Sanders *et al.,* 2013), recent sex with sex worker/casual partner (Sutcliffe *et al.,* 2009; Guy *et al.,* 2011) and unprotected sex with new sexual partners (Brown *et al.,* 2006; Guy *et al.,* 2011; Sanders *et al.,* 2013; Vandepitte *et al.,* 2013) were significant independent factors associated with new HIV infection. Surprisingly, incident HIV infection was not associated with baseline HSV-2 infection in our study. This finding was largely unexpected given the high baseline HSV-2 (56.3%) prevalence in this study of fishermen with high risk sexual behavior, and the strong association between HIV and HSV-2 at baseline. However, similar results were documented by Mehta *et al*. in their Kisumu circumcision trial (Mehta *et al.,* 2012). In contrast, other previous studies found baseline HSV-2 infection, a significant risk factor for HIV acquisition in men (Brown *et al.,* 2006; Tobian *et al.,* 2009b; Sobngwi-Tambekou *et al.,* 2009).

Although, evaluation of risk factors for HIV incidence had study power limitations which confined analysis to the eight seroconversion events that occurred in the study, the observed increased risk for new HIV acquisition among young single men was not surprising. In this study, single men were 3 times as likely as married men to report a new sexual partner(s) in the past 12 months. In the effort to provide explanation to why MSM are at increased risk for HIV compared to heterosexual men, Glick *et al*. (2012) found that 86% of young MSM (18 – 24 years) compared to 56% of young heterosexual men (18 – 24 years) acquired a new sexual partner in the year prior to study participation (Glick *et al.,* 2012). This was similar to 83% observed in this study among younger single fishermen compared to 57% among married fishermen who formed a new sexual partnership in the same period of time. This similarity in addition to low condom use with new sexual partners may partially explain the higher risk for HIV incidence among young single fishermen.

STIs inducing recruitment and activation of CD4+ T-lymphocytes into clinical (subclinical) mucosal lesions associated with disrupted mucosal membrane likely increase the risk of HIV acquisition (Gupta *et al.,* 2007; Rebbapragada *et al.,* 2007). Earlier studies observed that HIV seroconversion was associated with incident STI infections: syphilis (Xu *et al.,* 2010; Guy *et al.,* 2011), HSV-2 (Reynolds *et al.,* 2003; Brown *et al.,* 2007; Tobian *et al.,* 2009b; Mehta *et al.,* 2012), *Mycoplasma genitalium* (Vandepitte *et al.,* 2013) and gonorrhea (Sanders *et al.,* 2013). This suggests that these infections potentially increase susceptibility to HIV infection, but more importantly that they share a mode of transmission and thus are biological markers for exposure to unprotected sexual behavior. New, current, or history of STI are strong indicators of previous or current exposure to unsafe sex.

Subramanian *et al.* (2013) documented a significant decline in STI prevalence and stable HIV prevalence in India. This was due to a large scale HIV prevention program targeting safer sexual practices among high-risk MSM in high-prevalence state of Tamil Nadu (Subramanian *et al.,* 2013). Two recent randomized clinical trials successfully demonstrated that antiretroviral drugs can be used for HIV prevention. The Partners Pre-exposure prophylaxis (PrEP) trial showed that tenofovir-

emtricitabine (TDF-FTC) conferred 75% protection against HIV acquisition by the HIV negative spouses in HIV discordant heterosexual couples (Baeten *et al.*, 2012). Cohen *et al.* (2011) demonstrated a 96% reduction in linked HIV transmission due to the effect of early ART (irrespective of CD4 count level or WHO staging) compared to delayed therapy administered to HIV positive spouses among heterosexual HIV discordant couples (Cohen *et al.*, 2011). A global rollout of a multipronged program integrating PrEP, treatment for prevention (early ART) and safer sexual practices in groups with high-risk sexual behavior may reduce the incidence of HIV and other STIs. However, feasibility and acceptability studies for use of HIV antiretroviral drugs among fishermen and other subpopulations with high risk sexual behavior are urgently required. These will inform formulation of early ART and PrEP programs targeted at these key populations in the HIV transmission triad.

5.1.1.3 Prevalence, Incidence and risk factors for HSV-2 infections among Fishermen

Fishermen in this study had a higher burden of HSV-2 infection. The estimated baseline HSV-2 prevalence of 56% was over two times higher than the estimated HSV-2 prevalence of 26.3% for men drawn from the general national adult population in Kenya (KAIS, 2009; Mugo *et al.,* 2011), 26.5% among young men in Kisumu Kenya (Mehta *et al.,* 2012), 22% among high risk men for HIV in Mombasa Kenya (Okuku *et al.,* 2012) and 20.3% among MSM in the US (Brown *et al.,* 2006). Similar high prevalence (43-80%) was previously observed among fishermen along Lake Victoria in Kenya (Seeley *et al.,* 2012), patients attending STD clinic in India (Reynolds *et al.,* 2003), women at high risk for HIV infection in Mozambique (Meque *et al.,* 2014) and female sex workers in Mombasa Kenya (Chohan *et al.,* 2009; Okuku *et al.,* 2011). Risk factors for prevalent HSV-2 infection found in this study (older age, marital status, inconsistent condom use, history of STI, and HIV infection) were consistent with results from other studies (Reynolds *et al.,* 2003; Chohan *et al.,* 2009; Okuku *et al.,* 2012; Seeley *et al.,* 2012).

The HSV-2 incidence rate of 23.6/100 pyr found in this study, to our knowledge is the highest for HSV-2 among men observed anywhere in the world. Similar HSV-2 incident rates have only been documented among women with high risk sexual behavior. Recent studies found HSV-2 incidence rate of 23/100 pyr among female sex workers in Mombasa Kenya (Chohan *et al.,* 2009), 21.9 /100 pyr among female sex workers in China (Wang *et al.,* 2013), 22.1/100pyr among women at high risk for HIV in Mombasa Kenya (Okuku *et al.,* 2012) and 20.5/100 pyr among women with high risk sexual behavior in Beira Mozambique (Meque *et al.,* 2014). HSV-2 incidence of 11.4/100 pyr was shown among patients attending STD clinic in India (Reynolds *et al.,* 2003) and 9.0/100 pyr found among men at high risk for HIV infection in Mombasa Kenya (Okuku *et al.,* 2012).

The rate of new HSV-2 infection in this study was almost 3-5 times that observed (4.9 – 7.4 /100 pyr) in studies evaluating HSV-2 incidence among young men, adolescents and young men drawn from the general population (Tobian *et al.,* 2009a; Sudenga *et*

al., 2012; Mehta *et al.,* 2012). Additionally, this study found much higher HSV-2 incidence rate compared to 2.4/100 pyr (with HIV negative partner) – 5.0 /100 pyr (with HIV positive partner) for all anal intercourse observed among MSM in the US (Brown *et al.,* 2006) and 4.0/100 pyr among injection drug users (IDU) in Chiang Mai, Thailand (Sutcliffe *et al.,* 2009).

In this study, engagement in transactional sex had a borderline association (p=0.047) with HSV-2 incidence, although the majority of fishermen reported recent transactional sexual activity. Transactional sex work as a risk for HSV-2 acquisition was also observed in other studies (Reynolds *et al.,* 2003; KAIS, 2009). Moreover, recent sex with casual partners, multiple sexual partners and inconsistent condom use; found to be independently associated with HSV-2 incidence were also demonstrated elsewhere (Reynolds *et al.,* 2003; Brown *et al.,* 2006; Sudenga *et al.,* 2012; Meque *et al.,* 2014). A greater number (≥3) of sexual partners in the 12 months prior to study participation was a stronger risk factor for HSV-2 acquisition compared to higher number (≥6) of lifetime sexual partners. Similarly, Brown *et al*. (2006) found an increased risk of HSV-2 acquisition strongly associated with a higher number of sexual partners (≥6) and multiple (≥5) unprotected sexual acts, six months prior to study participation (Brown *et al.,* 2006). This emphasized that it is not just a temporary sequence of events cumulated in a lifetime but rather the intensity of high-risk sexual events occurring in the period immediately preceding HSV-2 seroconversion.

This is the first study to assess HPV infection as a risk factor for incident HSV-2 infection. Therefore, the association between persistent HR HPV and new HSV-2 infection observed in this study warrant further studies and should be interpreted with caution. However, several studies have found that HPV infection increases the risk for HIV acquisition by 1.8 – 3.5 times (Chin-Hong *et al.,* 2009; Averbach *et al.,* 2010; Smith *et al.,* 2010b). Lissouba *et al*. (2013) in their meta-analysis review found that HR HPV infection was associated with almost a 2-fold increased risk for incident HIV infection, while LR HPV infection had only a borderline association (Lissouba *et al.,* 2013). Hypothetically, persistent HR HPV infection is biologically necessary for development of HPV disease that may be associated with breach of epithelial

membranes and infiltration with pro-inflammatory cytokines and other immune cells. These potentially provide enabling environment for HSV-2 or HIV entry and replication. Just like HSV-2, HPV is believed to be more infectious than HIV with prevalence always exceeding that of HIV and the most infectious of the three viruses (Veldhuijzen *et al.,* 2010). Therefore, if the interaction between HSV-2 and HPV found in this study is confirmed by other studies, HPV vaccination would be a recommended strategy for HSV-2 prevention in addition to safer sexual practices.

HSV-2 and HIV have a common route for transmission and therefore shared common factors on high-risk sexual behavior. Numerous prevalence studies have shown a strong association between the two sexually transmitted viral infections (Mbopi-Keou *et al.,* 2003). Several incidence studies have found prevalent (baseline) HSV-2 infection an important risk factor for new HIV acquisition, with the strongest association emerging between HIV incident and recent or incident HSV-2 infection (Reynolds *et al.,* 2003; Ramjee *et al.,* 2005; Brown *et al.,* 2006; Sobngwi-Tambekou *et al.,* 2009; Okuku *et al.,* 2011). In these studies, the majority of incident HSV-2 infection events occurred concurrently with or close to, the HIV seroconversion events. Therefore, the high HSV-2 incidence rate observed in this study is likely a strong indicator for a rapidly growing HIV epidemic in this population.

5.1.2 Molecular characterization of genotypic HPV infections among Fishermen

High-risk HPV genotypes HPV-68b, HPV-16 and HPV-58 were the most prevalent oncogenic HPV detected among studied fishermen. Apart from HPV-68b, these results were consistent with other studies which found that HPV-16 (Ng'ayo *et al*., 2008a; Giuliano *et al*., 2008b; He *et al*., 2009; Vardas *et al*., 2011; Rositch *et al*., 2012a) and HPV-58 (He *et al*., 2009; Wei *et al*., 2016) were among the most prevalent high-risk HPV genotypes among men in Kisumu, Kenya and elsewhere. Different from this study, other studies found HPV-52 (Ng'ayo *et al*., 2008a; Wei *et al*., 2016), HPV-18 (Ng'ayo *et al*., 2008a; He *et al*., 2009), HPV-56 (Vardas *et al*., 2011; Rositch *et al*., 2012a), HPV-51 (Giuliano *et al*., 2008b; Vardas *et al*., 2011), HPV-59 & 66 (Giuliano *et al*., 2008b), HPV-35 (Ng'ayo *et al*., 2008a) and HPV-39 (Wei *et al*., 2016), also to be among the most common high-risk HPV genotypes detected in men. In this study, HPV-6 was among the most prevalent LR-HPV genotypes. This observation was consistent with other studies among fishermen (Ng'ayo *et al*., 2008a) and uncircumcised men (Rositch *et al*., 2012a) in Kisumu, Kenya.

Based on epidemiological studies assessing prevalence of different HPV genotypes in HPV-related cancers, HPV-16 and 18 have been associated with 70% of invasive cervical cancer (ICC) and in combination the 7 high-risk HPV genotypes included in the nonavalent HPV vaccine (HPV genotypes–16, 18, 31, 33, 45, 52 and 58) have been associated with 90% of ICC (de Sanjose *et al*., 2010; Mosciski *et al*., 2011; Serrano *et al*., 2012; Serrano *et al*., 2014). However, there is no direct biological data on the relative intrinsic viral oncogenic potential between different high-risk HPV genotypes that could suggest that HPV-16 and HPV18 are more oncogenic compared to other high-risk HPV genotypes.

Although genotypic HPV distribution documented among patients with HPV-related disease suggest that HPV-16 followed by HPV-18 and subsequently (HPV-45, HPV-31, HPV-33, HPV-35, HPV-52 and HPV-58) are the most dominant high-risk HPV genotypes detected in ICC patients, similar prevalence trends have been observed in women with normal cytology or in individuals without HPV-related malignancies (de

Sanjose *et al*., 2007; Bruni *et al*., 2010), except for HPV-58 that has been detected as the second commonest genotype among HIV infected women (Clifford *et al*., 2006). Therefore, immune evasion ability (viral persistence) or immune system susceptibility (viral clearance) differences between HPV genotypes rather than intrinsic oncogenicity could explain detection of some high-risk HPV genotypes more frequently than other genotypes among individuals with HPV disease.

Fishermen in this study were more likely to be detected with multiple HPV genotypes, consistent with other studies among men and women (Sjoeborg *et al*., 2010; Rositch *et al*., 2012a, Yang *et al*., 2014). This study and many others found that HIV infection was a strong risk factor for concurrent multiple HPV genotype infections, genotypic HPV persistence and have also documented increased risk for cancer and HPV-associated malignancies among HIV infected patients (Frisch *et al*., 2000; Moscicki *et al.,* 2001; Moscicki *et al.,* 2004a; Moscicki *et al.,* 2004b; Palefsky *et al.,* 2006; Palefsky *et al.,* 2009, de Pokomandy *et al*., 2009). These findings underscore the importance of robust immune responses against HPV: during infection, persistence and subsequent development of HPV disease.

The probability of genotypic co-infections among fishermen infected with 9vHPV vaccine genotypes was random and no specific pattern or genotypic clustering was observed. Infection with one genotype does not preclude infection with other HPV genotypes. Several positive interactions and no associations between HPV genotypes were observed in this study. Other studies reported similar findings among patients attending STI clinics or the general population (Chaturvedi *et al*., 2011; Rositch *et al*., 2012a; Rositch *et al*., 2012b; Vaccarella *et al*., 2013). However, these positive genotypic associations are of little consequence because they seldom result into type replacement.

Type-specific negative interactions between HPV genotypes were rare among fishermen, with only HPV-70 being negatively associated with HPV-52 and HPV-58. Therefore type competition between HPV genotypes is highly unlikely among fishermen. Similar findings have been documented in other studies (Chaturvedi *et al*., 2011; Rositch *et al*., 2012a; Palmroth *et al*., 2012; Rositch *et al*., 2012b; Querec *et al*.,

2013; Mollers *et al*., 2014; Yang *et al*., 2014; Nie *et al*., 2016). This suggests minimal potential of HPV genotype replacement with non-vaccine genotypes in the event of implementing a vaccination program among fishermen using the 9vHPV vaccine (which also covers 4vHPV and 2vHPV vaccine genotypes).

Despite this emerging evidence of no potential for genotype replacement following HPV vaccination, it is evident from this study and others (Sjoeborg *et al*., 2010; Rositch *et al*., 2012a; Yang *et al*., 2014) that HPV infection is characterized with simultaneous existence of multiple genotypes, and infection with one genotype does not reduce/eliminate the probability of infection with other genotypes. Studies evaluating cross-reactivity and cross-protection of immune responses to HPV infection against other heterologous HPV genotypes have yielded conflicting results (Wideroff *et al*., 1999; Marais et al., 2000; Combita *et al*., 2002; Rositch *et al*., 2012b), with those reporting cross-reactivity in natural HPV infection documenting dismal immune responses against phylogenetically related HPV genotypes (Wideroff *et al*., 1999; Marais *et al*., 2000; Combita *et al*., 2002).

