UNIVERSITY OF PUERTO RICO

MEDICAL SCIENCES CAMPUS

GRADUATE SCHOOL OF PUBLIC HEALTH

DEPARTMENT OF BIOSTATISTICS AND EPIDEMIOLOGY

CROSS-SECTIONAL STUDY OF GENITAL HPV INFECTION AMONG HIV POSITIVE WOMEN WHO ATTENDED AN INVESTIGATIONAL CLINIC AT THE UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCE CAMPUS, 2009-2010

ANN MARIE SCORSONE

JULY, 2012

Mentor Certification

We hereby certify that this original investigation entitled, *"Cross-Sectional Study of Genital HPV Infection among HIV Positive Women who Attended an Investigational Clinic at the University of Puerto Rico, Medical Science Campus, 2009-2010"* completed by Ann Marie Scorsone and presented in this document, serves as a partial requirement of the degree of Masters of Science in Epidemiology and meets all the requirements of the Department of Biostatistics and Epidemiology of the Graduate School of Public Health, from the Medical Sciences Campus of the University of Puerto Rico.

Ana Patricia Ortiz, PhD, MPH Date
Associate Professor
Department of Biostatistics and Epidemiology
Graduate School of Public Health
Medical Science Campus
University of Puerto Rico

Heidi Venegas, DrPh, MS Date
Assistant Professor
Department of Biostatistics and Epidemiology
Graduate School of Public Health
Medical Science Campus
University of Puerto Rico

A B S T R A C T

Background: Human Papillomavirus (HPV) is the most common sexually transmitted infection in the US. Infections with high-risk HPV genotypes are known to be strongly associated with cervical cancer. HPV-DNA has also been detected in the tissue of several other non-cervical cancers, including cancers of the vulva, penis, vagina, anus, oral cavity, pharynx, and larynx. Even though population-based data on HPV infection is currently nonexistent, the prevalence of HPV among US women ages 14-59 is an estimated 27%; the prevalence of high-risk HPV in this same population is 15%. Research shows that that the prevalence of HPV among HIV-infected individuals is even higher than in the general population. The risk of cervical cancer is also higher among HIV positive women. Common labs, like the CD4 counts and HIV viral load are not routinely utilized in the gynecological care of HIV positive women; despite research showing a link between these biomarkers and HPV infection, and its persistence in this population. The association between HPV and these immunological biomarkers has yet to be fully explained; and further research is needed to properly treat HPV infection in HIV positive women.

In Puerto Rico, there exists no population-based data of HPV incidence or prevalence. Despite the high burden of HIV/AIDS in Puerto Rico, information on HPV prevalence in this high risk population is also scant. This study aimed to describe the prevalence (overall and type-specific) of cervical HPV infection and its association with CD4 count, HIV viral load, and highly active antiretroviral therapy (HAART) among a clinic-based sample of adult HIV positive women living in Puerto Rico.

Methodology: Cross-sectional study with a sample size of 130 consecutive women, 21 and over, who attended the Maternal Infant Studies Center at the UPR-RCM, a longitudinal gynecological/obstetric clinic for HIV positive women, from September, 2009 to February, 2010. Data was collected during a routine scheduled clinical visit. Women who gave consent completed

two self-administered questionnaires; one collected mainly socio-demographic information, and the second focused on information related to factors known to be associated with HPV positivity. The women met with a clinician who completed a pelvic exam, collected HPV and cervical cytology samples. HPV samples were collected with an hc2 HPV DNA Collection Device. The cervical cytology sample was collected following the clinic's standard protocol; serological samples for CD4 count and viral load were collected by a study nurse, and these samples were sent to the Department of Health for laboratory analysis. HPV samples were sent to the Ponce School of Medicine for HPV-PCR Linear Array genotyping using Roche Linear Array HPV Genotyping kits. Data was abstracted from the study questionnaires, the clinical source documents, and patient's charts and entered into SPSS 18.0 for analysis.

Results: The mean age of participants was 39 years (± 10.8). The majority of the women had undetectable HIV viral load (51.5%), a mean CD4 count of 570 (± 535.1), 60% of the women had normal cervical cytology at the time of the visit; and 75.4% of the women reported using HAART to manage their HIV. The overall HPV prevalence of the study was 57.7% (95% CI: 49.07, 65.97). Among those HPV infected, the prevalence of high-risk HPV was 58.7% (95% CI: 47.29, 69.38); and the prevalence of low-risk HPV was 85.3% (95% CI: 75.94, 92.04), the prevalence of multiple HPV genotype infections was 66.7% (95% CI: 55.45, 76.62); and 50.7% of the HPV infected women had normal cervical cytology. The ten most common HPV genotypes found among the study participants were (in order of frequency) HPV-61 (11%), -66 (11%), 6 (9%), 53 (9%), 62 (9%), 81 (9%), 16 (8%), 18 (8%), 52 (8%), and 70 (8%). Those genotypes currently covered by FDA approved vaccines (-6, -11, -16, -18) accounted for 36% of all HPV infections. Multivariate analysis showed that HIV positive women who had detectable HIV viral loads and CD4 counts $\leq$ 350 were almost four times more likely to have HPV than their counterparts who had detectable HIV viral loads and CD4s $\geq$ 350, while adjusting for age, cervical cytology, and lifetime number of sexual partners

(OR_a=3.90, 95%CI 0.91, 16.78). Yet, these results did not reach statistical significance. Among women with undetectable HIV viral load, there was no statistical difference between these CD4 groups in terms of odds for HPV positivity (OR=1.81. 95% CI 0.24, 13.49). There was no association between use of HAART and HPV infection.

Conclusions: This is the first investigation, to our knowledge, that describes the prevalence and HPV genotype distribution among a sample of HIV positive women living in Puerto Rico. While the prevalence of HPV in our clinic-based study population (58%) is much higher than that of the US general population (27%), it is lower than several prevalence estimates documented in various international studies investigating HPV infections in HIV positive women. The wide ranges in prevalence of HPV infections in HIV positive women have been documented to be 36% - 97%, and dependent largely on the sample and geographical locale. Even though use of HAART was not associated to HPV infection in our study, we did see evidence that those women with CD4 counts below 350 and with detectable HIV viral load are more likely to be HPV infected, however our limitation in sample size did render a p-value of 0.068. Regardless, our findings support that these two variables as readily accessible biomarkers that should be considered and monitored in the gynecological care of women living with HIV. Public health efforts that target HIV positive women in Puerto Rico should be developed in order to have an impact on the prevention of HPV infection and related malignancies in this population. These efforts should include patient education, HPV vaccination and continued promotion of cervical cancer screening.

A C K N O W L E D E G E M N T S

First and foremost I would like to express my sincere gratitude to the women of CEMI. Without their commitment to the advancement of care for HIV positive women, this study would have never been accomplished. The women who go to CEMI for services, along with those who work there, will forever hold a special place in my heart. I am truly appreciative for the collaborative spirit with which took to see this study through.

I would especially like to thank Dr. Carmen D. Zorrilla, the Principal Investigator and Clinical Director of CEMI. She took me under her wing, and if it were not for all of her assistance this study would have never become a reality. If not for Dr. Zorrilla's support, guidance and wisdom this investigation would have not been successful. I learned so much by working with her over the last five years, and she has truly helped to forge me into a clinical researcher dedicated to the healthcare of HIV positive women.

I would also like to genuinely thank my dear friend, Dr. Vivian Tamayo, the sub-clinical director of CEMI. Vivian you are an amazing clinician, brilliant teacher, and a wonderful human being. Your passion for the care of HIV positive women is truly contagious, and I thank you for bringing such clinical insight and expertise to this investigation. Our friendship nurtured this research from concepts to completion. Thank you for welcoming me into your home and making me feel a part of your beautiful family.

I would like to acknowledge the Puerto Rico Comprehensive Center for the Study of HIV Disparities and the Mentoring Institute for HIV and Mental Health Related Research, for guidance and financial support for this investigative project. I would especially like to thank Dr. Lydia E. Santiago and Dr. Silvia E. Rabionet for all their guidance and support during my graduate education; and a special thanks to Dr. Yamamura and his team of the Ponce School of Medicine for their assistance with the PCR-Linear Array HPV Genotyping.

The clinical and administrative staff at CEMI assisted in several aspects of this study. Elizabeth “Eli” Navedo welcomed each woman into the clinic, and assisted in our consecutive sampling and screening process. She served a vital role in promoting the study among the women attending the clinic. The study nurses at CEMI are extraordinary. Jannette “Vale” Valentine and Brenda “Brendi” Beauchamp-Báez, your input into the flow of the study procedures was quintessential; the respect, dedication, and passion that you undertake your duties is evident in the high quality of care you provide these women; and is an example of good clinical practices. I am forever grateful to you all.

A special note of thanks to all the behind the scenes staff at CEMI: Omar Santiago, Lourdes de Jesus, Martha Pagan and Estrella. Your assistance and support of this project is very much appreciated and I thank you all for always welcoming me to CEMI. They are the oil that keeps this hard working machine running smoothly.

Thank you to Janice Perez and Isaedmarie Febo for all of you support and assistance throughout my career at the University of Puerto Rico Medical Sciences Campus. I don’t know if this project would have been possible without your unending show of morale support and guidance. Your friendship has meant so much to me during my time in Puerto Rico. You both are two remarkable women and epidemiologist. Thank you.

Thank you to my dearest Georgamaly “Geo” Estronza, my soul sister. Words could never express how indebt I am to you for years and years of friendship, professional guidance, and sisterhood. Your friendship, your family, and love have meant the world to me. The kindness and strength you have shown me and my family, made us all feel at home in Puerto Rico. You are truly an admirable woman, and I am so proud to have you as a friend. I will forever remember our office, your advice and all the hours of laughter we shared. You truly are the IRB queen, and I am eternally grateful for the epidemiological and regulatory wisdom you shone upon this research. You have taught me so much about myself, friendship, family, motherhood, and clinical research. You are my

touchstone, mi compinche, and a treasured part of my family. Thank you for always being there, for always being a true friend, and for always telling me like it is.

I would like to thank the Department of Biostatistics and Epidemiology of the University of Puerto Rico Medical Sciences Campus for always believing in me, even when I had my own doubts. Thank you to Professor Linnette Rodriguez for always helping me get back on track; to Dr. Carmen Velez for your long lessons in life and in epidemiological study design.

This thesis would never have been possible without my beloved and ever-patient mentors: Dr. Ana Patricia Ortiz and Dr. Heidi Venegas. Thank you Ana for inspiring me, for cultivating my passion for HPV and cancer research. Your constant supportive mentorship has meant the world to me; you expanded my love of epidemiology. Heidi, I want to thank you for infusing your love of biostatistics and solid clinical research, into the very fibers of my being. Your endless encouragement and expertise is what makes you a remarkable professor, colleague, and mentor. Thank you for always making biostatistics not only learnable, but enjoyable! You both have helped shape me, and I learned so much about professionalism, integrity, dignity, and perseverance through our work together. If it were not for your patience, encouragement, and belief in me this thesis would have died a long time ago. Your high standards and drive for excellence forever compel me to strive for more, and I hope I have done you all proud! Thank you from the very bottom of my heart.

D E D I C A T I O N

This work is dedicated to my beloved family both near and far; to my parents, Mary and Sam Scorsone who always encouraged me to discover myself and help other people. Mom, you are a strong, remarkable woman who has great depth to her soul and compassion; you are an extraordinary mother and dear friend. Dad, you have always pushed me to excel in my academics, to pursue my dreams to the farthest limits. To my brother and sister-in-law, Sam and Natalie Scorsone. Thank you for always believing in me and encouraging me to go after my dreams, I love you both very, very much! To Aunt Teresa, Uncle Richard, Michael and Eric, I love you all beyond words; and it has been your love and encouragement that have been the foundation of all my journeys. Thank you for always supporting me wherever the river of life should take me.

Gombal Javier Suarez, my love, this work is dedicated to you as well. Thank you for always encouraging me to see this dream through, for never letting me give up, for always helping me to awake early and for reminding me when it was late, and time for bed. You respect my being, care for me, and love me, more than I ever had hope. I never imagined that I could love someone as I do you, and I am so proud of the family we are creating together.

Finally, this work is dedicated to my daughters, Maya Sol and Emma Luna. You are my light and my love; you are my sun and my moon. I love you both more than anything I have ever loved on this earth. Your smiles bring joy, your laughter fills my soul, your arms around my neck- my sustenance. You are my purpose, my hopes, and my reason for being. I dedicate this to you, so that you will know that even when everything in the world feels upside down and inside out, anything and everything is possible.

TABLE OF CONTENTS

LIST OF GRAPHS AND FIGURES

LIST OF TABLES

CHAPTER ONE: INTRODUCTION

The intention of this work is to expand our understanding of the prevalence cervical Human Papillomavirus (HPV) infection in HIV positive women who attend the Center for Maternal Infant Studies (CEMI, for its Spanish acronym) of the University of Puerto Rico Medical Sciences Campus; and to analyze the HIV co-factors that are frequently associated with HPV infection in this population. As a part of this introduction, important epidemiological aspects of HPV and HIV will be presented, including the disease burden related to HPV and HIV infections.

Epidemiology of Human Papillomavirus (HPV)

HPV is a non-enveloped double stranded DNA tumor virus, which is sexually transmitted to both males and females. These viruses are termed Papillomaviruses because a large majority of the types are known to cause benign warts or papillomas. More than 100 types of HPV have been genetically identified, with approximately 40 genotypes infecting the mucosal surfaces of the genital areas (Muñoz, et al, 2006). Clinically, it manifests into commensal infections, genital warts, and intraepithelial lesions. To complicate the virology of HPV, it also has several subtypes and several variant genotypes (Scheurer, Tortolero Luna, Alder-Storthz, 2005).

The way in which HPV clinically manifests itself, is largely based on the type of HPV that a person is infected with. HPV types can either be cataloged as types that invade cutaneous tissue or types that attack mucosal tissue. Most researchers investigating HPV have historically used two categories to describe the genotypes

caused by HPV: high-risk and low-risk, each named specifically for its oncogenic potential to cause cancer. The low-risk HPV genotypes are those that are considered as having a low risk of causing cancer; and these are most frequently associated with genital warts and commensal infections. Those classified as high-risk genotypes are those which have been associated with various types of cancers, including cervical, anal, penile, anogenital, nasopharyngeal, and oral cancers, among others.