Evaluation of vaccine-induced immune response cross-reactivity and cross-protection against non-vaccine HPV genotypes provided inconsistent results associated with low levels of cross-neutralization by vaccine-induced antibodies that sometimes fall below the detection limit of the assays (Einstein *et al.*, 2011; Malagon *et al*., 2012). Bivalent vaccine (genotypes HPV16/18) has been suggested to provide a weak cross-protection against heterologous HPV genotypes: HPV-45 (Alpha 7) and HPV-31 (Alpha 9) (Harper *et al*., 2006; Einstein *et al.*, 2011; Wheeler *et al*., 2012; Harari *et al*., 2016); Alpha 9 HPV-33 and Alpha 5 HPV-51 (Wheeler *et al*., 2012), but not other genotypes including: Alpha 9 genotypes: HPV-33 (Harper *et al*., 2006; Harari *et al*., 2016), HPV-35 (Wheeler *et al*., 2012; Harari *et al*., 2016), HPV 52 and HPV-58 (Harper *et al*., 2006; Wheeler *et al*., 2012); Alpha 5 genotype HPV-51 (Harari *et al*., 2016); Alpha 7 genotypes (HPV-39, HPV-59 and HPV-68) and Alpha 6 genotypes (HPV-56 and HPV-66) (Wheeler *et al*., 2012). Additionally, the 2vHPV vaccine did not provide cross-protection against Alpha 10 HPV genotypes (HPV-6 and HPV-11) (Woestenberg *et al*., 2017) and neither did vaccination with genotype HPV-16 (Alpha

9) L1 vaccine cross-react nor confer cross-protection against Alpha 7 genotype HPV-18 (Pastrana *et al*., 2004).

Although these contradictory results suggest existence of selected genotypic cross-protection, the documented evidence is for non-2vHPV vaccine genotypes that are currently covered in the 9vHPV vaccine, except for genotype HPV-51. Nonetheless, the nonavalent HPV vaccine includes only seven (28%) HPV genotypes of the 25 characterized high-risk HPV genotypes and 5% (2/37) low-risk HPV genotypes (Harari *et al*., 2014). The majority (>70%) of high-risk HPV genotypes are not covered by any of the three HPV vaccines. Indeed, results from this study indicated that 80% of fishermen were infected with at least one non-vaccine high-risk HPV genotype. If this population would have been vaccinated (using 9vHPV vaccine), only 20% would have benefited from vaccine-related protection against infection with any of the 7 high-risk HPV genotypes included in the vaccine. Therefore, if a vaccination program is rolled out, the vaccinated population will still remain at risk for HPV infection with non-vaccine HPV genotypes and subsequent development of HPV-related disease. In order to reduce the risk of genotypic HPV infections and eliminate the burden of HPV-related disease, it would be necessary to expand the current 9vHPV vaccine to cover even more HPV genotypes or explore additional molecular remedies that could be synergized with 9vHPV vaccination to ensure greater impact.

5.1.3 Cell-mediated Cytokine Immune response to HPV infections among Fishermen

Significant upregulation of IFN-γ and IL-2 observed in fishermen with HPV clearance suggest that induction of immune response biased towards Th1 pathway is necessary for rapid clearance of natural HPV infection in men. In support of this hypothesis, Chan *et al*. detected INF-γ T-cell responses against L1 peptides in PBMCS collected from women with a recently cleared HPV-58 infection (Chan *et al*., 2010). A significant proportion of women clearing HPV-16 (compared to women with persistent HPV-16), mounted cell-mediated IFN-γ responses against E6 HPV-16 protein (Farhat *et al*., 2009). Additionally, increased expression of IFN-γ (in absence of IL-4) was observed to precede HPV clearance among women participating in a study exploring the natural history of cervical HPV infections (Scott *et al*., 1999). Detection of IFN-γ in women with mild to normal cytology was independently associated with high-risk HPV clearance (Song *et al*., 2008). IL-2 was significantly induced against HPV-16 peptides in PBMCs collected from women with normal cytology previously positive for HPV-16, compared to cytologically abnormal (LSIL, HSIL or ICC) patients (Tsukui *et al*., 1996).

Unlike in HPV clearance group, IL-4 and IL-10 cytokines were upregulated among men with HPV persistence, compared to media from unstimulated cultures. Similar results have been shown elsewhere among women. In a longitudinal study of university students, increased levels of IL-10 in cervical secretions were found to be associated with diminished likelihood of HPV clearance (Scott 2013). Azar and colleagues reported a higher secretion of IL-6 and IL-10 inversely correlating with IFN-γ and TNF-α among HPV positive women with normal cytology compared to normal HPV negative women (Azar *et al*., 2004). Although, IFN-γ and IL-2 were upregulated in fishermen with persistent HPV (compared to media) suggesting a mixed (Th1 and Th2) immune activation that was unable to clear HPV infection, it corroborates the findings that exclusive Th1 responses are necessary for HPV clearance (Scott *et al*., 1999; Song *et al*., 2008; Farhat *et al*., 2009, Chan *et al*., 2010,). Put together, these data suggest that cell-mediated immune activation biased towards Th2 cytokine responses creates immunological-tolerance to HPV infection.

Alternatively, HPV (together with other co-factors) modulate cellular immune responses, dampening Th1 pathway in favor of Th2. This likely promotes a microenvironment suitable for HPV persistence as a means of viral survival.

Cell mediated immune responses observed in 22% of HPV uninfected men in this study, suggests immune priming from past HPV infections prior to study participation. Several studies among women have also reported cytokine response believed to have resulted from previous exposure to natural HPV infection. Pinto and her colleagues detected IFN-γ and IL-10 cytokine response in PBMCs from healthy women prior to vaccination or in placebo group, suggesting immune priming resulting from past HPV infections (Pinto *et al*., 2003, Pinto *et al*., 2006). Additionally, García-Piñeres and others reported increased levels of induced IFN-γ , IL-2, IL-4, IL-6 and IL-10 cytokine response in L1 VLP treated PBMCs from healthy women prior to vaccination (García-Piñeres *et al*., 2007).

Higher mean counts of platelets and CD8+ T cells observed among fishermen with persistent HPV infection, suggest activation of immune-modulated inflammatory responses. Although it is difficult to associate these responses to the persisting HPV infection because men with persistent HPV had increased odds of being HIV co-infected, some studies have suggested that a CTL response to HPV 16 E6 is important for elimination of infected host cells (zur Hausen, *et al.,* 1999; Nakagawa *et al.,* 2000). Therefore, increased activation of cytotoxic T-lymphocytes reported in this study, could be as a result of either chronic HIV infection, persistent HPV infection or both. In as much as the role of platelets in the activation process of acquired immune response against HPV infections remains unclear, there is increasing evidence that platelets play an important role in both innate and adaptive immune responses against infections (Morrell *et al*., 2014, Bhanu *et al*., 2017; Ozge *et al*., 2017; Koupenova *et al*., 2018); including trafficking and activation of CD4+ T cells, and enhancing B-cell signaling (Elzey *et al*., 2003; Danese *et al*., 2004).

Notably, majority of men in this study who cleared new HPV-16 infection within 3 months after infection lacked evidence of adaptive cellular immune activation as detected by IFN-γ assay (Table 4.21). Additionally, absence of cytokine responses

was observed in 36% of men with HPV clearance. This suggests that factors other than adaptive cell-mediated immune response also play a role in clearing HPV infection. Potentially, innate immune activation against the virus rather than self-limiting factors could be involved in HPV viral elimination during acute infection among immunocompetent individuals. Although the role of host's innate immune responses in HPV clearance is largely un-documented, a recent study suggested that reduced expression of Toll Like receptors (TLRs) play a significant role in HPV-16 persistence among women (Daud *et al.*, 2011).

Therefore, Th1 cell-mediated immune response was associated with HPV clearance in fishermen. Findings from this study confirm that cellular immune priming by natural HPV infection occurs among men. However, it is difficult to speculate on whether these immune responses result in naturally acquired immunity, conferring future protection against re-infection. HPV infection in men occurs in keratinized epithelial cells that have poor systemic immune vigilance compared to mucous membranes among women (Franceschi *et al*., 2014). The extent, to which immune system is induced among men following natural HPV infection, could be much lower compared to women. Therefore, the magnitude of naturally acquired protection against future HPV infections is likely to be much higher in women as opposed to men. Indeed, several studies have reported higher HPV seroprevalence among women than men (Clifford *et al*., 2007; Markowitz *et al*., 2009; Tiggelaar *et al*., 2012) and a significant reduction in HPV prevalence by age among women (Castle *et al*., 2005; Smith *et al*., 2008) but not men (Smith *et al*., 2011). These observations suggest that, although immune priming occurs in a proportion of men with HPV infection, development of acquired immunity is weak or totally absent. Alternatively, acquired immunity in a subset of HPV infected men could be weak and wans-off quickly due to lack of constant boosting of immune memory (in cases of repeated exposure) resulting from poor systemic immune surveillance in keratinized epithelium.

5.1.4 Overall Study Summary

HPV, HIV and HSV-2 infections are highly prevalent among fishermen along beaches on the Kenyan side of Lake Victoria in Kisumu County, compared to the general population. These fishermen comprise a highly mobile sub-population that is significantly at increased risk for new infection with these three sexually transmitted viruses. High-risk sexual behavior: inconsistent condom use with new sexual partners, concurrent sexual partnerships, high number of sexual partners, sexual partnerships with sex workers/casual partners were found to be primarily the most important risk factors for acquisition of incident HPV, HIV and HSV-2 infections (Table 5.1). These unsafe sexual practices are in total disregard of the ABC approach (Abstinence, Being faithful to one sexual partner and Condom use). The prevalence and incidence rate of these viral STIs reported in this study in comparison to those found in well-defined most-at-risk populations (MAPs) for HIV, strongly supports the classification of fishermen as a key population for HIV and STIs.

Table 5.1: Summary of risk factors associated with HPV, HIV and HSV-2 infections among Fishermen

	HPV			HIV		HSV-2	
Characteristic	**Prevalence**	**Incidence**	**Persistence**	**Prevalence**	**Incidence**	**Prevalence**	**Incidence**
Age				Risk factor		Risk factor	
Number of sex partners >2		Risk factor		Risk factor			Risk factor
Marital status (married)	Risk factor		Risk factor			Risk factor	
Marital status (single)					Risk factor		
Condom use	Protective	Protective	Protective	Protective	Protective	Protective	Protective
Circumcision	Protective						
History of STI	Risk factor			Risk factor		Risk factor	
Most recent sexual act was with casual/sex worker			Risk factor		Risk factor		Risk factor
Transactional sex				Risk factor			Risk factor
CD4 <500	Risk factor						
Baseline HIV	Risk factor		Risk factor			Risk factor	
Baseline HSV-2				Risk factor			
Baseline HPV		Risk factor	Risk factor	Risk factor			
Persistent HR HPV							Risk factor

HPV infection was the commonest of the three viral STIs evaluated in this study. The high HPV prevalence in these fishermen and the low level of condom use among these men suggested even higher rates of HPV infection among their regular sexual partners. Low condom use, multiple sexual partners, high number of sexual partners and sex with casual partners/sex workers were the key risk factor for new HPV acquisition. Conversely, HIV infection was the main risk factor for multiple genotypic HPV infection and persistence of HR-HPV genotypes. Given that HIV rates are higher among women than men, HPV persistence is likely higher among the female sexual partners to these fishermen, suggesting much higher risk for cervical cancer in these women compared to women from the general population. HPV vaccination among fishermen is therefore needed to prevent new HPV acquisition and persistence among fishermen, consequently mitigating HPV transmission to their female sexual partners.

This study demonstrated that fishermen experience a higher burden of HIV infection. Due to compounding and overlapping behavioral and sexual risk factors for STIs and HIV, findings from this study suggest that high-risk unsafe sex could be the most important predisposing factor for HIV acquisition. The success in stopping the HIV epidemic potentially lies in a renewed call for innovative revamping and strengthening of safer sex HIV preventions strategies fortified with the use of antiretroviral drugs. Well-targeted risk reduction interventions for high risk groups such as fishermen are urgently needed to prevent new HIV infections among most at risk subpopulations and the general population.

Highly active antiretroviral therapy (HAART) is effective in suppressing the HIV virus and significantly preventing transmission to new sexual partners. Antiretroviral pre-exposure prophylaxis (PrEP) has also been found to prevent acquisition of new HIV infection among HIV discordant couples. Provision of universal early HAART (irrespective of CD4 level or WHO staging) to all HIV infected individual is highly recommended. More specifically targeting HIV positive men and women in MAPs (or key populations) will substantially reduce new HIV transmission to the general population. Provision of PrEP to HIV negative individuals in sub-populations at

increased risk for HIV infection (those who belong to defined MAPs and key populations) is a suggested major public health intervention that could avert acquisition of new HIV infections.

Notwithstanding the parallel HPV and HIV epidemics, fishermen are highly burdened and at increased risk for HSV-2 infection characterized by the highest incidence rate ever reported among men that is only comparable to rates documented among female sex workers. Persistent HR-HPV infection was independently associated with increased risk for HSV-2 acquisition in addition to factors related to high risk sexually behavior. Without an efficacious HSV-2 vaccine in sight, well-tailored intervention programs promoting safer sex for prevention of HSV-2, HPV and subsequently HIV infections are urgently needed. Moreover, use of acyclovir therapy among patients with clinical herpes would limit herpetic lesions (which serve as portal of entry for HIV and other infections), and reduce viral shedding for both HIV and HSV-2 among co-infected individuals.

There is clear evidence that HIV infection totally changes the natural history of HPV and HSV-2 infections. The risk of infection with multiple HR-HPV genotypes that are persistent is significantly elevated among HIV infected individuals. This provides an explanation to the high rates of HPV-related cancers among people living with HIV. Evidence is mounting that underlying HSV-2 infection and acquisition of incident HSV-2 infection is a major risk factor for HIV acquisition. Clinical/subclinical herpetic lesions/ulcerations and immune activation associated with active HSV-2 infection provide biological plausibility explaining this risk. The novel finding of this study that persistent HR-HPV infection increases the risk for incident HSV-2 acquisition suggest that the three viruses form a triad of interaction raising the risk of either; infection, activation or diseases progression of the other viruses in the triad.

Efficacious HPV vaccines against 2-7 important HR-HPV genotypes and 2 LRHPV genotypes are available. This study has characterized genotypic HPV infections among fishermen and documented that negative genotypic HPV type-specific interactions were rare. Moreover, the study found no genotypic type competition or potential for genotype replacement, if an HPV vaccination program was to be

implemented among fishermen. Additionally, this study demonstrated that genotypic co-infections of HPV had no specific clustering pattern and infection with one genotype had no effect on the probability of infection with other HPV genotypes among fishermen detected with vaccine genotypes.

However, due to cost implications in resource limited countries, the vaccine which is highly recommended for implementation among young pre-adolescent children (girls and boys) before sexual debut is largely inaccessible. With the high HIV burden in Africa and specifically in Sub-Saharan Africa where there is a parallel epidemic of HPV-related cancers, it is prudent to provide HPV vaccination to both men and women. Indeed, this study has demonstrated that men require a robust Th1 cell-mediated immune response in order to clear HPV infection. Immunocompromised individuals are less likely to mount natural Th1 cytokine response necessary for HPV clearance that was demonstrated in this study. Targeted early immunization especially among sub-population of men and women at highest risk for HIV infection and STIs should be a public health policy to prevent HPV infection, persistence and disease progression upon HIV infection. Although efficacy data on HPV vaccination among PLWH is still scarce, two HPV vaccines are safe and immunogenic among individuals already infected with HIV. Therefore, HPV vaccination for PLWH including young women and men at increased risk for HPV infection and related diseases should be made a universal immunization requirement, integrated in programs for HIV prevention, HIV treatment and continuum of care.

Indispensably, programs promoting safer sexual practices as the primary mode of transmission/acquisition of these viruses are urgently needed. Clearly, human behavior is highly dynamic, controlled by social norms, work-related mobility, profession/industry, technology, and peer pressure among other factors. Therefore, interventions promoting safer sex must be well-tailored to the sub-population being targeted. The approach should be multidisciplinary in nature, integrating all available prevention interventions including sexual behavior modification and biomedical tools (Figure 5.1).

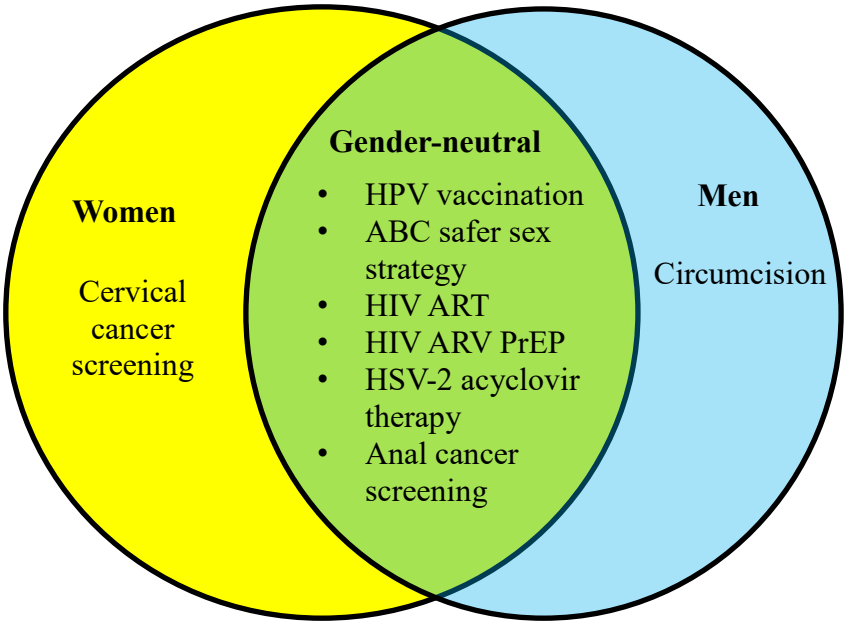


Figure 5.1: Prevention approach for HPV/HIV/HSV-2 infection and HPV-related diseases among Fishermen and their sexual partners.

5.1.5 Study Limitations

This study had limitations. Analysis of genital swabs collected at baseline, six-month and 12-month intervals for HPV infection may have underestimated the incidence and overestimated the duration of infection since individuals may have both acquired and cleared HPV genotypes between these study visits. Therefore, this study was conservative in the estimates of HPV prevalence, incidence, clearance and persistence, which were restricted to sequential samples with amplifiable cellular or viral DNA assuring sample adequacy. Additionally, HPV molecular assays utilized for HPV diagnosis were unable to distinguish between incident, reactivating and persistent HPV infections. Detection of new HPV genotypes that had not been detected earlier could probably be completely newly acquired HPV genotypes or a possible reactivation of latent HPV infections. Similarly, detection of the same HPV genotype on subsequent visits could probably be the same genotype detected at the prior visit (meaning a persistent infection) or a re-infection with the same genotype after clearing the initial infection that was detected at a previous visit. Therefore, interpretation of incident and persistent HPV infections was elusive.

Despite this study providing empirical data expanding the limited knowledge on cell-mediated immune response against natural HPV infections among men, it has several additional limitations: First, the study could not exclude possibility of immune responses resulting from past HPV infections or cross-reactions from homologous HPV genotypes in multi-genotypic infections. Therefore, natural priming of these induced responses may not solely be attributed to genotypic HPV infections detected in the current study or those in Gardarsil®9 only. Second, the reported immune responders had induced cytokine responses to at least one of the 9 HPV genotypes. Therefore in this analysis, it was not possible to report singled out cytokine responses against specific individual genotypes but rather combined response against any of the 9 L1 VLPs included in Gardersil®9 VLP pool. Third, cytokines reported in this study are not exclusively secreted by CD4 T- cell subpopulation. There is a possibility that without cell sorting/depletion, other cell types might have contributed to immune reposes observed in this study.

5.2 Conclusions

With the available data, the following deductions are plausible:

1. Fishermen along beaches of Lake Victoria in Kisumu County have a higher burden of HPV, HIV and HSV-2 infections and are at increased risk of incident infection with these three sexually transmitted viruses, compared to the general population. The rates of these three infections are similar to those observed among most-at-risk populations (MARPs) such as female commercial sex workers and men who have sex with men (MSM). HPV, HIV and HSV-2 share risk-factors for prevalence and incidence among fishermen. The main independent factors include: high-risk sexual behavior, age and baseline co-infection with any of the three viruses. Marital status, high-risk sexual behavior, and underlying HIV and HPV infections are major risk-factors for high-risk HPV persistence among fishermen. Infection with HR-HPV genotypes is a major risk factor for incident HSV-2 and acquisition of new HPV genotypes. Findings from this study suggest that HPV, HIV and HSV-2 are co-factors to each other forming a viral infection triad.
2. There was no evidence for competitive interactions between HPV genotypes among fishermen and thus no potential for genotype replacement if nonavalent HPV vaccination program is introduced among men. Therefore, with the limited evidence of cross-protection, expanding the HPV vaccine to cover more HPV genotypes or exploring additional molecular remedies for HPV prevention is necessary.

3. Th1 cell-mediated immune response is associated with HPV clearance in fishermen, confirming that adaptive cellular immune priming by natural HPV infection occurs among men.

5.3 Recommendations

1. There is need for targeted safer sexual practice interventional programs among fishermen to address the primary risk factor for these infections among fishermen – high-risk sexual behavior characterized by very low condom use, concurrent multiple sexual partners and constantly changing/expanding sexual networks.
2. There is need to evaluate acceptability and uptake of immediate HAART for HIV treatment among HIV positive fishermen as a transmission prevention tool and Pre-Exposure prophylaxis for the HIV negative fishermen.
3. Need to vaccinate both girls and boys against HPV to alleviate the high burden of HPV, persistence of high-risk HPV, and subsequently HPV disease among fishermen and their female sexual partners.
4. There is need for operational research evaluating well-tailored programs for safer sexual practices to avert STIs including HPV, HSV-2 and HIV among fishermen.
5. There is need for continued search for antivirals and molecular interventions against non-vaccine high-risk HPV genotypes, including studies expanding the number of HPV genotypes covered by the HPV vaccine strategy beyond the current 9vHPV vaccine, due to limited cross-protection from vaccine induced immunity against non-vaccine genotypes.
6. Need to continue search for vaccine against HSV-2 and HIV infections, as well as microbicides against these three viruses.

7. There is need for future studies exploring potential biological interactions among HPV, HIV and HSV-2 viruses to inform biological interventions that would disrupt the viral triad synergy.

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APPENDICES

Appendix I: English Participant Enrolment informed consent form

Kenya Medical Research Institute (KEMRI), Jomo Kenyatta University of Agriculture and Technology (JKUAT)

Evaluation of the Dynamics and Immuno-Virology of Human Papillomavirus (HPV) Infections among Fishermen in Kisumu, Kenya

WRITTEN CONSENT FORM

Enrolment

Researchers:

Name	Role	Institution	Telephone number
Raphael Ondondo BSc, MSPH	Principal Investigator	JKUAT and KEMRI	Tel: 0733133141
Elizabeth Bukusi, MMBChB, M.Med, MPH, PhD	Lead KEMRI Supervisor	Center for Microbiology Research, KEMRI	Tel: 0733617503
Solomon Mpoke BSc, MSc, PhD	KEMRI Co-Supervisor	KEMRI	Tel: 0202722541
Prof Zipporah Ng'ang'a	University Supervisor	JKUAT	Tel: 0722794883

24-hour Emergency Telephone Number: Raphael Ondondo, PI, Tel: 0733133141

Researchers' Statement

I would like to tell you about a study being conducted by researchers from the Kenya Medical Research Institute (KEMRI), and Jomo Kenyatta University of Agriculture and Technology (JKUAT), I would like to tell you about the study to find out if you would be willing to participate. The purpose of this consent form is to give you the information you will need to help you decide whether to be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, and if you qualify based on the screening criteria, you can decide if you want to be in the study or not. This process is called 'informed consent.' We will give you a copy of this form for your records.

PURPOSE

This is a research study. The purpose of this research study is to gather information about factors associated with infection and persistence or clearance of a sexually transmitted virus called Human papillomavirus (HPV) This virus is the cause of many types of cancer of the sexual organs of both men and women. The researchers hope that the information from this study would be useful in designing

intervention strategies that would limit acquisition and transmission of HPV such as exploring the use of a microbicide gel to protect men against HPV and other sexually transmitted infections (STIs), including HIV.

STUDY PROCEDURES

In this study, we hope to recruit up to 300 men in the Kisumu fishing community and follow them up for a total of 5 visits (every three months) for a period of 1 year. At all the visits: enrollment, month 3, moth 6, month 9 and month 12, a clinician will conduct a physical examination, including a detailed examination of your genital area with a magnifying glass. He will draw 4 tablespoons of blood (38ml) to test for HPV, HSV-2 and HIV infections, as well as immune response to these infections. The clinician will also take a penile swab to test for presence of HPV. Some of these samples will also be send to international laboratories at University of Washington and University of California San Francisco (UCSF) in the USA for specialized confirmatory testing and quality assurance after ethical clearance from KEMRI ERC is granted. All left over specimens will be stored for 2 years after the study end to address any issue requiring retesting and discarded thereafter. We will offer you risk reduction counseling, such as abstaining from sex until the right time, being faithful to one uninfected partner and using a condom each time you have sex with a person whose health status is unknown to you as well as availability of voluntary medical male circumcision (VMMC)

RISK, STRESS OR DISCOMFORT

It may be embarrassing for you to have us examine your genital area with a magnifying glass. Some of the questions about your sexual and bathing practices may be embarrassing, but you will have a choice not to answer any question you are not comfortable with. Examples of these questions are 'How many times a week do you wash your genitals?', 'Did you ejaculate during all the rounds' and 'How many minutes did you take before ejaculating during the first round?' Some people experience discomfort when blood is drawn. Some may feel dizzy or even faint and may have a bruise or swelling where the needle goes into the arm. Learning results of STI tests may be stressful.

If you think you have an injury or illness related to this study, contact the study staff right away. The study staff will treat you for minor conditions or refer you for treatment for conditions that require more extensive care. You will not lose any of your legal rights if you sign this form.

BENEFITS

You may benefit by receiving free STI/HIV testing and treatment, if necessary. We will also counsel you on abstinence, being faithful to one uninfected partner and using a condom each time you have sex. Your participation in this study could benefit society when the information gathered from this study translates into a strategy that could prevent transmission of these sexually transmitted infections including HIV.

ALTERNATIVES

Your participation in this study is voluntary. You may refuse to participate and you may withdraw from the study at any time without penalty. You are also free to ask questions of the investigators both before consenting to participate and any time thereafter. If you decide not to participate or stop participating after the study has begun, it will not affect your ability to obtain services from this health center, including STI/HIV prevention and STI/HIV counseling and testing services; or any other benefit to which you are entitled. You just need to let the study staff know that you are terminating your study participation. The investigator may also terminate your participation in the study if there is any

evidence that your continued participation in the study may result in harm or for any reason you loose ability to act rationally.

OTHER INFORMATION

Data, including questionnaires and files from the study, will be kept in locked cabinets during the study. Study documents will be kept for a maximum of five years, until the study is completed. Your data will be labeled only with your study code, not your name. We will keep your identity as a research participant confidential. However, no system of protecting your confidentiality can be completely secure so it is still possible that someone could find out you were in this study and could find out information about you.

Consortium for National Health Research (CNHR) or any other sponsor or a designee/contractor on behalf of the sponsor, KEMRI, JKUAT or University of Washington staff sometimes review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

There is no cost to participate in this research study. Recently concluded studies in South Africa, Uganda and Kenya show that circumcision can protect men from getting HIV infected. We will provide detailed information on this in a flyer and let you decide whether you want to get circumcised or not. If you join the study while uncircumcised and later on decide to get circumcised, we will terminate your participation in the study to give you time to heal. Should any new information that is important for your participation in the study becomes available, we will promptly let you know so that it can assist you in making a decision about continuing with the study. We will compensate you for your transport to the clinic for up to a maximum of Kshs 500.

Do you have any questions? Do you agree to participate in the study?

_____ _____ _____ _____

Printed name of researcher Signature of researcher Date Time

Participant's Statement

I have read this form/this for has been read and explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have future questions about the research, I can ask one of the investigators listed above. If I have questions about my rights as a research participant, I can contact the chairperson of the KEMRI Scientific Steering Committee at 020-272-2541, the chairperson of the KEMRI Ethical Review Committee at 020-272-2541.

_____ _____ _____ _____

Printed name of participant Signature/thump print of participant Date Time

_____ _____ _____ _____

Printed name of witness Signature of witness s Date
Time

Copies to: Investigator’s files, study participant

Appendix II: English Participant HIV testing consent form

Kenya Medical Research Institute (KEMRI), Jomo Kenyatta University of Agriculture and Technology (JKUAT)

Evaluation of the Dynamics and Immuno-Virology of Human Papillomavirus (HPV) Infections among Fishermen in Kisumu, Kenya

WRITTEN CONSENT FORM

HIV

Researchers:

Name	Role	Institution	Telephone number
Raphael ondondo BSc, MSPH	Principal Investigator	JKUAT and KEMRI	Tel: 0733133141
Elizabeth Bukusi, MMBChB, M.Med, MPH, PhD	Lead KEMRI Supervisor	Center for Microbiology Research, KEMRI	Tel: 0733617503
Solomon Mpoke BSc, MSc, PhD	KEMRI Co-Supervisor	KEMRI	Tel: 0202722541
Prof Zipporah Ng'ang'a	University Supervisor	JKUAT	Tel: 0722794883

24-hour Emergency Telephone Number: Raphael Ondondo, PI, Tel: 0733133141

Introduction

A virus called HIV (Human Immunodeficiency Virus) causes the disease AIDS (Acquired Immunodeficiency Syndrome). Anyone with HIV can spread it to others. It is spread through unsafe sex, sharing needles, or donating blood or other tissues. Infected mothers can spread HIV to their babies. The test for HIV detects the body's reaction to the virus (antibody). It does not detect the virus itself. The decision to be tested for antibody to the virus that causes AIDS is voluntary; you are not required to have the test. This test is being done for a research study. You should know the advantages and disadvantages of testing before you decide to take the test. Please read this consent form with care so that you can make an informed choice about having the blood test.

What the test means

If you test POSITIVE, you have the HIV virus. That means you can pass it to others. The test cannot tell how long a person has been infected. It does not mean that you have AIDS, which is the most advanced stage of HIV infection.

If the test is NEGATIVE you probably do not have the HIV virus. It may mean that you have the virus, but your body has not yet made antibody to fight the virus. It could take up to six months after infection for the test to turn positive. False results are rare. Unclear results are also rare. When a test result does not seem to make sense, we do the test again. We might do another kind of blood test to find out if you are infected or not.