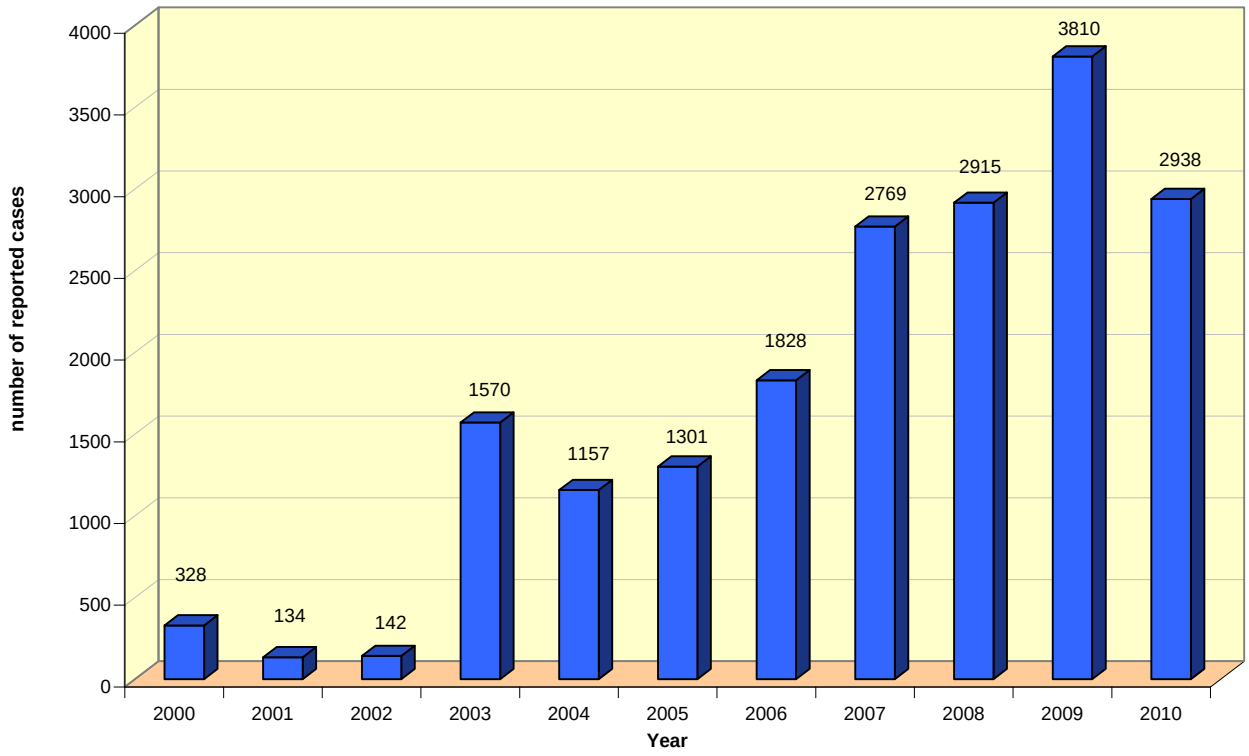
The World Health Organization (WHO) has estimated that the global prevalence of HPV infection among women with normal cervical cytology is 11.4% at any given time. The WHO also estimates that 75.3% of women with abnormal cervical cytology (LSIL/CIN 1, HSIL/CIN 2&3, CIS) test positive for HPV-16 and HPV-18 genotypes (WHO/ICO, 2010), the two most common high-risk genotypes. Worldwide, an estimated 291 million women are carriers of HPV (Burchell, et al, 2006; de Sanjose, et al., 2007).

According to the Centers for Disease Control and Prevention (CDC), HPV is the most common sexually transmitted infection in the United States, affecting some 20 million people each year with active infections; with approximately 6.2 million Americans newly diagnosed each year (CDC, 2006). As reported by Dunne et al., the prevalence of HPV among US women ages 14-59 years is estimated at a staggering 26.8 %; and the prevalence for high-risk HPV types was 15.2% (Dunne, et al., 2007). It is believed that by the end of reproductive age, nearly 70% of US women will have been exposed to at least one type of HPV in their lifetime, and more than 30% of all US women will be infected with multiple HPV types during their lifetime (Franco, Durate-Franco, Ferenczy, 2001). While these statistics are daunting, they fail to describe the

true magnitude of this health problem within marginalized segments of the population, that remain undetected as a consequence of inadequate access or utilization of healthcare services.

No population-based data exist of the incidence and or prevalence rates of HPV in Puerto Rico, as surveillance of HPV infection is not routinely done in this population, and population-based studies on disease burden are lacking. Nonetheless, in the last five years, Puerto Rico has seen a rise in the incidence rates of genital warts (PRDH, 2007). According to the Puerto Rico Department of Health, in the year 2002, the incident rate for genital warts was 3.68 cases per 100,000 habitants; while in 2005, the incident rate spiked to 33.26 cases per 100,000 habitants (PRDH, 2007). Graph 1, demonstrates this dramatic rise in incidence. For example, in 2000, the Puerto Rico Department of Health reported that there were 328 cases of genital warts detected in the population; in 2010 this same statistic jumped to 2,938 reported cases of genital warts (PRDH, 2011). This striking increase in reported HPV genital warts is not believed to be a result of a recent outbreak if HPV, but rather a reflection of stronger, more effective disease surveillance efforts.

Graph 1: Reported Cases of HPV-Genital Warts in Puerto Rico, by Year



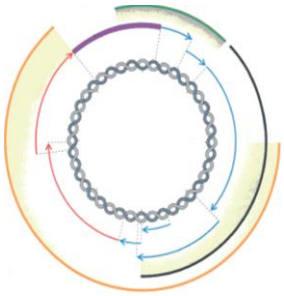
Puerto Rico Health Department, Division of Prevention of Sexually Transmitted Diseases, HIV/AIDS, Office of Sexually Transmitted Diseases Surveillance (2011). Datos Generales de las ETS 2000-2010.

Over the last 20 years, epidemiological evidence of the causal relationship between HPV and cervical cancer has clearly been established; and is considered to be a gold standard in Bradford-Hill Causality (IARC, 2005). The WHO International Agency for Research on Cancer (IARC) has concluded that HPV is a necessary cause of cervical cancer. HPV-DNA has been detected in over 90 - 100% of cervical malignant tumors (Muñoz, et al., 2006; Bosch, 2007; IARC 2012). Nevertheless, the simple presence of HPV in cancerous cervical tissue is not the only indicator of a causal relationship. There is a distinct uniform sequence in HPV disease progression that has been shown to lead to cervical carcinogenesis; regardless of the population under investigation (Moscicki, Schiffman, Kjaer, and Villa, 2006).

Infectious Agent

HPV is a non-enveloped circular double-stranded DNA tumor virus, infecting the differentiating epithelial cells of the skin and mucosae, see figure 1 (WHO, 2011; Nelson & Masters, 2007; CDC 2006). It is a small virus, containing only 8000 base pairs in length. The early region proteins, E1–E7, encode genes mainly aimed at DNA replication; while the late region proteins, L1 and L2, code the encasing protein shell of the genome, so that the virus can be recognized by the host's immune system (WHO, 2011). In the cervix, HPV is produced and assembled into highly immunogenic virions. These viral particles are then released into the outer epithelial layer, far from the submucosa, the host's primary site of immune surveillance. HPV DNA can either remain in an episomal form, as is common in genital warts and commensal infections; or HPV can integrate into host DNA, as is seen in most, if not all, of cervical cancer cells (WHO, 2011)

Figure 1: HPV Genome



Blue regions are the early regions (E).