Procedures

This is what will happen if you decide to have the test. First, you will meet with a counselor. The counselor will give you more information about the risks and benefits of the test. They will explain the meaning of test results. They will teach you how to reduce the chance of spreading HIV. They will explain the dangers of HIV infection. Less than 2 teaspoons (approximately 8 mls) of blood will be taken from a vein with a sterile needle for this antibody test as well as for other serology test discussed in the enrolment consent form. We will test your blood in the laboratory. We will be able to tell you your results at your next visit. When you learn the test results, you will also be counseled to increase your understanding of HIV transmission and how to reduce your risks of getting or transmitting sexually transmitted diseases by being faithful to one uninfected partner, abstaining from sex if you are diagnosed with a sexually transmitted disease while on treatment, and using a condom consistently and correctly each time you have sex. You will also be counseled about how to notify your sexual partners if your test result is positive.

Benefits of being tested

The benefits of being tested are very personal. If you are worried about AIDS, you might feel better if you have a negative test. Sometimes knowing that the test is positive can relieve stress. You may want to know your test result before you have sex with a partner. In some cases, test results may help diagnose a medical problem or help you make decisions about your future or on health care. Those who test positive for HIV will be referred to the FACES clinic to receive subsidized ARV treatment. There may be other benefits of testing that we don't know about now.

Risks of being tested

Learning test results may cause you and your partner severe stress, anxiety and depression. This may result into blaming each other and even cause separation or divorce. Other people learning about your HIV status may lead to discrimination in travel, work and insurance. You might be tempted to have unsafe sex if the result is negative. This would increase your risk of getting AIDS. If the results of the test get into the wrong hands, prejudice, discrimination, risk to employment, travel restrictions, and other adverse effects could result. There may be other risks and stresses of being tested that we don't know about now.

You may get a bruise where the needle enters the vein and there is a small risk of infection. You may feel some pain as the needle enters your vein.

Information about confidentiality

Your AIDS antibody test results will be held in the strictest confidence, and no identifying information of any kind will be released to any other person or agency without your specific permission in writing. We will not publish or discuss in public anything that could identify you.

Do you have any questions? Do you agree to participate?

_____ _____ _____ _____

Printed name of researcher Signature of researcher Date Time

Participant's Statement

I have read this form/this for has been read and explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have future questions about the research, I can ask one of the investigators listed above. If I have questions about my rights as a research subject, I can contact, the chairperson of the KEMRI Scientific Steering Committee at 020-272-2541 or the chairperson of the KEMRI Ethical Review Committee at 020-272-2541.

_____ _____ _____ _____

Printed name of participant Signature/thump print of participant Date Time

_____ _____ _____ _____

Printed name of witness Signature of witness s Date Time

Copies to: Investigator's files, study participant

Appendix III: English Structured Demographic Questionnaire

Kenya Medical Research Institute (KEMRI), Jomo Kenyatta University of Agriculture and Technology (JKUAT)

Evaluation of the Dynamics and Immuno-Virology of Human Papillomavirus (HPV) Infections among Fishermen in Kisumu, Kenya

DEMOGRAPHIC QUESTIONNAIRE

DEMOGRAPHIC INFORMATION

PTID **FM2011 H P V** ☐☐☐ **Beach** _____

1. Date of interview (day/month/year) ☐☐-☐☐-☐☐

2. Date of birth (month/year) ☐☐-☐☐

3. What is your ethnic group?

_____Luo

_____Luhya

_____Kikuyu

_____Kisii

_____Kamba

_____Kalenjin

_____Kuria

_____Decline

_____Other

(specify)_____

4. What is your religion?

_____Muslim

_____Protestant

_____Catholic

_____Independent churches

_____Traditionalist

_____Decline

_____Other

(specify)_____

5. What is your marital status

_____Single

_____Married

_____Divorced

_____Widowed

_____Seprated

6. How many years of school have you completed (_____)

7. Have you had any vocational or professional training? (_____)

8. How many years did you take for the training? (_____)

9. What is your main occupation?

_____Fisherman

_____Boat owner

_____Fish trader

_____Fish agent (buys fish in small quantities and sells in large quantities)

_____Truck driver

_____Truck turnboy

_____Farmer

_____Mining

_____Other (specify) _____

10. What is your monthly income (from all your sources)? Kshs. (_____)

MIGRATION

11. How many different beaches have you worked in? _____
12. Did you change your residence every time you moved to a new beach
    Yes No

13. How many times have you changed your residence during your entire fishing experience?

14. Have you traveled and spent a night away from your current house in the last one month?
    Yes No

15. If yes, how many times have you travelled and spent a night away from your current house in the last one month? (_____)

16. How many nights did you spend away from your current house?(_____)

17. How many times did you have sex during this travel? (_____)

18. With whom did you have sex during this travel?
    _____Wife

    _____Girlfriend

    _____New casual partner

    _____New commercial sex partner

19. For how long had you known this person? (_____)

20. Did you use a condom?
    Yes No

SEXUAL HISTORY

21. Are you circumcised?
Yes No

22. When did you get circumcised? (_____)

23. Why did you decide to get circumcised?
_____Disease prevention
_____Cultural reasons
_____Accident/medical reasons
_____Parents decided
_____Other (specify) _____

24. How long after circumcision did you resume sex? _____
25. How old were you when you first had sexual intercourse? (_____)

26. How old was your partner? (_____)

27. Did you use a condom during this sexual intercourse
Yes No Do not remember

28. Do you think this was also his/her first sexual encounter?
Yes No

29. How many sexual encounters did you have with this partner? (_____)

30. How long did this relationship last? (_____)

31. What ended this relationship?
_____I got a new partner

_____We fought/ disagreed

_____She cheated on me

_____I got married

_____Distance (she/ I went far from me/her

_____Parents/relatives separated us

_____Relationship still on

32. How often did you use a condom during sex with this partner?

_____100% of the time

_____75% of the time

_____50% of the time

_____Less than 25% of the time

_____Never at all

33. How many sexual partners have you had in the last one month
(_____)

34. How many sexual partners have you had in the last six months
(_____)

35. How many sexual partners have you had in the last one year
(_____)

36. How many sexual partners have you had in your lifetime?
(_____)

37. Have you ever paid someone, given gifts, fish, favors or the right to buy your fish in exchange for sex?

Yes No

LAST THREE SEXUAL PARTNERS

Now I would like to talk with you about your sexual partners. Think back to the person with whom you had sex most recently….

38. When did this sexual experience occur?
(___/___/___)

39. For how long did you know this person before first sex? (_____)

40. Did you do foreplay (caressing) before actual sex?

Yes No

41. Did you stimulate her genital using your fingers? (_____)

Yes No

42. Did you stimulate her genital using your mouth/tongue? (_____)

Yes No

43. Did she stimulate your genital using her fingers? (_____)
Yes No

44. Did she stimulate your genital using her mouth/tongue? (_____)
Yes No

45. When you inserted your penis, was she lubricated?
Yes No

46. How old was this person? (_____)

47. Some women are believed to spurt some fluid during sexual intercourse, do you think you/your partner spurted during this encounter?
_____Yes

_____I don't think so

_____Maybe

_____No

48. How often do you have sex with this partner?
(_____)

49. Was this person your?
_____Girl friend

_____Casual partner

_____Prostitute

_____Male partner

_____Other (specify) _____

50. Had this partner ever been married before?
Yes No

51. Was this partner married at the time of this sexual intercourse?
Yes No

52. Did you put on a condom before you started having sex?
Yes No

53. After sexual encounter, did you give or promise to give anything to your partner (money, favor, or gift)?
Yes No Decline

54. If yes, what was it?

_____Money

_____Promise to sell her fish

_____Take her for outing

_____Buy her shoes or clothes

_____Marry her

_____Other (specify) _____

55. Do you expect to have sex with this partner again?
Yes No

Now, think back to the person with whom you had sex prior to the person you had sex with most recently….

56. When did this sexual experience occur?
(___/___/___)

57. For how long did you know this person before first sex? (_____)

58. Did you do foreplay (caressing) before actual sex?
Yes No

59. Did you stimulate her genital using your fingers? (_____)
Yes No

60. Did you stimulate her genital using your mouth/tongue? (_____)
Yes No

61. Did she stimulate your genital using her fingers? (_____)
Yes No

62. Did she stimulate your genital using her mouth/tongue? (_____)
Yes No

63. When you inserted your penis, was she lubricated?
Yes No

64. How old was this person? (_____)

65. Some women are believed to spurt some fluid during sexual intercourse, do you think you/your partner spurted during this encounter?

_____Yes

_____I don't think so

_____Maybe

_____No

66. How often do you have sex with this partner?
(_____)

67. Was this person your?

_____Girl friend

_____Casual partner

_____Prostitute

_____Male partner

_____Other (specify) _____

68. Had this partner ever been married before?
Yes No

69. Was this partner married at the time of this sexual intercourse?
Yes No

70. Did you put on a condom before you started having sex?
Yes No

71. After sexual encounter, did you give or promise to give anything to your partner (money, favor, or gift)?
Yes No Decline

72. If yes, what was it?

_____Money

_____Promise to sell her fish

_____Take her for outing

_____Buy her shoes or clothes

_____Marry her

_____Other (specify) _____

73. Do you expect to have sex with this partner again?
Yes No

Now think about the person you had sex with prior to the second-to-last partner.

74. When did this sexual experience occur?
(___/___/___)

75. For how long did you know this person before first sex? (_____)

76. Did you do foreplay (caressing) before actual sex?
Yes No

77. Did you stimulate her genital using your fingers? (_____)
Yes No

78. Did you stimulate her genital using your mouth/tongue? (_____)
Yes No

79. Did she stimulate your genital using her fingers? (_____)
Yes No

80. Did she stimulate your genital using her mouth/tongue? (_____)
Yes No

81. When you inserted your penis, was she lubricated?
Yes No

82. How old was this person? (_____)

83. Some women are believed to spurt some fluid during sexual intercourse, do you think you/your partner spurted during this encounter?
_____Yes

_____I don't think so

_____Maybe

_____No

84. How often do you have sex with this partner?
(_____)

85. Was this person your?
_____Girl friend

_____Casual partner

_____Prostitute

_____Male partner

_____Other (specify)
_____

86. Had this partner ever been married before?
Yes No

87. Was this partner married at the time of this sexual intercourse?
Yes No

88. Did you put on a condom before you started having sex?
Yes No

89. After sexual encounter, did you give or promise to give anything to your partner (money, favor, or gift)?
Yes No Decline

90. If yes, what was it?
_____Money
_____Promise to sell her fish
_____Take her for outing
_____Buy her shoes or clothes
_____Marry her
_____Other (specify) _____

91. Do you expect to have sex with this partner again?
Yes No

CONDOM USE AND STIs/HIV PREVALENCE

92. When did you first use a condom? (_____)

93. Generally, how often do you use a condom during sex with your wife (regular partner)?
_____All the time (100%)
_____Most of the time (>75%)
_____Half of the time (50%)
_____Some of the time (25-<50%)
_____Rarely (<25%)
_____Not at all (0%)

94. Generally, how often do you use a condom during sex with a new partner?
_____All the time (100%)

_____Most of the time (>75%)

_____Half of the time (50%)

_____Some of the time (25-<50%)

_____Rarely (<25%)

_____Not at all (0%)

95. Have you ever been tested for HIV?
Yes No

96. Have you ever had what you thought was an STI?
Yes No

97. How old were you when you first had what you thought was an STI?
(_____)

98. How many episodes of STIs have you had since the first time you had one?
(_____)

HYGIENE BEHAVIORS

99. How many pieces of underwear do you own?
(_____)

100. How many times in a week do you change your underwear?
(_____)

101. How many times do you bathe in one week? (_____)

102. Do you focus to wash your genitals ("private parts") during bathing?
Yes No

103. Do you ever wash your genitals ("private parts") only apart from when you are bathing?
Yes No

104. If yes, how many times in a week? (_____)

105. Have you ever washed your genitals immediately before sex?
Yes No

106. If yes, how often do you wash your genitals before sex?

_____Always

_____More than half of the time

_____Half of the time

_____Less than half of the time

107. Have you ever washed your genitals immediately after sex?

Yes No

108. If yes, how often do you wash your genitals after sex?

_____Always

_____More than half of the time

_____Half of the time

_____Less than half of the time

Appendix IV: Dholuo Participant Enrolment informed consent form

Kenya Medical Research Institute (KEMRI), Jomo Kenyatta University of Agriculture and Technology (JKUAT)

Nono weche matut etudruok gi rageng mag kute makelo tuo mar (Human Papillomavirus -HPV) kuom jolupo ma chuo ei Kisumu, Kenya

Oboke ma indikoe yie mari.

Yiero joma nyalo donjo e nonro

Jotim Nonro

Nying	Tich	Migaw	Namba Simo
Raphael Ondondo BSc, MSPH	Principal Investigator	JKUAT and KEMRI	Tel: 0733133141
Elizabeth Bukusi, MMBChB, M.Med, MPH, PhD	Lead KEMRI Supervisor	Center for Microbiology Research, KEMRI	Tel: 0733617503
Solomon Mpoke BSc, MSc, PhD	KEMRI Co-Supervisor	KEMRI	Tel: 0202722541
Prof Zipporah Ng'ang'a	University Supervisor	JKUAT	Tel: 0722794883

Namba simo ma seche te: Raphael Ondondo, jatend jo tim nonro, Namba: 0733133141.

Wach moa kuom jotim nonro

Aduaro ni mondo anyisi weche ma nitie kuom nonroni ma itimo kod jononro moa KEMRI kod Mbalariany mar Jomo Kenyatta mar Pur gi teko ma sani(ji) (JKUAT), Aduaro ni mondo anyisi wechegi mondo ang'e ka inyalo yie donjo e nonroni. Tich mar obokeni en miyi weche duto ma diduar ng'eyo mondo okonyi ng'ado wach ka idonjo kata ok idonji e nonroni. Yie isom obokeni maber. Inyalo penjo weche duto ma odok korka gima omiyo itimo nonroni, gik ma wabiro kwayi mondo itim, rach kata ber madibedie, ratiro mari kaka jachiwre, t kod weche duto kuom nonroni kata obokeni ma ok winjoreni maber. Ka waseduoko penjoni duto,kendo ka ikalo rapiedhi marwa mar donjo e nonro, eka inyalo ng'do ka idonjo kata ok idonjie. Ma iluongo ni chiwo thuolo mari ka isewinjo weche duto manitie. Wabiro miyi oboke machielo machal kama ma ikano in iwuon.