Purple region is the upstream regulatory region.

Red areas encode for the late regions (L).

World Health Organization: The Department of Immunization, Vaccines and Biologicals (2011). Immunological Basis for Immunization Series. Module 19: Human Papillomavirus. WHO Press, Geneva.

HPV initially enters the host through a microscopic abrasion or break in the squamous epithelium, binding to the basement membrane. Then through a complex

interaction of HPV with the host's squamous epithelium, the basal cells are then infected. As HPV continues viral replication, so does the frequency by which HPV replicates in a single cell. After initial basal cell infection, HPV can replicate at a low rate of a hundred copies per cell, but after differentiation and mitigation it can increase to several thousand copies per cell. This variable replication rate is mostly based upon the availability of the host's replication enzymes (WHO, 2011).

The host's immune system is slow to detect HPV; mainly because of its localization in the epithelium and specific viral properties related to how HPV interacts with the epithelium. HPV has no viremic phase, as is seen with most blood -borne pathogens (WHO, 2011). In the absence of a viremic phase, HPV doesn't generate the needed innate immune response to target HPV for destruction. HPV is then able to replicate with relatively low levels of viral proteins. While typically it is an unexpected high level of a viral protein that would activate an innate immune response. The WHO, as part of its vaccine series, has the most extensive description of HPV immunology. While the particulars of the exact immunological properties and processes of HPV are still under investigation; investigators regularly research HPV immunology in immuno-suppressed populations, like those living with HIV. This sub-population has yielded the most advances in HPV immunological research (WHO, 2011).

HPV infection is not cytolytic; meaning it does not cause cellular death. In the cases of HPV-related cancer, it stops cellular death and causes uncontrolled cellular growth, sometimes referred to as cellular immortality. The adaptive immune response is slow to develop because of the delayed innate response. It is believed that when a person has been infected with HPV, that isn't eliminated by the innate immune response, the

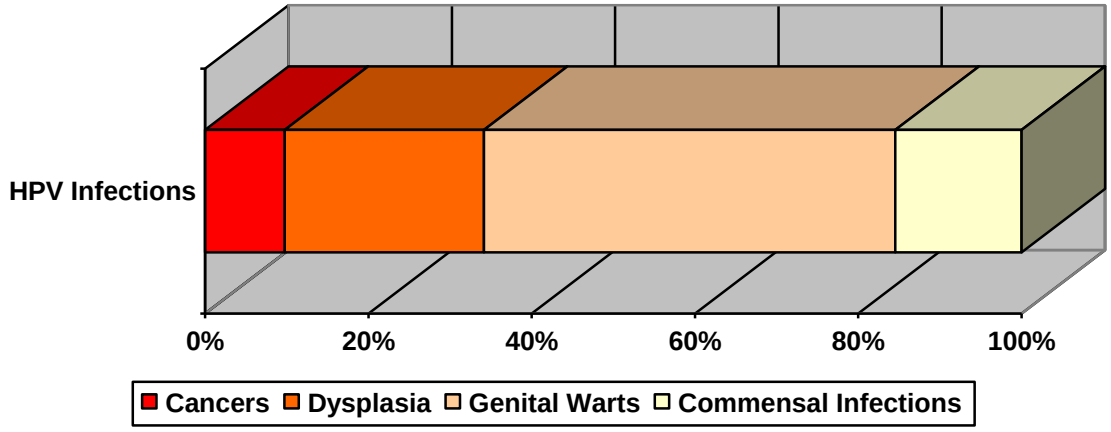
adaptive immune mechanisms are then called upon to develop specific t-cells (WHO, 2011). The specific t-cell lymphocytes that target HPV infections are known as CD4s; and in normal healthy individual will produce in abundance given an increase in levels of HPV viral proteins. Should this scenario play out, HPV will self-clear; and this is the case for the majority of infections. If CD4 cells are not activated, then clearance of HPV will be hindered and this may be what opens the door to persistent infection (WHO, 2011).

Given that genital HPV infection is extremely common in women, acquiring HPV is not the most critical step in cervical carcinogenesis. It has been estimated that merely 10% of all HPV infections leading to persistent infections and only a small percentage of all persistent infections eventually lead to precancerous cellular development (CDC, 2006). It is the *persistence* of HPV infections that augments the risk for cervical cancer, not an infection of HPV. The probability of being HPV infected and it developing into cancer is quite small, as demonstrated in Graph 2. The majority of HPV infections will remain subclinical or are related to genital wart and commensal infections, and will self-clear typically within 1 - 2 years (Nelson & Masters, 2007).

Aside from a persistence of high-risk HPV infection, several other factors may directly or indirectly affect the transition from viral infection, reoccurrence, and to the development of malignant tumors. Smoking, active Chlamydia infection, limited access to healthcare services, infrequent cervical screenings (Pap smears), long-term use of oral contraceptives, presence of high risk HPV types (specifically, HPV-16), and increasing age are all thought to be factors that may influence HPV's likelihood of initiating cervical

carcinogenesis (Crum, 2000; Franco et al, 2001; Castellsagué, Díaz, de Sanjose, et al., 2006).

Graph 2: Clinical Manifestations of HPV Infection



Adapted from Nelson KE, & Masters CF (2007). Infectious Diseases Epidemiology: Theory and Practice, (2nd Edition). Sudbury, Massachusetts: Jones and Bartlett Publishers.

Natural History of HPV

Mode of Transmission: The primary route of genital HPV infection is through vaginal or anal sexual intercourse, but frequently it is transmitted through direct skin-to-skin contact (genital-genital, oral-genital, manual-genital contacts). In rare cases, HPV can be passed from mother-to-child transmission. This has been confirmed by the presence of both HPV-DNA and HPV serum antibodies in newborns (Burchell, et al., 2006).

Communicability and Transmissibility: Understanding transmissibility is one of the most important components in developing public health policies and health promoting strategies to prevent new infections. There are several factors that increase an individual's risk of HPV infection, including young sexual debut, multiple sex partners, unprotected sex, frequent anal sexual intercourse, among other factors like previous

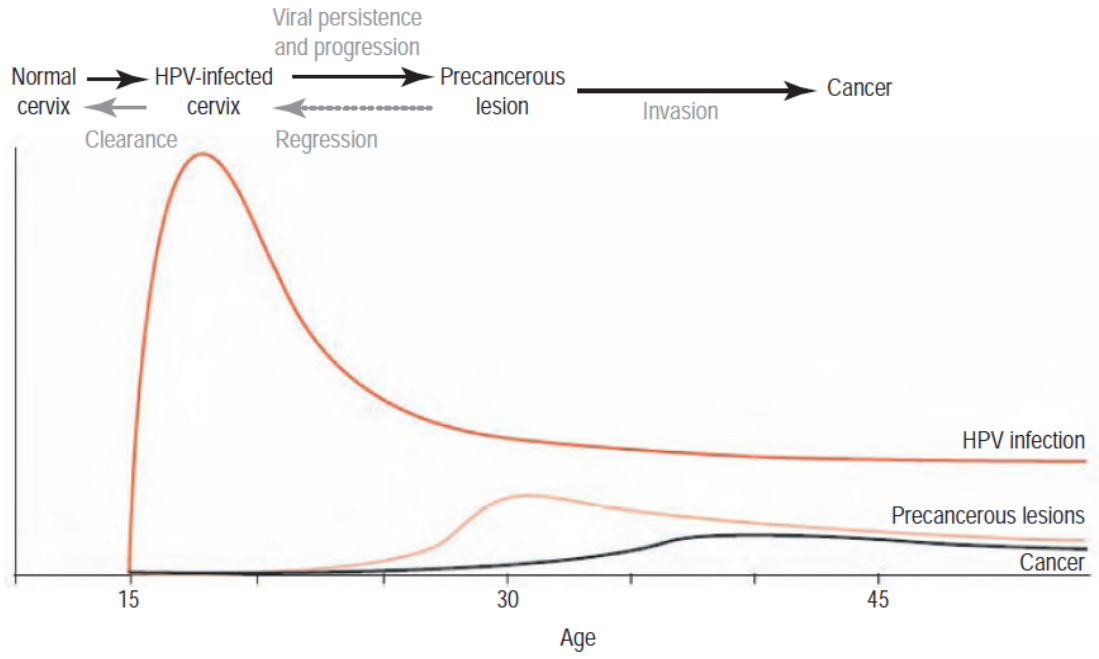
history of sexually transmitted infections and sexual partners with multiple sex partners (CDC, 2006; CDC, 2000; Franco et al, 2001).