Gima omiyo itmo nonroni

Ma en nonro. Gima omiyo itimo nonroni en mondo wayud weche modokHPV. Kudni ni e makelo achiel kuom adhola ma mako duong joma chuo kod mine . Jotim nonrogi geno mar tiyo gi weche mowuok ka mondo gitgo nonro maduong' mondo mabiro duoko piny ngamo kendo landruok HPV kaka manyo chako tiyogi mor awira mondo okony joma chuo mondo kik gam tuo mar HPV kod nyaye mamoko(Sti's) ka achiel kod kute makelo tuo mar Ayaki

Kaka ibiro time

Enonroni,, wageno mar ruako chuo 300 moa e oganda mar joma tiyo ei nam e Distrkt ma Kisumo kendo ibiroluwogi kuom limbe dibich (bang duoche adek ka duoche adek) e higa.E limbe duto:e enrollment,bang duoche adek,bang duoche auchiel,bang duoche ochiko kod bang duoche apar kod ariyo,Daktari biro rangi ma otingo nyaka rango duong ni matut kod glass ma neno gigo matindo.Obiro golo remo madirom ojiko 4 mar mesa(38ml) mondo opim HPV,HSV-2,kod HIV kod teko mar dend kuom kutegi..Daktari bende biro golo ondoyo moa e duong jomachuo mondo opim ka HPV nitiere.sample gi ibiro ter e labaratory ma pinje maoko e Mbalariany mar Washington kod mbalariany mar Califonia San Francisco(UCSF) manie piny mar America mondo pim moyiedhi mar yango adier mar pim.Wabiro miyi hocho ma duoko rach chien kaka rito dendi kuom gombo kod bedo ja adiero kindi gi jaherani achiel maonge kod kute mar HIV kod tiyo kod rabuyunga saa moro amora ma uriworu kod ngato ma chal mare ok ingeyo ka achiel gi tero joma chuo nyange e yor hmoko

Rach kata chandruok ma nyalo betie

Inyalo bedo kod wich kuot ka wangi'yo kar duongni gi kiyo. Penjo moko ma wabiro penji kor ka wuodhi gi mon/nyiri kod kaka iluokori nyalo kuodo wiyi, katakamano ibiro bedo gi thuolo mar tamori duoko penjo moro amora ma ok mori. Gin penjo kaka: 'Iluoko ga duong'ni nyadidi e juma?', to gi 'Bende ne ichopo e akirikiki saa ka saa mane iriwori gi ng'to?' Kod ' Nokawi dakika adi mondo ichopi e akirikiki e riwruok u mokwongo?'. Jomoko bedo gi chandruok esa ma ikawo rembgi. Jomoko wang'gi nyalo lil kendo podho. Inyalo ridhori matin kama riw donjogo e ler mari kendo nitie thuolo matin ni onyalo kuot kata ridhore. Yudo duoko mar pim mar nyachnyalo buogi. Kapo ni in kod hinyruok kata tuo ma iparo ni owuok kuom bedoni e nonroni to tudri kod jotij nonro mapiyo. Gibiro thiedhi ka en gimatin ma ginyalo kata ori kar thieth maduong' ka en gima ok ginyal. Ok ibiwito ratiro mari moro amora ka iketo lueti e obokeni.

Ber ma nyalo betie

Ber ma ibiro yudo en pimo kendo thieth manono mar tuo mar nyach to gi tayo tuo mar ayaki ka nitie. Komedore gi tiyo kod yath ma wabiro miyi, wabiro bende miyi hocho eyore mag tamruok bedo e achiel nyaka chopi saa ma owinjore, bedo kod jahera achiel kende maonge kod kute mag ayaki kendo gi tiyo kod rabo yunga e kinde duto ma ibedo e achiel kod ng'ato. Donjoni enonroni nyalo konyo oganda kuom temo yath manyalo geng'o touché mag nyach kod ayaki.

Yiero mamoko

Donjoni e nonroni en yiero mari. Inyalo tamori donjo kata inyalo wuokie saa asaya maonge kum moro. Inyalo bende penjo gimoro amora kapok iyie donjo e nonro kata bang'e. Kapo ni idagi donjo kata iwuok ka nonro pok orumo, mano ok bi moni yudo thieth mapile kar thiethni kata mana kodok korka pimo kata geng'o ayaki/nyach to gi hocho ewi wechegi. Nyis mana jatij nonro kuom dwaro mari mar wuok e nonro. Jatich nonro nyalo chungi kuom bedo e nonroni kaponi bedo jakanyo mari e nonro nyalo keloni hinyruok kata kaponi e yore mamoko omk inyal timo tijeni/yoreni kaka dwarore.

Weche ma moko

Gik ma ooyan'go weche duto moa kuomi e nonroni ibiro kan kama ogo kiful e kinde mar nonroni. Bang' ka osetiek nonroni, to ibiro kano wechegi kuom higni abich. Weche duto moa kuomi ibiro ketie mana nambani ma ok nyingi. Wabiro kano malingling mari kaka jachiwre. '. To kata wabiro temo pando wechegi kamano, nyaka wang'e ni nitie jomoko ma nyalo minore kodwa

Consortium for National Health Research (CNHR) kata jomamoko ma ochiwo nyalo mar timo nonroni mani e KEMRI, JKUAT, kata Mbalariany ma Washington samoro nyalo ng'iyo weche mag nonroni mondo gibed gi adiera ni itime eyo makare. Ka gima kama otimore, to gibiro ng'eyo wecheni ma owuok e nonroni. Jogi biro pando siri mari. Wecheni mani e nonro ok bitigodo kuom ketho ratiro mari kata hinyi.

Onge chudo kuom bedo jakanyo mar nonroni. Machigni ni nonro mane orumo Afrika ma Milambo, Uganda kod Kenya noyudo ni tero nyangu nyalo geng'o chuo kuom yudo kute mag ayaki. Wabiro miyi weche adimba e oboke kendo weyi mondo iyier ka idwaro ni oteri nyange kata ooyo.Ka idonjo e nonro ka pok oteri nyange to beng'e oteri nyange to ibiro chungi e nonro mondo omiyi thuolo mar change. Kopo ni wach moro amora amnyien owuok maber mondo ing'e nikech bedo ni e nonroni, wabiro nyisi mondo okonyi ng'ado kopod did hi nyime gi bedo e nonroni.Wabiro duokoni pesa moromo siling 500 mar wuoth kibiro kar thieth.

Bende in kod penjo moro? Iyie donjo e nonroni?

_____ _____ _____ _____

Nying Jatim Nonro Luet Jatim Nonro Tarik Saa

Wach moa kuom jachiwre

Nonro ma wachne ni malokani oselerna. Achiwora mondo adonjie. Osemiya thuolo mar penjo. Kapo no an kod penjo moko bang'e to anapenji jotim nonro ma nying'gi ni maloka. Kapo ni an kod penjo ma odok korka ratiro mara kaka jachiwre to abiro penjo jakom marKEMRI scientific steering committee e namba 020-272-254**1**, jakom mar ng'yo chike mag nonro ma KEMRI e namba 020-272-2541.

_____ _____ _____ _____

Nying Jachiwre Seyi/Luet Jachwre Tarik Saa

_____ _____ _____ _____

Nying Janeno Seyi Janeno Tarik Saa

Obokeni moro imiyo jachiwre to moro iketo kama okanie wechene.

Appendix V: Dholuo Participant HIV testing consent form

Kenya Medical Research Institute (KEMRI), Jomo Kenyatta University of Agriculture and Technology (JKUAT)

Nono weche matut etudruok gi rageng mag kute makelo tuo mar (Human Papillomavirus -HPV) kuom jolupo ma chuo ei Kisumu, Kenya

Oboke ma indikoe yie mari.

Jolueny mag del ma goyore gi ayaki.

Jotim Nonro

Nying	Tich	Migaw	Namba Simo
Raphael Ondondo BSc, MSPH	Principal Investigator	JKUAT and KEMRI	Tel: 0733133141
Elizabeth Bukusi, MMBChB, M.Med, MPH, PhD	Lead KEMRI Supervisor	Center for Microbiology Research, KEMRI	Tel: 0733617503
Solomon Mpoke BSc, MSc, PhD	KEMRI Co-Supervisor	KEMRI	Tel: 0202722541
Prof Zipporah Ng'ang'a	University Supervisor	JKUAT	Tel: 0722794883

Namba simo ma seche te: Raphael Ondondo, jatend jo tim nonro, Namba: 0733133141.

Weche motelo

Kudni moro ma iluongo ni ayaki (ma chamo jolueny mag del ma goyore gi tuoche) kelo tuo ma landore ma roko dend dhano ka osenego joluenygi moduoko kuangi chien. Ng'atno ma ni gi ayaki nyalo keye ni joma moko. Olandore e yo mar riwruok kod ng'ato, maok iritori chuoruok gi sandan ma ng'ato osechuorego, medo remo kata chiwo ni ng'ato lemo moro amora ma oa ei del mar ng'at machielo. Mine ma nigi tuoni nyalo miye nyithindgi. Pimo mar ayaki neno mana kaka del timore ka kudni mar ayaki odonje. Ok one kudni owuon. Ng'ado wach mondo opim ng'ato ka en gi kute mag ayaki en kuom chwruok; ok chuni mondo opimi. Pimoni itimo mar nonro moro. Onego ing'e ber kod rach mar pim ka pok inga'do wach mondo opimi. Yie mondo isom otasni maber mondo ing'ad wach mar pimo rembi ka iseng'eyo weche duto manitie.

Gima pimoni nyiso

Ka opimi ma oyudi ni in kod ayaki, ma nyiso ni in kod kute mag ayaki ei dendi. Mano nyiso ni inyalo miye jomoko. Pimni ok nyal nyiso ni ng'ato osebedo gi ayaki kuom kinde maromo nadi. Ma ok nyisi ni kutegi osenyoro dendi nikech ma timore e okang' moseniang' ka ng'ato osebet gi kutegi.

Ka opimi ma oyud ni ionge kod ayaki, ma samoro nyalo nyiso ni ionge kute mag ayaki ei dendi. Samoro nyalo bedo ni in kod kute mag ayaki to dendi pod ok oloso jolueny ma goyore kodgi. Nyalo kawo madirom dueche auchiel bang' ka iseyudo kutegi mondo pimo ogolgi.

Tek mondo ka opimi to duoko owuog ataro. Duoko ma ok nen maler bende tek mondo owuogi. Ka opimi to duoko ok winjrenwa maler to wanuoye kendo. Bende wanyalo timo kit pim machielo kod rembi mondo wang'e ka in gi kute mag ayaki kata iongego.

Kaka ibiro time

Ma e gima biro timore ka ing'ado wach ni ipimori. Mokuongo, ibiro romo kod jahocho. Jahocho biro miyi weche moko kendo mang'eny manyiso ber kata rach mar pimni. Gibiro leroni kaka isomo duoko mar pim. Gibiro puonji kaka iduoko chien thuolo mar keyo/lando ayaki. Gibiro leroni chandruok ma wuok ka ng'ato oyudo kute mag ayaki. Ibiro kaw remo matin madirom kijiko mesa ariyo (nyirri 8) e ler mari gi sandan maler ma ok oting'o kudni moro amora ma nyalo kelo tuo kendo remoni ibiro pimgo ka dendi ni kod jolueny ma goyore gi kute mag ayaki. Wabiro pimo rembi e kama oyang mana mar pimo. Wabiro miyi duoko mar pimni ka iduogo kendo. E kinde ma iyudo duokoni, ibiro hoyi bende mondo winjoni omedre e yore ma ayaki nyalo ago kuom ng'ato ka odhigo kuom ng'at machielo, to gi kaka bende thuolo ma inyalo yudogo tuoche mag nyach nyalo dok chien ka ibedo kod jahera achiel kende maonge kod kute mag ayaki, ibedo mabor kod nindruok e kinde ma ithiedhori kapo ni oyudi ni in kod nyach, to gi ka itiyo kod rabo yunga eyo ma kare sa asaya ma iriwori kod ng'ato**.** Bende ibiro puonji kaka inyalo nyiso joherani koponi duoko owuok ni in kod kute mag ayaki.

Ber mar pim

Ber mag pim en gima ng'ato winjo owuon. Ka wach ayaki osebedo ka thagi to inyalo winjo maber ka iyudo ni ionge kod kute mag ayaki. Samoro yudo ni in kod kute mag ayaki bende nyalo miyo dang'ni. Samoro inyalo duaro ng'eyo kit ngimani kaka chal ka pok iriwori gi ng'ato. Seche moko ka opimi to duoko nyalo konyo mondo onuang' kaka ithiedhi kata ng'ado wach kaka irito ngimani e kinde ma biro. Jogo moyudi ka nigi kute makkelo ayaki ibiro chwal e klinic mar FACES mondo oyud yadhe madwoko kwiri mag ayaki chien gi nengo man piny. Bende nyalo bedo ni nitie ber moko mag pim ma ok wang'eyo sani.

Chandruok ma nyalo wuok e pimruok

Ng'eyo duoko mar pim nyalo miyi gi jaherani paro mang'eny, dang'ni, to gi achiedh nadi. Ma nyalo miyo uyuaru gi wach kuom ng'ano ma nene okele ma upog kar dak kata uweru. Jomoko ma biro ng'eyo kit ngimani kaka chal nyalo tuoti sama ji dhi wuoth, kar tich to gi ka iduaro nyiewo 'bima'. Gimoro nyalo temi ni mondo iriwri gi ng'ato ka iyudo ni ionge gi kute mag ayaki. Ma nyalo medo thuolo mari mar yudo kute mag ayaki. Ka duoko mari oluar e luet jomaricho, achae, akwede, duaro riembi e tich, tami wuotho kuonde moko to gi gik moko maricho ma chal kama nyalo timoreni. Nyalo bedo ni nitie chandruok moko ma nyalo wuok ka opimi ma ok wang'eyo sani.

Inyalo ridhori matin kama riw donjogo e ler mari kendo nitie thuolo matin ni onyalo kuot kata bedo gi tutu. Inyalo winjo rem matin e sama riw donjo e ler mari.

Pando wechegi

Duoko mari mar pim mar kute mag ayaki wabiro pando madiny kaka nyalore, kendo onge gimoro amora ma nyalo miyo ong'eyi ma wabiro miyo ng'ato ang'ata ma ok in iwuon ichiwo thuolo ka indiko piny. Ok wabi ndiko kata wacho e yo moro amora ma oganda nyalo somo kata winjo gima nyalo miyo ji fuenyi.

Bende in kod penjo moro amora? Bende iyie donjo e nonroni?

_____	_____	_____	_____
Nying Jatim Nonro	Luet Jatim Nonro	Tarik	Saa

Wach moa kuom jachiwre

Nonro ma wachne ni malokani oselerna. Achiwora mondo adonjie. Osemiya thuolo mar penjo. Kapo no an kod penjo moko bang'e to anapenji jotim nonro ma nyinggi ni maloka. Kapo ni an kod penjo ma odok korka ratiro mara kaka jachiwre to abiro penjo jakom mar tayo nonro e namba 020-272-2541, jakom mar ngi'yo chike mag nonro ma KEMRI e namba 020-272-2541.

_____	_____	_____ _____
Nying Jachiwre	Seyi/Luet Jachwre	Tarik Saa

_____	_____	_____ _____
Nying Janeno	Seyi Janeno	Tarik Saa

Obokeni moro imiyo jachiwre to moro iketo kama okanie wechene.

Appendix VI: Dholuo Structured Demographic Questionnaire

Kenya Medical Research Institute (KEMRI), Jomo Kenyatta University of Agriculture and Technology (JKUAT)

Evaluation of the Dynamics and Immuno-Virology of Human Papillomavirus (HPV) Infections among Fishermen in Kisumu, Kenya

DEMOGRAPHIC QUESTIONNAIRE

DEMOGRAPHIC INFORMATION

PTID **FM2011 H P V** ☐☐☐ **Beach** _____

1. Odiechieng limbe (tarik/dwe/higa) ☐☐-☐☐-☐☐

2. Tarik mar nyol (dwe/higa) ☐☐-☐☐

3. In ja dhoot mane/ Ogandani/dhou en mane?

_____Luo

_____Luhya

_____Kikuyu

_____Kisii

_____Kamba

_____Lang'o

_____Kuria

_____Decline

_____Mamoko

(Dimbi)_____

4. In ja din mane?

_____Muslim

_____Protestant kaka Angilikan, AIC, PAG

_____Katholik/ Kopere

_____Independent churches kaka Hera, Roho, Israel, Nomiya

_____Jaramogi

_____Tamruok hulo

_____Mamoko (dimbi)_____

5. Bei se kendo?

_____Ok okendi

_____Okendi

_____Uweru chuth

_____Chiegi osetho

_____Uweru

6. Isetieko higni adi mag skul ka isomo? (_____)

7. Bende isebedo kod tiegruok moro amora modok korka eche mag tich? (_____)

8. Nene ikao higni adiwa e tiegruogni? (_____)

9. Tiji maduong' makeloni yuto en mane?

_____Lupo/ luwo rech

_____Loko/ uso rech

_____Jaohand rech (nyiewo rech matintin kendo usogi e rapim madongo)

_____Jariemb loch rech

_____Tand-boyi mar loch rech

_____Japur

_____Kunyo fedha

_____Mamoko (dimbi) _____

10. I yudo pesa adi e due kuom nue ng"oni duto? Kshs. (_____)

DAR/WUOTH E DHO WEDHE

11. Isetiyo e dho nembe adi?_____

12. Be ne iloko kari mar dak ekinde mane idar ka idhi e dho nam machielo?
    Ee Ooyo

13. Iseloko kuondegi mag dak nyadidi nyaka ne ichak tiyo e dho nembe kilupo?

14. Bende isedhi e wuoth kia e odi masanini ma inindo oko ei dwe achiel mokalo?
    Ee Ooyo

15. Ka ee, ndalo adi ma isedhi e wuoth kia e odi masanini ma inindo oko ei dwe achiel mokalo? (_____)

16. Otieno adi mane inindo oko ma ok iduogoe e odi masanini?(_____)

17. Nyadidi mane iriwori/ ibedoe achiel kod ng'ato mane in ei wuodhni?(_____)

18. Ng'awa mane ibedoe godo e achiel mane in ei wuodhni?
    _____Chiegi

    _____Jaherani

    _____Jaherani manyien mapok ne ibedogodo e achiel

    _____Ochot manyien mane pok ibedogodo e achiel

19. Kuom kinde marom nade mane iseng'eyoe ng'atni? (_____)

20. Bende ne itiyo kod rabo oyunga?
    Ee Ooyo

SIGAND TER RUOK

21. Bende oteri nyange?
    Ee Ooyo
22. Karang'o manene oterie nyange? (_____)

23. Ang'o mane omiyo iyiero ni mondo oteri nyange?
    _____Geng'o tuoche

_____Kitwa gi timbewa
_____Ajali/ nikech yore mag thieth
_____Jonyuol ne ong'ado kamano
_____Mamoko (dimbi) _____

24. Bang' kinde maromo nade bang'dhi nyange mane ichakoe bet e achiel?
_____

25. Ne in jahigni adi mane ikwongo bet ei achiel mar ringruok?
(_____)

26. Ng'ama ne iterorigo ne ja higni adi? (_____)

27. Be ne itiyo gi kondom esa mane uteroru?
Ee Ooyo Ok apar

28. Iparo ni ma ema ne odiochienge mokuongo mar ter ruok?
Ee Ooyo

29. Ne uteroru kode nyadidi? (_____)

30. Winjruok u ni ne okawo kinde marom nade?

31. Ang'owa mane otieko/oketho osiepuni?
_____Ne ayudo jahera machielo manyien
_____Ne adhaw/ chwanyore
_____Ne ochodo
_____Ne akendo
_____Distance (Ne odhi/adhi mabor koda/ko)
_____Jonyuol nene opogowa
_____Pod wadhi mbele

32. Nyadidi ma ne utiyo gi kondom ka uriworu gi osiepini?
_____100% mar saa
_____75% mar saa
_____50% mar saa
_____Matinne 25%mar saa
_____Onge kata

33. Ji adi ma iseriwori godo ei de achiel mokalo (_____)

34. Ji adi ma iseriwori godo ei deche auchiel mokalo (_____)

35. Ji adi ma iseriwori godo ei higa achiel mokalo?(_____)

36. Ji adi ma iseriwori godo ei ngimani duto? (_____)

37. Bende isegachulo ng'ato, chiwo mich, rech, ber kata ratiro mar nyiewo/ng'iewo reyi ni mondo uriuru kode?
    Ee Ooyo

JI ADEK MOGIK MA IBEDOGO E ACHIEL E RINGRUOK

Koro adwa wuoyo kodi kuom ji mamoko ma ise terrorigo ma opogre gi jaodi ii dweche adek ma okalo. Parane ng'ato ma nyocha iriwori godo….

38. Karang'o mane tim mar betie achielni nene otimore?
    (___/___/___)

39. Kuom kinde madirom nade mane ing'eyoe jali kapok ubedo kode e achiel chieng' mokwongo?? (_____)

40. Bende ne imule/inyodhe ka iike ne bet e achiel kodi kane pok uchako bet e achielni??
    Ee Ooyo

41. Be ne Ichiege kimulo gire mar nyuol gi lueti?
    Ee Ooyo

42. Be ne ichiege kimulo gire mar nyuol gi lewi/dhogi?
    Ee Ooyo

43. Be ne ochiegi komulo giri mar nyuol gi lwete?
    Ee Ooyo

44. Be ne ochiegi konyodho giri mar nyuol gi dhoge?
    Ee Ooyo

45. Kane isoyo duong'ni be ne odhiek?
    Ee Ooyo

46. Ng'atni ne ja higni adi sechego? (_____)

47. Mon moko ong'ere ni chwero pige moko ka gitimo osiep, iparo ni in/jaherani nene ochuero pigego ka uteroru?
    _____Ee

_____Ok apar kamano

_____Samoro

_____Ooyo

48. Mang'eny marom nade ma ijariworigani kod jaheranini?(_____)

49. Be ng'atni ne en?

_____Jaherani

_____Osiepni mawiyewiye

_____Opamo/ochot

_____Jaherani ma dichwo

_____Mamoko (dimbi) _____

50. Bende jaheranini ne osetedo/osekendo chon kapok iromo kode?
Ee Ooyo

51. Bende jaheranini nene ni ei keny/okende/otedo e sama ne uriworu kode?
Ee Ooyo

52. Bende nene irwako rabo oyunga kapok uchako bedo e achiel mar ringruok?
Ee Ooyo

53. Bang' bedoe e achiel, bende nene ichiwo kata chiko ni ibiro chiwo gimoro ne jaheranino (pesa,ber, kata mich)?
Ee Ooyo Tamruok duoko

54. Ka ee, ne en ang'owa?

_____Pesa/omuom/manyonge/rupia

_____Singruok mar usone rech

_____Tere bayo /aut

_____Nyiewone wuoche kata lewni

_____Kende

_____Mamoko (dimbi) _____

55. Bende igeno mar bedo e achiel kod jaheranini kendo?
Ee Ooyo

Koro adwa wuoyo kodi kuom ji mamoko ma ise terrorigo ma opogre gi jaodi ii dweche adek ma okalo.parane ng'ato ma nyocha iriwori godo….

56. Karang'o mane tim mar betie achielni nene otimore?
(___/___/___)

57. Kuom kinde madirom nade mane ing'eyoe jali kapok ubedo kode e achiel chieng' mokwongo? (_____)

58. Bende ne imule/inyodhe ka iike ne bet e achiel kodi kane pok uchako bet e achielni?
Ee Ooyo

59. Be ne Ichiege kimulo gire mar nyuol gi lueti? (_____)
Ee Ooyo

60. Be ne ichiege kimulo gire mar nyuol gi lewi/dhogi? (_____)
Ee Ooyo

61. Be ne ochiegi komulo giri mar nyuol gi lwete? (_____)
Ee Ooyo

62. Be ne ochiegi konyodho giri mar nyuol gi dhoge? (_____)
Ee Ooyo

63. Kane isoyo duong'ni kuome, bende ne osegolo pi nyodo?
Ee Ooyo

64. Ng'atni ne ja higni adi sechego? (_____)

65. Mon moko ong'ere ni chwero pige moko ka gitimo osiep, iparo ni in/jaherani nene ochuero pigego ka uteroru?

_____Ee

_____Ok apar kamano

_____Samoro

_____Ooyo

66. Mang'eny marom nade ma ijariworiga kod jaheranini?
(_____)

67. Be ng'atni ne en?

_____Jaherani

_____Osiepni mawiyewiye

_____Opamo/ochot

_____Jaherani ma dichwo

_____Mamoko (dimbi) _____

68. Bende jaheranini ne osetedo/osekendo chon kapok iromo kode?
Ee Ooyo

69. Bende jaheranini nene ni ei keny/okende/otedo e sama ne uriworu kode?
Ee Ooyo

70. Bende nene irwako rabo oyunga kapok uchako bedo e achiel mar ringruok?
Ee Ooyo

71. Bang' bedoe e achiel, bende nene ichiwo kata singo ni ibiro chiwo gimoro ne jaheranino (pesa,ber, kata mich)?
Ee Ooyo Tamruok duoko

72. Ka ee, ne en ang'owa?
_____Pesa/omuom/manyonge/rupia

_____Singruok mar usone rech

_____Tere bayo /aut

_____Nyiewone wuoche kata lewni

_____Kende

_____Mamoko (dimbi) _____

73. Bende igeno mar bedo e achiel kod jaheranini kendo?
Ee Ooyo

Koro par ng'ato ma nene iriworigo mokuongo kapodi ok iriwori go ng'ama mararіyo nyaka ng'ato mogik.

74. Karang'o mane tim mar betie achielni nene otimore?
(___/___/___)

75. Kuom kinde madirom nade mane ing'eyoe jali kapok ubedo kode e achiel chieng' mokwongo? (_____)

76. Bende ne imule/inyodhe ka iike ne bet e achiel kodi kane pok uchako bet e achielni?
Ee Ooyo

77. Be ne Ichiege kimulo gire mar nyuol gi lueti? (_____)
Ee Ooyo

78. Be ne ichiege kimulo gire mar nyuol gi lewi/dhogi? (_____)
Ee Ooyo

79. Be ne ochiegi komulo giri mar nyuol gi lwete? (_____)
Ee Ooyo

80. Be ne ochiegi konyodho giri mar nyuol gi dhoge? (_____)
Ee Ooyo

81. Kane isoyo duong'ni kuome, bende ne osegolo pi nyodo?
Ee Ooyo

82. Ng'atni ne ja higni adi sechego? (_____)

83. Mon moko ong'ere ni chwero pige moko ka gitimo osiep, iparo ni in/jaherani nene ochuero pigego ka uteroru?
_____Ee
_____Ok apar kamano
_____Samoro
_____Ooyo

84. Mang'eny marom nade ma ijariworiga kod jaheranini? (_____)

85. Be ng'atni ne en?
_____Jaherani
_____Osiepni mawiyewiye
_____Opamo/ochot
_____Jaherani ma dichwo
Mamoko (dimbi) _

86. Bende jaheranini ne osetedo/osekendo chon kapok iromo kode?
Ee Ooyo

87. Bende jaheranini nene ni ei keny/okende/otedo e sama ne uriworu kode?
Ee Ooyo

88. Bende nene irwako rabo oyunga kapok uchako bedo e achiel mar ringruok?
Ee Ooyo

89. Bang' bedoe e achiel, bende nene ichiwo kata singo ni ibiro chiwo gimoro ne jaheranino (pesa,ber, kata mich))?
Ee Ooyo Tamruok duoko

90. Ka ee, ne en ang'owa?
_____Pesa/omuom/manyonge/rupia

_____Singruok mar usone rech

_____Tere bayo /aut

_____Nyiewone wuoche kata lewni

_____Kende

_____Mamoko (dimbi) _____

91. Bende igeno mar bedo e achiel kod jaheranini kendo?
Ee Ooyo

TICH GI RABUO YUNGA KOD VYACH KOD NG'ENY MAR KUTE MAG AYAKI

92. Karang'o mane ihangoe tiyo kod rabo oyunga? (_____)

93. Koriwote to nyadidi ma itiyo gi raboyunga kiriwori gi jaodi??

_____Seche te(100%)

_____Thoth seche(>75%)

_____Nus seche(50%)

_____Seche moko(>25%)

_____Seche matin(<25%)

_____Ok kata (0%)

94. Koriwo te to nyadidi ma itiyo gi rabo yunga ka iterori gi ng'at ma wendo?

_____Seche te(100%)

_____Thoth seche(>75%)

_____Nus seche(50%)

_____Seche moko(>25%)

_____Matin (<25%)

_____Ok kata(0%)

95. Bende osegapimi pim mar ng'iyo ka in kod kute mag ayaki?

Ee Ooyo

96. Bende isegabedo kod gima ne iparo ni en tuo mar nyach?

Ee Ooyo

97. Nene in kod higni adi kane ibedo kod gima ne iparo ni en tuo mar nyach? (_____)

98. Isebedo kod tuoche mag nyeye nyadidi chakre ibed kod mokwongo chande? (_____)

TIMBE MAG LER

99. In-gi opete (anda) adi? (_____)

100. Ilokoga inawea ndalo adi ei juma? (_____)

101. Iluokoriga nyadidi ei juma achiel? (_____)

102. Be ikawo thuolo mar luoko duong'ni samiluokori?
Ee Ooyo

103. Be iluokoga duong'ni kopogore gi saa ma iluokori?
Ee Ooyo

104. Ka ee, nyadidi ei juma? (_____)

105. Be iseluoko duong'ni sano sano kapok inyemo?
Ee Ooyo

106. Ka ee to didi mailuoko duong'ni ka pok inyemo?

_____Seche te

_____Moloyo nus seche

_____Nus seche

_____Mathin ne nus seche

107. Bende isegaluoko duong'ni e sama eka itieko bet e achiel?
Ee Ooyo

108. Ka ee, nyading'eny marom nade ma ijaluokoga duong'ni bang' bedoe e achiel?

_____Seche te

_____Mokalo nus mar seche

_____Nus seche

_____Matinne nus mar seche

Appendix VII: KEMRI Scientific Aprroval Letter

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: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/9478 **13th June, 2011**

Raphael O. Ondondo

Thro'
Director, CMR
NAIROBI

Forwarded
14-06-2011

REF: SSC No.2014 (Revised) – Evaluation of the dynamics and immune-virology of human papillomavirus infections among fishermen in Kisumu, Kenya

Thank you for your letter dated 30th May, 2011 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

FOR: **Sammy Njenga, PhD**
SECRETARY, SSC

Appendix VIII: KEMRI Initial Ethical Aprroval Letter

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: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **July 29, 2011**

TO: **RAPHAEL O ONDONDO,**
PRINCIPAL INVESTIGATOR

THRO': **DR. SAMUEL KARIUKI,**
THE DIRECTOR, CMR,
NAIROBI

RE: **SSC PROTOCOL NO. 2014 (*RE-SUBMISSION*): EVALUATION OF THE DYNAMICS AND IMMUNE-VIROLOGY OF HUMAN PAPILLOMAVIRUS INFECTIONS AMONG FISHERMEN IN KISUMU KENYA.**

Reference is made to your letter dated July 21, 2011.

The Committee is satisfied that the issues raised at the initial review are adequately addressed. The study is granted approval for implementation effective this **29th day of July 2011**. Please note that authorization to conduct this study will automatically expire on **28th July 2012**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **29th May 2012**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC.

You are also required to submit any proposed changes to this protocol to the ERC to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Sincerely,

Caroline Kithinji,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix IX: KEMRI Ethical Aprroval Renewal Letter – 2012

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: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **July 11, 2012**

TO: **MR. RAPHAEL O ONDONDO (PRINCIPAL INVESTIGATOR)**

THROUGH: **DR. SAMUEL KARIUKI, THE DIRECTOR, CMR, NAIROBI** forwarded 13/7/2012

Dear Sir,

RE: **SSC PROTOCOL No. 2014 (*REQUEST FOR STUDY RENEWAL*): EVALUATION OF THE DYNAMICS AND IMMUNE-VIROLOGY OF HUMAN PAPILLOMAVIRUS INFECTIONS AMONG FISHERMEN IN KISUMU KENYA**

Thank you for the Continuing Review Report for the period starting 29th July 2011.

This is to inform you that at the 204th meeting of the KEMRI Ethics Review Committee held on 10th July 2012, the Committee reviewed the above referenced application and made note of the following:

(a) 300 fishermen are enrolled in the study and are in their 6 to 9 month of follow up.
(b) The planned activity for the next project period is to continue with follow-up through their 12 month follow-up visit as well as laboratory sample testing and analysis.