The incubation period of HPV is not clearly defined. However it is believed to be within a few months of infection up to a year, however some evidence supports the idea that the incubation period can last up to several years (Crum, 2000; WHO, 2010). More studies into the natural history of HPV infection in both females and males would divulge more light on this important area. Similar to the investigative debate that surrounds HPV's incubation period, the duration of HPV infectivity is still not well understood. Most infections last one to two years and are self-clearing as people gradually develop an immunological response; yet for some it seems that HPV enters a dormant state. It is unclear if people are infectious during this dormant state (CDC, 2006; WHO, 2011). Laboratory studies have revealed that most infections are undetectable after this timeframe and it is unclear if individuals are infectious throughout the whole period of a detectable infection (Burchell, et al., 2006). See Figure 2.

The reactivation vs. dormant stage, and the co-factors associated with reactivation remain relatively unclear. The role that the human adaptive immune system plays in the suppression and/or clearance of HPV infections needs further explanation. People can become re-infected with the same types of HPV several times, and HPV type specific infections offer no immunity to other HPV types, as demonstrated in individuals with multi-type infections (WHO, 2011).

Diagnosis: Genital warts caused by the common low-risk HPV's are almost always diagnosed by visual inspection and occasionally followed by confirmatory biopsies (CDC, 2006). Nonetheless, the majority of HPV infections have asymptomatic clinical presentation, are often unrecognized, and there are no routine methods for detecting these infections (CDC, 2006). Most cervical high-risk HPV infections are recognized through an abnormal Pap test showing the presence of atypical squamous cells of undetermined significance (ASC-US) which warrant HPV-DNA testing, yet several restrictions govern the use of this form of diagnosis (CDC, 2006). Nevertheless, recent meta-analyses and review articles show that an estimated 11% of women worldwide with normal pap smears were found to have HPV infections (de San Jose, 2007; Bosch, Burchell, Schiffman, Guiliano, de San Jose, et. al 2008). HPV-DNA genotyping tests are

Figure 2: The natural history of HPV & Age-Adjusted Prevalence of HPV-associated Disease in Women



World Health Organization: The Department of Immunization, Vaccines and Biologicals (2010). Human Papillomavirus Laboratory Manuel, 1st Edition. WHO Press, Geneva.

available mostly only in clinical research settings. There is also a growing body of research suggesting that the limitations in liquid based cervical cytology (Pap Smears) and inadequate availability of HPV-DNA testing, may lead to delays in detection of precarious squamous cell intraepithelial lesions and adenocarcinomas of the cervix (Castellasgué, et al, 2006; Wright, 2007, Cuzick, Arbyn, Sankaranarayanan, Tsu, Ronco, Mayrand et al, 2008).

Treatment: There is no cure for HPV, and there is no recommended treatment for most genital HPV infections due to their self-limiting nature. However, treatments are available for genital warts. Two types of treatment are available to treat genital warts: patient-applied and provider administered treatments (CDC, 2006). Patient-applied treatments include gels or creams; these are typically the preferred course of treatment as they are discreet in nature. Nevertheless proper treatment adherence is important in order to effectively treat the warts.

Researching HPV Genotypes and Species

Low-risk HPV types most commonly cause common or genital warts, and commensal gynological infections. The HPV types that cause such common skin warts are different from those warts found in the genital area or throat which are known as, condylomata acuminate (NCI, 2008). It is believed that low-risk HPV types-6 and -11 are responsible for an estimated 90% of all genital warts. The CDC, in 2009, estimated that 1% of all sexually active adults will have had genital warts at least once in their lifetime. The warts associated with HPV may appear as flat, raised or shaped like a cauliflower and may appear in small clusters or as individual skin abnormalities. These

may decrease in size and numbers on their own after several weeks, a few months or a few years depending on the host's immune response to the HPV infection. They may need medical attention, treatment, or removal; they may never go away; however they will not become cancerous (CDC, 2009).

There are 14 fairly common high-risk HPV genotypes, however type -16 and -18 are considered by far the most common in general populations worldwide, accounting for some 80% of all HPV infections (WHO, 2006; Muñoz, Bosch, et al, 2003). Commonly high-risk HPV infections are asymptomatic or have sub-clinical presentations for years before they are detected through abnormal "Pap" smears

Since 1995, the WHO International Agency for Research on Cancer (IARC) has published information establishing the causal relationship between HPV and cancer in humans. The IARC has frequently updated its findings and monographs concerning HPV and in 2007, published volume 90 of the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, which addressed all current information known about Human Papillomaviruses and their association to cancers (IARC, 2007).

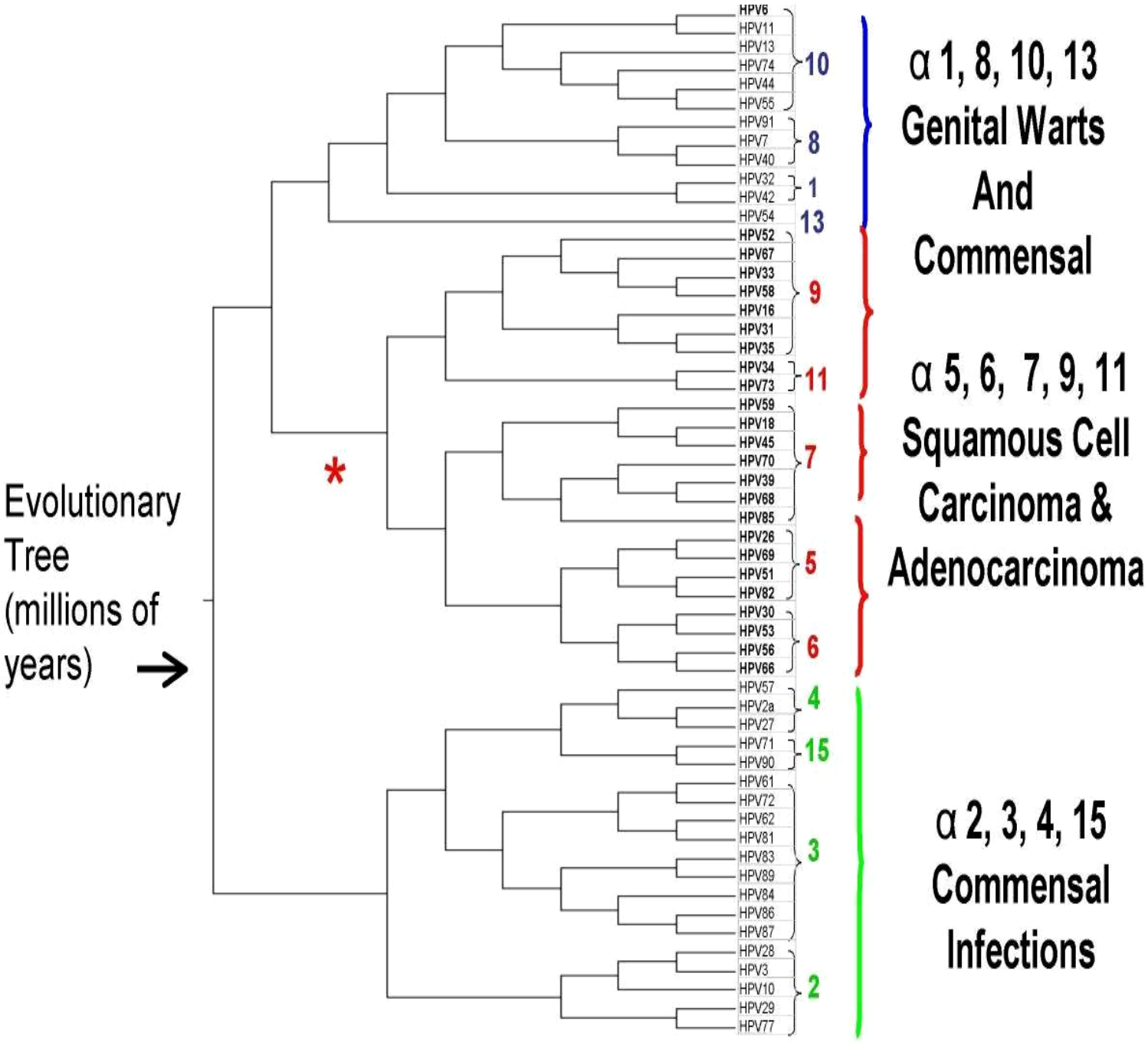
The WHO/ICO (Institut Català d'Oncologia) Information Centre on HPV and Cervical Cancer, in 2010 outlined the 10 most frequent worldwide HPV types found in women according to cervical cytology. Women with normal cytology were most frequently infected with HPV types -16, -31, -18, 52, -51, -58, 56, -39, -45, and -33; while women with low grade lesions were infected with HPV types -16, -51, -31, -52, -56, -58, -66, -18, -6, and -39. On the contrary, women with high grade lesions were mostly infected with HPV types -16, -31 , -58 , -33 , -52 , -18 , -51 , -35 , -45 , and-39. In women

with cervical cancer the most common HPV types found were -16, -18, -58, -33, -45, -31, -52, -35, -39, and -59 (WHO/ICO, 2010). All of these, except HPV-6 and HPV-66 are high-risk HPV types.

More recently, the Monograph working groups began discussing re-categorizing HPV types and their association to cancer (IARC, 2012). It has become clear, through their compilation of research over the last 15 years, that grouping HPV types into high-risk and low-risk for their presence in cervical cancer was problematic because of difficulties in epidemiological surveillance and analysis of individual HPV genotype analysis was resulting in (Schiffman, Clifford, and Buonaguro, 2009). This new movement among HPV experts, and the IARC, to explore HPV according to HPV phylogenetic species is based on three categories: those causing genital warts and genital commensal infections, those causing squamous cell carcinoma and adenocarcinomas, and those causing commensal infections, see figure 3.

Commensal infections are those who have very little clinical significance and are frequently called innocent infections because of the often symbiotic nature maintained between the virus and host. Commensal infections consist of HPV species alpha-4, -15, -3, and -2. Genital warts and genital commensal infections include alpha-10, -8, -1, and-13, and this group includes such HPV genotypes like HPV-6, 11, 40, 42, 54 among others. Those HPV types causing squamous cell carcinoma and adenocarcinomas include HPV species alpha-5, -6, -7, -9, and -11. Alphas -7 and -9 include HPV types that are most strongly associated to cervical cancer (Schiffman, Clifford, and Buonaguro, 2009; IARC, 2012).

Figure 3: Phylogenetic Analysis of Anogenital HPV Species



Phylogenetic analysis of anogenital HPV types. Branches determined by 100 bootstrap estimations using each of the methods in the following order: Bayesian credibility value, parsimony bootstrap percentage based on nucleotide alignment, and parsimony bootstrap percentage based on amino acid alignment. All definitely, probably, and possibly carcinogenic HPV types belong to one phylogenetic clade of the alpha genus. Schiffman et al. Infectious Agents and Cancer 2009 4:8

IARC concluded that there are eight HPV species that are crucial in cervical carcinogenesis; they are: HPV 18 and 45 (from alpha-7); HPV 16, 31, 33, 35, 52, and 58 (from alpha-9). They also found that HPV 51 (alpha-5), HPV 56 (alpha-6), HPV 39 and 59 (both from alpha-7) are also powerful carcinogens. Despite the high prevalence of HPV 66 and HPV 53, there has not been largely conclusive epidemiological evidence of their association to cancer. Schiffman et al, concluded that if they were to be included in screening assays it could lead to decreased specificity and poor predictive value in HPV testing, since they are rarely found in cancerous tissue (Schiffman, Clifford, and Buonaguro, 2009).

HPV as a Carcinogen

HPV is an established necessary risk factor for cervical cancer (IARC, 2012; IARC, 2007; and Schiffman, 2009). HPV-DNA has also been detected in the tissue of several other non-cervical cancers, including cancers of the vulva, penis, vagina, anus, oral cavity, pharynx, and larynx (Moscicki, et al, 2006; Muñoz, et al, 2006; Parkin and Bray, 2006; Zhao, Rasmussen, Perry, and Kiev, 2006; Coissard, Besson, Polette, Monteau, Birembaut, and Clavel, 2005; Karagas, Nelson, Sehr, Waterboer, Stukel, Andrew, et al, 2006; Kan, Iacopetta, Lawson, and Whitaker, 2005). Despite the fact that HPV –DNA can be found in many different types of cancerous tissue, HPV seems to be more strongly associated with ano-genital and oral cancers (IARC, 2012; IARC, 2007; and Schiffman, 2009).

The Bradford-Hill criteria of causation are typically used in epidemiology to summarize the causal relationship between a risk factor and a disease. Bradford-Hill

consists of nine criteria: temporal relationship, strength of association, dose-related relationship, and consistency in research, plausibility of pathological association, reversibility, specificity, and coherence with accepted theories. HPV successfully meets the Bradford-Hill criteria so strongly as a causal factor for cervical cancer, that it now serves as a point of reference for other Bradford-Hill criteria comparisons (Nelson & Masters, 2007).