The Committee was of the opinion that the progress made in the reporting period is satisfactory and that the risk/benefit status of the study remains favourable. Consequently, the study was granted approval for continuation effective the **10th day of July 2012.**

Please note that authorization to conduct this study will automatically expire on **July 9, 2013**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **May 28, 2013**.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the conduct of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

You may continue with the study.

DR. CHRISTINE WASUNNA,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

Appendix X: KEMRI Ethical Aprroval Renewal Letter – 2013

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: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **June 20, 2013**

TO: **MR. RAPHAEL O. ONDONDO (PRINCIPAL INVESTIGATOR)**

THROUGH: **DR. SAMUEL KARIUKI,**
THE DIRECTOR, CMR,
NAIROBI

Forwarded 2/7/13

Dear Sir,

RE: **SSC PROTOCOL No. 2014 (*REQUEST FOR STUDY RENEWAL*): EVALUATION OF THE DYNAMICS AND IMMUNO-VIROLOGY OF HUMAN PAPILLOMAVIRUS INFECTIONS AMONG FISHERMEN IN KISUMU, KENYA.**

Thank you for the Continuing Review Report for the period **May 2012 to May 2013**. This is to inform that during the 216th meeting of the KEMRI/ERC meeting held on the 20th of June 2013, the Committee **conducted the annual review and approved** the above referenced application for another year.

This approval is valid from today, **20th June 2013** through to **19th June 2014**. Please note that authorization to conduct this study will automatically expire on **19th June 2014**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the **ERC** secretariat by **8th May 2014**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Yours faithfully,

MR. AMBROSE RACHIER,
CHAIR,
KEMRI ETHICS REVIEW COMMITTEE

Appendix XI: UCSF Ethical Aprroval letter

UCSF
University of California
San Francisco

Human Research Protection Program
Committee on Human Research

Notification of Expedited Review Approval

Principal Investigator	*Co-Principal Investigator*
Anna-Barbara Moscicki	Raphael O Ondondo

Type of Submission: Initial Review Submission Packet

Study Title: Evaluation of the Dynamics and Immuno-Virology of Human Papillomavirus Infections among Fishermen in Kisumu, Kenya

IRB #: 12-08590
Reference #: 040563

Committee of Record: Parnassus Panel

Study Risk Assignment: Minimal

Approval Date: 03/15/2012 **Expiration Date:** 03/14/2013

Regulatory Determinations Pertaining to this Approval:

This research is not subject to HIPAA.

A waiver or alteration of informed consent is acceptable because, as detailed in the application: (1) the research involves no more than minimal risk to the subjects; (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects; (3) the research could not practicably be carried out without the waiver or alteration; and (4) whenever appropriate, the subjects will be provided with additional pertinent information after participation. The waiver or alteration of informed consent applies to all subjects.

IRB Comments:

All changes to a study must receive CHR approval before they are implemented. Follow the modification request instructions. The only exception to the requirement for prior CHR review and approval is when the changes are necessary to eliminate apparent immediate hazards to the subject (45 CFR 46.103.b.4, 21 CFR 56.108.a). In such cases, report the actions taken by following these instructions.

Expiration Notice: The iMedRIS system will generate an email notification eight weeks prior to the expiration of this study's approval. However, it is your responsibility to ensure that an application for continuing review approval has been submitted by the required time. In addition, you are required to submit a study closeout report at the completion of the project.

Approved Documents: To obtain a list of documents that were approved with this submission, follow these steps: Go to My Studies and open the study – Click on Submissions History – Go to Completed Submissions – Locate this submission and click on the Details button to view a list of submitted documents and their outcomes.

Appendix XII: UCSF Ethical Aprroval Renewal Letter– 2013

UCSF
University of California
San Francisco

Human Research Protection Program
Committee on Human Research

Notification of Expedited Review Approval

Principal Investigator	*Co-Principal Investigator*
Anna-Barbara Moscicki	Raphael O Ondondo

Type of Submission: Submission Correction for Continuing Review Submission Form
Study Title: Evaluation of the Dynamics and Immuno-Virology of Human Papillomavirus Infections among Fishermen in Kisumu, Kenya

IRB #: 12-08590
Reference #: 062225

Committee of Record: Parnassus Panel

Study Risk Assignment: Minimal

Approval Date: 02/25/2013 **Expiration Date:** 03/14/2016

Regulatory Determinations Pertaining to this Approval:

- This research is not subject to HIPAA rules.
- A waiver or alteration of informed consent is acceptable because, as detailed in the application: (1) the research involves no more than minimal risk to the subjects; (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects; (3) the research could not practicably be carried out without the waiver or alteration; and (4) whenever appropriate, the subjects will be provided with additional pertinent information after participation.
- The waiver or alteration of informed consent applies to all subjects.

All changes to a study must receive CHR approval before they are implemented. Follow the modification request instructions. The only exception to the requirement for prior CHR review and approval is when the changes are necessary to eliminate apparent immediate hazards to the subject (45 CFR 46.103.b.4, 21 CFR 56.108.a). In such cases, report the actions taken by following these instructions.

Expiration Notice: The iMedRIS system will generate an email notification eight weeks prior to the expiration of this study's approval. However, it is your responsibility to ensure that an application for continuing review approval has been submitted by the required time. In addition, you are required to submit a study closeout report at the completion of the project.

Approved Documents: To obtain a list of documents that were approved with this submission, follow these steps: Go to My Studies and open the study – Click on Submissions History – Go to Completed Submissions – Locate this submission and click on the Details button to view a list of submitted documents and their outcomes.

For a list of all currently approved documents, follow these steps: Go to My Studies and open the study – Click on Informed Consent to obtain a list of approved consent documents and Other Study Documents for a list of other approved documents.

Appendix XIII: KEMRI Specimen shipment Export permit Aprroval – 2012

SSC 2014 (SHIPMENT)

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Microbiology Research, P.O. Box 19464 - 00202, NAIROBI - Kenya,
Tel: (254) (020) 2720794, 2720038, Nairobi E-mail: cmr@insightkenya.com Website: www.kemri.org

KEMRI / ERC RECEIVED 04 FEB 2013 P.O. Box 54840-00200, NAIROBI

REF: KEMRI/CMR/RCTP/FM HPV/SP 2013/01

Monday ,4th February, 2013

THE CHAIRPERSON
KEMRI SSC
NAIROBI

Through
Director,
CMR

Forwarded 04/02/2013
Prof. B.M. NZUGI
for DCMR

RE: SSC Protocol No. 2014 – Evaluation of the Dynamics and Immuno-virology of Human papillomavirus infection among Fishermen in Kisumu Kenya by Raphael Ondondo (CMR) approval to export the study subjects' specimens

Thank you for your letter approving the implementation of the above protocol. As part of participant follow up, several specimens were collected from fishermen in this study. These include, genital swabs for HPV molecular testing and genotyping, serum for HPV antibody testing and Peripheral Blood mononucleated cells (PBMC) for HPV immunology experiments. Subjects' specimen's aliquots were archived for use at UCSF for confirmatory testing and training purposes for the PI who is a PhD student at JKUAT.

- Genital Swabs for HPV molecular and genotypic testing
- Serum aliquots for HPV antibody testing
- PBMC aliquots in cryovials for HPV immunology experiments

We will be shipping a total of 2740 specimens in **Dry ice** and **Dry shipper**. The shipping will be done just once at the end of participant follow up. The attached form requesting for approval to export these biological materials for research (Form SSC 1/91d) shows the individual specimens, the quantity and the recipient institution for these specimens as required.

We hope our request for an approval covering three months to facilitate our screening process will be met by your most sincere consideration

Yours faithfully,

Raphael Ondondo
Research Scientist (KEMRI CMR RCTP), PhD student JKUAT
PI, HPV STUDY

Cc Secretary SSC

APPENDIX VI **Form SSC 1/91d**

KENYA MEDICAL RESEARCH INSTITUTE

SCIENTIFIC STEERING COMMITTEE (SSC)

REQUEST FOR EXPORTATION OR STORAGE OF HUMAN BLOOD AND OTHER BIOLOGICAL MATERIALS FOR RESEARCH

PART A: Project Information

i. **Project Title:** Evaluation of the Dynamics and Immuno-virology of Human papillomavirus infection among Fishermen in Kisumu Kenya. SSC No.2014 Approval date 29th July 2011

ii. **KEMRI Centre of Affiliation:** Centre for Microbiology Research (CMR)

iii. **Principal Investigator(s):**

1. Raphael Ondondo – PhD Student JKUAT

iv. **Other Investigators: Supervisors**

Dr. Elizabeth Bukusi (**Supervisors)**

1. ----------

Dr. Solomon Mpoke (**Supervisors**)

2. ----------

Prof. Zipporah Ng'ang'a (**Supervisors**)

3. ----------

4. ----------

5. ----------

PART B: Specimen Details:

i. Is the request for specimen exportation or storage or both?

For both Exportation and for participants consenting for long term storage

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(Request for storage is necessary if the samples are to be stored beyond the duration of the present study)

ii.Description of specimen(s) to be exported/stored:

Genital swabs, serum PBMCS, for confirmatory genotypic HPV and immunology testing as well as quality control to be exported to UCSF for further analysis and enable PhD training for the PI

iii. Reason(s) for exportation/storage of samples:

1. This tests require sophisticated and advanced laboratory facilities and for training of the PI to enable technology and expertise transfer. The PI is a PhD student in Molecular Medicine at JKUAT and is traveling to receive advance laboratory mentorship and training at UCSF
2. The findings in the study site laboratories will be verified at the central labs for quality controls.

iv. Duration of specimen storage: The study will run for Three years, thereafter all confirmatory evaluation have been done the specimens will be destroyed as per the study protocol and local guidelines on specimen destructions.

v. For samples originating from human subjects, state whether or not written consent for specimens exportation or storage: All the specimens were collected for this study had consents obtained from the participants prior to study participation for exportation of samples for confirmatory and quality assurance testing. Also enabling training for the PhD student..

vi. Name and address of recipient institution/department responsible for the specimens:

Anna-Barbara Moscicki University of California San Francisco, 513 Parnassus, HSW 1419, San Francisco, CA 94143, Tel: +1 415 476-3260), Fax: +1 415-502-1222

vii. Name(s) and address of person(s) responsible for the specimens in the recipient institution:

1. **Prof Anna-Barbara Moscicki** , University of California San Francisco, CA USA

2.

vi. Name and role in the project of the Kenyan investigator(s) expected to carry out investigations on the specimens in the overseas institution:

Raphael Ondondo, is traveling with the samples to UCSF in the USA to perform all the investigation as part of his PhD training and will ensure that all specimens sent to the above mentioned institutions are used for the stated research and specimens destined for destruction are destroyed as per the study protocol and local guidelines.

PART C: Declarations: (To be completed at the time of shipping samples)

i. Declaration by the person requesting exportation/storage of research specimens:

I certify that the information provided in this request form is true and correct to the best of my knowledge, and I hereby declare that the specimens referred to herein will be utilized for the stated purpose only

Name: Raphael Ondondo **Role in the Project:** PI

Signature: _____ **Date:** 22 November, 2012

ii. Declaration by Recipient Institution:

This is to certify that the specimens referred to herein being sent to **University of California San Francisco, 513 Parnassus, HSW 1419, San Francisco, CA 94143, Tel: +1 415 476-3260), Fax: +1 415-502-1222** (Name of Institution) for further analyses/experimentation will be in the custody of the Department of ***Global Health UCSF***, and I hereby confirm that they will be utilized for the purpose stated in this request form, and I accept full responsibility and control over the usage of these samples

Name of Department/Institution Head: Prof. Anna-Barbara Moscicki, UCSF Mentor for Raphael Ondondo who is the Principal Investigator

Signature: _____ **Date:** 24th November, 2012

iii. Declaration by Centre Director:

I certify that the protocol SSC No **2014** referred to in this request was approved by the Centre's Scientific Committee on **29th July 2011** and that the request to export the biological specimens referred to in this request was found to be valid and justifiable. I further confirm that the study participants in this project have consented in writing to the exportation/ storage of samples taken from them, for use in further research.

Name: Dr. B.M. Ngugi & D/CMR **Signature:** _____

Centre: CMR **Date:** 04/02/2013

PART D: (For SSC and ERC Use Only)

i. Request Considered and Approved by the SSC during its Meeting Held on

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ii. Request Forwarded to the Ethical Review Committee (ERC) for consideration on

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iii. Request Approved by the ERC on ----------

iv. Request Considered and Deferred Due to the Following Reasons:

1) ----------

2) ----------

3) ----------

4) ----------

5) ----------

PART E: Approvals

Request Approved By:

i. Chairman, SSC: ---------- **Date:** 25/2/2013

ii. Chairman, ERC: ---------- **Date:** 26/2/2013

iii. Director, KEMRI: ---------- **Date:** 27/2/2013

KENYA MEDICAL RESEARCH INSTITUTE

KENYA MEDICAL RESEARCH INSTITUTE SCIENTIFIC STEERING COMMITTEE (SSC)

AUTHORITY TO EXPORT BIOMEDICAL RESEARCH MATERIALS*

This is to certify that **Raphael Ondondo**

Principal Investigator/Co-Principal Investigator/Investigator in the research project titled:

Evaluation of the Dynamics and Immuno-virology of Human papillomavirus infection among Fishermen in Kisumu Kenya **SSC No**.2014 ***undertaken in collaboration with the Kenya Medical Research Institute (KEMRI)*** has been granted permission to send out:

The shipment will contain 2, 740 specimens. 1400 genital swabs, 670 (2ml vials) each cryovials containing 0.5mls of serum and 670 (2ml vials) each vial containing 1 ml of PBMC aliquots, at this shipment scheduled. (Number and Description of the Samples)

To: Anna-Barbara Moscicki University of California San Francisco, 513 Parnassus, HSW 1419, San Francisco, CA 94143, Tel: +1 415 476-3260), Fax: +1 415-502-1222
(Name of Department and/or Institution)

In **USA** (Country of Destination) for the purpose of: *Genital swabs for HPV genotypic confirmatory testing, Serum for confirmatory testing of antibody repose to HPV infection and PBMC aliquots for immunology experiments.*
(Description of the type(s) of investigations or analysis to be conducted on the samples)

This certificate is issued with the understanding that the investigator will not use the samples for purposes other than those stated above. The investigator will submit a copy of the results of the investigations/analyses undertaken on these samples to the Director, KEMRI; and will ensure that KEMRI's intellectual property rights arising from work on the stated samples will be protected and safeguarded, and the findings thereof are published with the approval of the Director, KEMRI.

Recommended by: DR B-M. NGUGI 04/02/2013

Name and Signature of Centre Director **Date**

Authorized by: Solomon Mpoke 27/2/2013

Name and Signature of Director, KEMRI **Date**

KENYA MEDICAL RESEARCH INSTITUTE

***This certificate is valid for a period of 30 (thirty) days with effect from the date of authorization. Please direct any queries to the Director, KEMRI, PO Box 54840-00200 Nairobi, Kenya; Phone:**
(254-20) – 2722541; Fax: (254-20) – 2720030; E-mail: director@kemri.org.

Appendix XIV: USA CDC Specimen shipment Import permit Approval

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
Centers for Disease Control and Prevention
Office of Health and Safety, MS A-46
Atlanta, Georgia 30333
TEL: 404-718-2077; FAX: 404-718-2093

CDC
SAFER·HEALTHIER·PEOPLE™

Permit to Import or Transfer Etiological Agents or Vectors of Human Disease

In accordance with 42 CFR Section 71.54 of the Public Health Service Foreign Quarantine Regulators, cited on the bottom of this permit, permission is granted the permittee to import into any port under control of the United States, or to receive by transfer within the United States, the material described in Item 1 below.