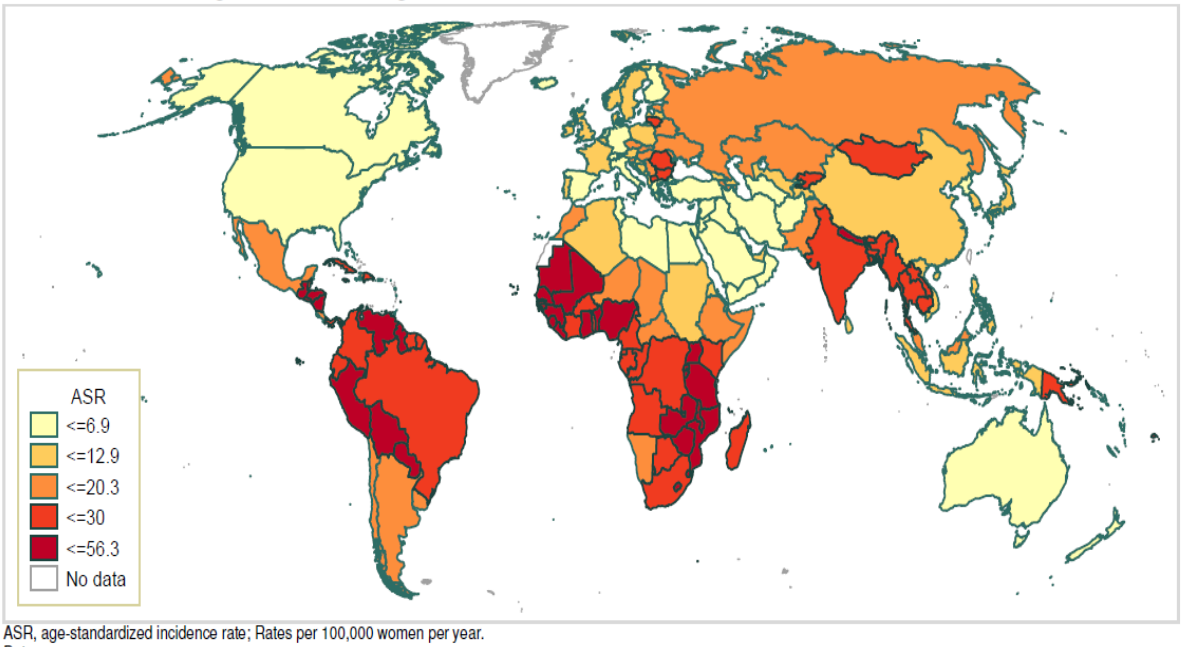
HPV must be present in order for cervical cancer to develop (temporal relationship). According to IARC, HPV can be found in 99.7 % of cervical malignant tumors (strength of association) (IARC, 2007; IARC, 2012). The HPV research regarding the causal relationship with cervical cancer has resulted in consistent findings across several large-scale studies over the last two decades (consistency) (Muñoz et al, 2006; Wright et al, 2006; Scheurer, Tortolero-Luna, 2005). HPV is a powerful carcinogen that has the capacity to stop programmed cell death, immortalizing human keratinocytes (biological plausibility) (Burchell, et al, 2006). Also, both *in vitro* and *in vivo* research has provided scientific evidence to support HPV and cervical cancer's causal relationship. Scientific research has been able to repeatedly demonstrate that the end result (cervical cancer) can be altered by the presences of HPV or by the absence of HPV (reversibility) (Munoz, 2006; Munoz, 2003). In addition, specific genotypes of HPV are strongly related to certain cancers, for example HPV-16 is most closely linked to cervical cancer, vaginal, anal cancers (specificity) (Munoz et al, 2006; IARC, 2007; IARC, 2012). Finally, epidemiological investigations of HPV's role in cervical cancer do not conflict with known natural history of cervical cancer (coherence) (Moscicki, et al, 2006).

Burden of Cervical Cancer

Cervical cancer is the second most common cancer, after age-standardization, found in women worldwide with more than 85% of all cases occurring in the developing world (Parkin, 2004; WHO/ICO, 2010). Worldwide, over 15 women per 100,000 (age adjusted rate) will be newly diagnosed with cervical cancer (WHO/ICO, 2010).

According to the WHO, worldwide, an estimated 529,828 women are newly diagnosed with cervical cancer each year; and 275,128 women worldwide will die from cervical cancer. Worldwide, the 2008 age-adjusted mortality rate for cervical cancer was estimated at 7.8 cases per 100,000 women. Also in 2008, the WHO estimated that in Latin America and in the Caribbean, the estimated age-adjusted incidence rate for cervical cancer was 23.5 cases per 100,000 women; and the mortality rate was 10.8 cases per 100,000 women, see Figure 4 (WHO/ICO, 2010). The National Cancer Institute estimates, that in the US, 12,170 women will become newly diagnosed and some 4,220 American women will die from cervical cancer in 2012 (NCI, 2012).

Figure 4: World Age-Standardized Incidence Rate of Cervical Cancer, 2008



ASR, age-standardized incidence rate; Rates per 100,000 women per year.
Data sources:
IARC, Globocan 2008.

In Puerto Rico, cervical cancer is the fifth most commonly found cancer among women, and caused 4% of cancers affecting women between the years 1993-2003 (PRDH, 2008). According to the Puerto Rican Department of Health (2008), approximately 50 women die because of cervical cancer, and some 200 women are newly diagnosed each year in Puerto Rico. The age-adjusted incident rate for Puerto Rico in 2003 was 7.75 cases per 100,000 women; and the mortality rate was 2.5 for every 100,000 women (PRDH, 2007). In addition, the burden of this and other HPV related cancers remains high in this population (Ortiz, 2010; Colon, 2010; Suárez, 2009).

Nonetheless, we won't know the true estimate of HPV prevalence in Puerto Rico until routine surveillance is firmly established for both genital warts and intraepithelial lesions; and further epidemiological research is conducted at a population level. For

Puerto Rico, the significant rise in the incidence of HPV-related genital warts, coupled with the high incidence of HPV-related cancers (particularly cervical and oropharyngeal cancer) surely warrant further epidemiological research.

HPV Vaccines

In June of 2006, the Food and Drug Administration approved the use of Gardasil® (Merck & Co.) as a quadrivalent HPV Vaccine. Gardasil® provides protection against the four most common types of HPV related to disease in humans, HPV-6, -11, -16, and-18. While there is regional variation among common HPV types, it is believed that Gardasil® will be able to prevent an estimated 70% of cervical cancers and 90% of genital warts in females with no prior exposure to these types of HPV; in addition to offering some additional protection against vulvar, vaginal, and anal cancers as well (CDC, 2006; WHO, 2006).

This vaccine is a prophylactic vaccine, rather than a therapeutic vaccine; and it will only help prevent new infections for the specific HPV designated above. Recent research found that up to 10 other HPV types may experience cross-immunization from this vaccine; nonetheless, these results are not conclusive (Smith JF, Brownlow M, Brown M, Kowalski R, Esser MT et al., 2007). This vaccine was originally approved for females of ages 9-26 years. In late 2009, the FDA granted approval for males ages 9-26 as well, citing its potential to prevent genital warts and anal cancers, in addition to those cancers mentioned above. The vaccine is administered in a series of three injections given at months 0, 2, and 6. The vaccine is estimated to have effectiveness for at least five years, if not more (CDC, 2006; FDA, 2009, CDC, 2010).

In October of 2009, a second prophylactic vaccine, Cervarix® (GlaxoSmithKline), was also approved by the Food and Drug Administration to be used in the US. Cervarix® is a bivalent HPV vaccine that provides protection again HPV 16 and HPV 18. The research leading up to the approval by the FDA found that this vaccine also offers some limited cross immunization for HPV types 45 and 31. This vaccine is approved for use in females' ages 10-25 years for the prevention of cervical cancer and precancerous lesions. Similar to Gardasil®, Cervarix® is administered by three intramuscular injections at months 0, 1, and 6 and while provide slightly over seven years of coverage. Both Gardasil® and Cervarix® were added to the CDC's schedule for immunizations in 2010 (CDC, 2010).