PHS PERMIT NO.	2013-01-010	
DATES	ISSUED: Tuesday, January 08, 2013	EXPIRES: Wednesday, January 08, 2014
1. DESCRIPTION OF MATERIAL	HUMAN BLOOD AND SWABS THAT MAY CONTAIN HUMAN PAPILLOMA VIRUS (HPV).	
2. PERMITTEE (NAME, ORGANIZATION, ADDRESS)	ANNA-BARBARA MOSCICKI UNIVERSITY OF CALIFORNIA SAN FRANCISCO 513 PARNASSUS, HSW 1419 SAN FRANCISCO, CA 94143	TEL: 415-476-3260 FAX: 415-502-1222
3. SOURCE OF MATERIAL (NAME, ORGANIZATION, ADDRESS, COUNTRY)	RAPHAEL O. ONDONDO KEMRI-UCSF-RCTP, RESEARCH CARE AND TRAINING PROGRAM LUMUMBA HEALTH CENTER, AGOI ST. KISUMU, KENYA	
4. TYPE OF PERMIT AND INSTRUCTIONS FOR USE	☑ Multiple Importation into the US ☐ Single Transfer Within the US A. Record of each importation shall be maintained on permanent file by permittee. B. Enclosed label(s) must be forwarded to the shipper(s). C. One label shall be affixed to shipping container. Enclosed labels may be photocopied.	
5. CONDITIONS OF ISSUANCE ITEMS APPLICABLE WHEN CHECKED	☐ A. Subsequent distribution, within the U.S., of the material described in this permit is prohibited without prior authorization by the Public Health Service. ☑ B. All material is for laboratory use only - Not for use in the production of biologics for humans or animals. ☑ C. All material is free of tissues, serum and plasma of domestic and wild ruminants, swine and equines. ☐ D. Additional Requirements: ☐ File APHIS/CDC Form 2 for select agents as defined in 42 CFR 73 ☐ IATA Packaged to preclude escape. ☐ USDA permit may be required (Telephone: 301-734-3277). ☑ E. Work with the agent(s) described shall be restricted to areas and conditions meeting requirements in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories." ☑ F. Packaging must conform to 49 CFR Sections 171-180. ☐ G. Select Agent. Receiving facility must be registered under 42 CFR Part 73.	
6. COPY SENT TO ☑ U.S. QUARANTINE STATION	7. Signature of issuing officer Robbin S. Weyant, PhD, RBP (ABSA) Captain, USPHS (Ret.) Etiologic Agent Import Permit Program	

CDC 0728 (F 13.40) REV. 2-91

42 CFR 71.54. Etiological agents, hosts, and vectors

(a) A person may not import into the United States, nor distribute after importation, any etiological agent or any arthropod or other animal host or vector of human disease, or any exotic living arthropod or other animal capable of being a host or vector of human disease unless accompanied by a permit issued by the Director.

(b) Any import coming within the provisions of the section will not be released from custody prior to receipt by the District Director of the U.S. Customs Service of a permit issued by the Director.

Note: Other permits may be required.

Appendix XV: PBMC Processing and Cryopreservation Protocol

Definitions:

PBMC – Peripheral blood mononuclear cells
FBS – foetal bovine serum (Gibco, Invitrogen)
v/v – volume/volume
rpm – rotations per minute
DMSO – dimethylsulfoxide (Sigma Aldritch)
Ficoll – Density gradient media (Histopaque, Sigma Aldritch)
PBS – Phosphate buffered saline (Gibco, Invitrogen)
CPS – Cell preservation solution
PTID – Participant identification number

Procedure:

Whole blood drawn in acid citrate dextrose tubes(ACD TUBES-BD Vacutainer®Blood CollectionTube)

1. Prepare and chill the CPS. (FBS+DMSO 9:1)
2. Ensure that empty Mr. Frosty's are in the 4 degree refrigerator at start.
3. Take out Ficoll at start of the day and let it warm to room temperature. Prepare 15 mL aliquots in 50 mL conical tubes ahead time and store in fridge – each day, take out enough for the day; smaller volumes warm up to room temperature more quickly than the full Ficoll bottle.
4. Label each 50 mL tube containing 15 mL of Ficoll with the right PTID
5. Carefully and slowly overlay the whole blood (~ 25 mL) on top of the Ficoll in 50 mL tubes.
6. Centrifuge at 2000RPM for 20 minutes at Room Temperature (22-25°C) with the Brake OFF.
7. From each Ficoll tube, aliquot 4 plasma cryotubes of 1mL each per Ficoll tube. This should be done with a 5mL pipette. After plasma has been aliquoted, aspirate the remaining plasma without disturbing the buffy coat.
8. With a 10mL pipette, harvest each buffy coat into a corresponding, single, labelled 50mL conical centrifuge tube.
9. Add sufficient quantity of PBS to a total volume of 45 mL and mix gently.
10. Wash #1—centrifuge at 1500RPM for 10 minutes at 22-25°C. Set brake to high. Check for the cell pellets!

Gently remove the supernatant without disturbing the cell pellet.

11. Re-suspend the cell pellet in small amount of PBS making a homogenous cell suspension.
12. Add PBS to approximately 45 mL to the harvested cell tube.
13. Wash #2— centrifuge at 1500RPM for 10 minutes at 22-25°C. Set brake to high. Check for the cell pellets!

Gently remove the supernatant without disturbing the cell pellet.

14. Re-suspend the cells pellets in 10mL volume (V) of PBS. This is the volume on which the cell count is based. Count the cells as follows:
    A. Hemacytometer Viability cell counting
        i. NOTE: Trypan Blue is one of several stains recommended for use in dye exclusion procedures for viable cell counting. This method is based on the principle that live (viable) cells do not take-up certain dyes, whereas dead (non-viable) cells do.
        ii. Add 20 uL of 0.4% trypan blue in a microplate well and then add 20 ul of cell suspension and mix.
        iii. Apply 10 uL of the trypan blue/cell mixture to a hemacytometer, using a cover slip. This volume will ensure that the hemacytometer is not overfilled. Place the hemacytometer on the stage of a binocular microscope and focus on the cells using a 10X objective. Pick one large outer corner, which have a fairly even distribution of cells.
        iv. Use a 10X objective to count cells in that large outer corner (which contain 16 smaller squares [4 x 4]). Include cells either touching the top line or left vertical perimeter line of any square. Do NOT count any cells, which touch either the bottom line or right vertical perimeter line of any corner square.
    B. Load onto the hemacytometer and view at 10X.
    C. Count the unstained (viable) and stained (non-viable) cells. Observe the cell morphology. Note counts on specimen processing worksheet
        Note: if the cells in one large corner square are less than 100, count cells in the diagonal corner as well.

15. Calculate the total number of viable cells

    T=[N/S]xDFx104xV

    T=Total cell count/ml

    N=number of viable cells counted

    S=number of large squares counted

    DF= Stain /cells dilution factor

    V=PBS resuspension volume

CF=Hemocytometer volume correction factor = 10^4

16. Calculate the percentage of viable cells as follows:

    Viable cells (%) = (viable cells/total cells counted) x 100

    Record the viability on the processing worksheet

17. Based on the total number of cells, determine the concentration of cells/mL in resuspension volume.

    Concentration (cells/mL) = Total # of cells/resuspension volume (10mL)

18. Centrifuge at 1500 RPM for 10min at 22-25°C. Set brake to high
19. Gently remove the supernatant without disturbing the cell pellet.
20. Gently re-suspend the pellet in 2 mL of cold CPS while swirling the tube for even distribution.
21. Gently make 2 CPS-cell aliquots.
22. Immediately (< 3 minutes) transfer all cryovials to the controlled rate freezing equipment (Mr. Frosty) and begin freezing.

NB WARNING-DMSO is highly toxic to cells. Minimize time that cells are on DMSO at room temperature.

23. Store the samples in Mr. Frosty overnight at 80°C.
24. The following morning remove the samples from Mr. Frosty and place the samples in pre-labeled and pre-chilled 10x10 cryoboxes. If not transferred the following morning, ensure that specimens are transferred from Mr. Frosty within 3 days maximum.
25. Transfer cryovials to the onsite liquid nitrogen storage tanks (LN_2).

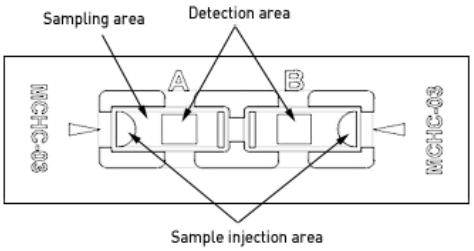
MANDATORY rules to follow for freezing samples into LN_2:

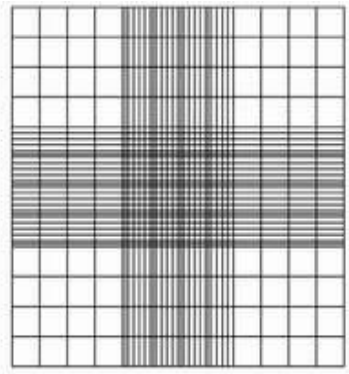
1. *If transferring to empty box*: place empty box into LN_2 tank for 10-15 minutes to allow box to cool down to temperature. Box must be at LN_2 temperature before being filled with samples.
2. Only transfer specimens from one Mr. Frosty at a time. Move Mr. Frosty to -80 freezer in the lab with the LN_2 tank so that there is minimal time between transferring of vials.
3. Place samples from one Mr. Frosty into LN_2 box in no more than 2-3 minutes.
4. After transfer to LN_2, replace LN_2 rack into the tank and wait for 15-20 minutes to allow vials to cool down to LN_2 temperature. Only after these 15-20 minutes should the next Mr. Frosty be pulled out of -80 freezer and its contents transferred.

5. Repeat steps 4 and 5 until all samples from previous day have been transferred to LN_2.
6. Once a Mr. Frosty has been used, place at room temperature for it to thaw and then put back into 4 degree refrigerator.

PBMC Cell counting: hemocytometer view at 10X

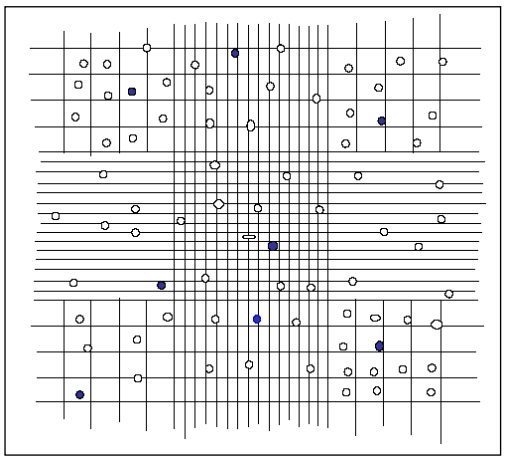
Blank Hemocytometer





PBMC counting – Loaded hemocytometer view at 10X

Count the number of live cells in all the four outer diagonal corner 4x4 squares using the 40x objective.



Appendix XVI: IFN-Gamma EliSpot Protocol

1. Coat a sterile Multi-Screen plate (Millipore, MAHA S4510- 0.45 µM {Millipore Ireland BV, Tullagreen, Carrigtwohill, County Cork, Ireland}) with 100 µL/well, mouse anti-human IFN-gamma antibody: Mab 1-D1K (Mabtech, 3420-3-250 {Anti-human IFN-g mAb 250ul: 1 µg/mL, Mabtech USA) at 16 µg/mL in PBS w/o Ca++, Mg++) overnight at 4 °C. (To prepare Ab solution, use 32 µL/2mL PBS). (Avoid making bubbles in wells and pop bubbles with needle.)
2. Decant the plate in a waste container, blot a few times on a paper towel and wash 4 times with PBS, 200 µL/well.
3. Block in 50 µL/well RPMI/5% filtered pooled human serum (PHS). Block for at least 1 hour at 37 °C.
4. Thaw the cells and wash them in 5 mL RPMI/10% FBS.
5. Resuspent the cells in mL RPMI/10% FBS
6. Count the cells using Trypan Blue exclusion.
7. Resuspend the cells in RPMI/10%FBS in volume needed for $3x10^5$ cells/100 µL. Count of the cell / mL=total count x 10^4 x 4 chambers x 2 x number of mL
8. Add 5 µL per well of each rIL2 (at 40 U/mL) and rIL7 (at 5 µg/mL) to the PBMCs to be seeded (at a total volume of 110 µL PBMC-IL2-IL7 mixture/well)
9. Distribute cells to duplicate wells using 110 µL ($3x10^5$ cells containing IL-2 and IL-7) per well. See table for ELISPOT plate template below.
10. Add 100 µL of the respective antigens to the test wells (for E6 that has 22 peptide pool; 1100 µg/mL = 50 µg/mL of each peptide)
11. Return the plate to the 37 °C, 5% CO2 incubator for overnight incubation.
12. The following day, decant the plate in a waste container, blot a few times on a paper towel and wash 4 times with 0.05%Tween-20/PBS.
13. Incubate with 100 µL/well of 5 µg/ml 7-B6-1 (Mabtech, 7-B6-1-Biotin {Mabtech USA, Biotin mouse anti- human IFN-γ mAb at 250

µL:1mg/mL)/PBS (no Tween) for 2 hours at 37 °C. (To prepare the Ab, add 20 µL/2mL of PBS).

Plate template for the ELISPOT IFN-gamma assay: Wells in Duplicate

Well 1	Well 2
Pos. Control 10ul of PHA 5ul of rIL-2 5ul of rIL-7	**Pos. Control** 10ul of PHA 5ul of rIL-2 5ul of rIL-7
E6-peptide pool 100ul (12.5 ug/ml) 5ul of rIL-2 5ul of rIL-7	**E6-peptide pool** 100ul (12.5 ug/ml) 5ul of rIL-2 5ul of rIL-7
E6-peptide pool 100ul (6.25 ug/ml) 5ul of rIL-2 5ul of rIL-7	**E6-peptide pool** 100ul (6.25 ug/ml) 5ul of rIL-2 5ul of rIL-7
E6-peptide pool 100ul (3.125 ug/ml) 5ul of rIL-2 5ul of rIL-7	**E6-peptide pool** 100ul (3.125 ug/ml) 5ul of rIL-2 5ul of rIL-7
Negative Control 100ul (R10) 5ul of rIL-2 5ul of rIL-7	**Negative Control** 100ul (R10) 5ul of rIL-2 5ul of rIL-7

Increasing concentration of the HPV E6 peptide pool

14. Twenty minutes before the end of this incubation, prepare the Vectastain Avidin-Peroxidase Complex (Vector lab, Elite PK-6100) by adding 1 drop each of solution A and solution B to 5 mL 0.1% Tween-20/PBS.

15. Decant the plate in a waste container, blot a few times on a paper towel and wash 4 times with 0.1%Tween-20/PBS.
16. Incubate with 100 µL/well Avidin-Peroxidase Complex, prepared above, for 1 hour in the 37 °C, 5% CO2 incubator
17. Repeat step 16.
18. Add 100 µL/well stable DAB (Invitrogen, 750118) for 10 minutes at room temperature. DAB is light sensitive, so cover the plate with paper towel to keep the light away.
19. Decant the plate in a waste container, blot a few times on a paper towel and wash 4 times with deionized water and allow to dry overnight.
20. Count spot manually, using a dissection microscope or by automated reader (EliSpot Series 4 Analyzer, Cell Technology, Inc, Jessup, MD).
21. Calculate the average spot-forming cells (SFC) per antigen.
22. The test is scored positive if the mean SFC of each antigen in twice the mean SFC of the background.
23. Adjust the SFC per $1x10^6$.

Appendix XVII: Publications

This page was intentionally left blank. Please see next page for Publications