It has been recommended that both of these vaccines be administered before onset of sexual activity, in order to offer the 100% efficacy provided for HPV naïve individuals (CDC, 2006; WHO, 2006). Yet evidence suggest that it can be administered to those already sexually active in the hopes of preventing these very common HPV infections (among those naïve to any of the HPV types included in the vaccine); but its efficacy will be altered. This stipulation should prove difficult to determine due to the lack of standardized screening. This restriction also generates the need for therapeutic vaccines for women who already have these or other HPV types.

The approved vaccines were tested in thousands of females worldwide, with very minimal side-effects (CDC, 2006). The most common adverse event among test subjects was soreness in the injection site. These vaccines are made of virus-like particle (VLP) resembling HPV. VLPs do not contain any viral genetic material; therefore they are non-infectious (WHO, 2006). Cervarix® comes in vials and two kinds of prefilled syringes.

The prefilled syringe tip caps may contain natural latex or their derivatives; use of these may cause allergic reactions in persons with latex sensitivities. The vial stoppers have no latex in them (GlaxoSmithKline, 2011). Neither vaccine contains any thimerosal, or mercury; and Gardasil® is latex-free (CDC, 2006).

The HPV vaccines, Gardasil® and Cervarix®, mark for the first time in vaccine history that a first generation vaccine has 100% efficacy. Not only are they very safe and clinically effective; the vaccines are extremely cost-effective. The whole series is listed in the private sector as costing around $360, to complete the whole series. A handful of organizations are subsidizing these vaccines in developing countries. The organizations include UNICEF, GAVI Alliance, and Pan American Health Organization Revolving Fund to name a few. These programs aim to reduce the price for governments and the private sector to assist with their vaccination programs development (IAVI and PATH, 2007). Aside from saving the lives of thousands of women, these vaccines may be able to prevent the $50,000 price tag associated with treating cervical cancer (WHO, 2006).

Despite the scientific advancements of obtaining a successful HPV vaccine and establishing vaccine programs, several issues still remain unresolved. Marketed as prophylactic vaccines, neither vaccine has been significantly researched to demonstrate any therapeutic efficacy. There is also a great need for more research that can expand the protection offered by vaccines, to cover more common high-risk HPV types in a vaccine. This would increase the cost-effectiveness of a single vaccine, and perhaps be fodder for insurance companies to expand their coverage to include regular HPV testing. However the high cost of the complete series, may be a barrier for people without adequate health insurance coverage and those living in developing nations.

Epidemiology of Human Immunodeficiency Virus

The world was first introduced to the Human Immunodeficiency Virus (HIV) in a MMWR publication in June of 1981. HIV is the virus that causes the life threatening condition called Acquired Immunodeficiency Syndrome (AIDS). Three decades ago HIV was considered an unknown viral killer of mostly homosexual men; and is now a manageable chronic condition for those with access to good HIV primary care and treatment. Nonetheless, there is still a great deal left to be discovered in our understanding of HIV.

The Joint United Nations Programme on AIDS (UNAIDS), estimated that for the year 2009, there were some 33.3 million people living with HIV/AIDS worldwide; with 22.5 million of these infected individuals from Sub-Saharan Africa alone (See figure 5 for further details of the global distribution of HIV prevalence). UNAIDS also estimates that there are 15.9 million women and 2.5 million children living with HIV (<15 years of age) worldwide. In 2009, it was estimated that there were a total of 2.6 million new HIV infections, with over 7000 new HIV infections a day in 2009; and an estimated 1.8 million AIDS deaths worldwide for the year 2009 (UNAIDS, 2010).

Figure 5: HIV Global Prevalence, 2009

Adults and children estimated to be living with HIV | 2009

Total: 33.3 million [31.4 million – 35.3 million]

World Health Organization UNAIDS

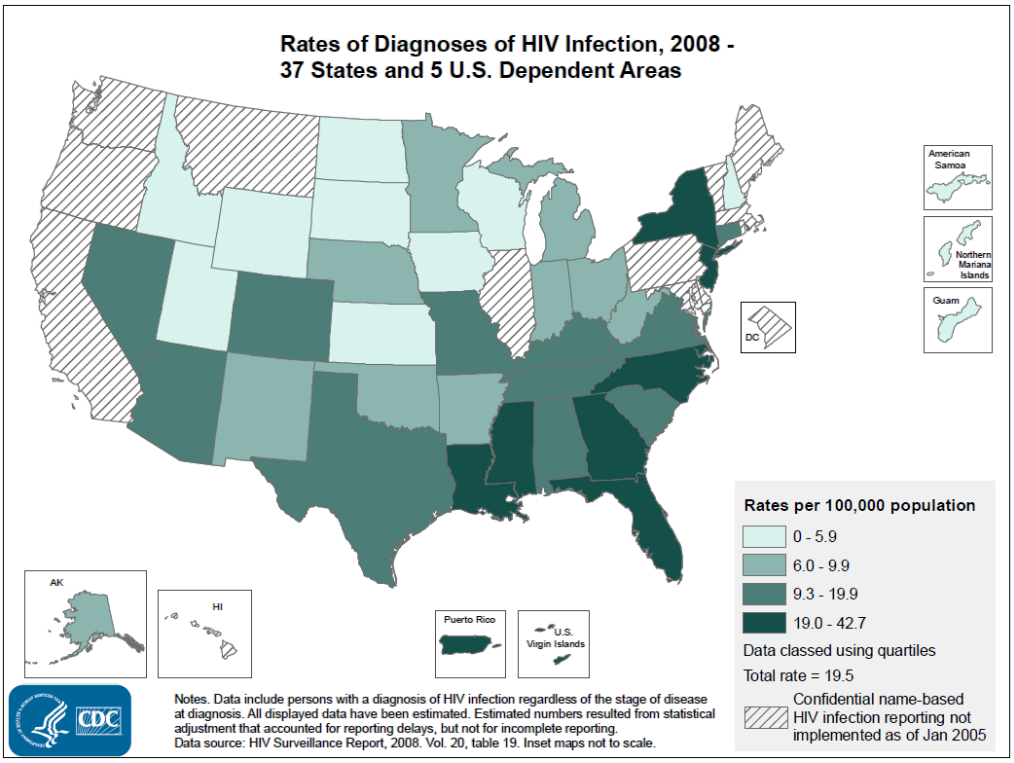
Joint United Nations Programme on HIV/AIDS (UNAIDS) (2010). Global report: UNAIDS report on the global AIDS epidemic 2010. Last accessed on 7/7/11 from http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf

In the US, the CDC estimated that 1,106,400 (95% CI: 1,056,400-1,156,400) persons were living with HIV infection at the end of 2006; with an estimated 21% of all cases being undiagnosed. In 2008, the CDC also estimated that the number of newly diagnosed infections was 56, 300 (95% CI: 48,200 – 645000) at the end of 2006; the 2006 estimated incidence rate was 22.8 new HIV infections per 100,000 persons in the US, including all 50 states and 5 US Dependent Areas. It was estimated that 53% of these new infections in the US occurred in gay and bisexual men. While Hispanics account for 16% of the total US population, they accounted for 20% of all new HIV infections in 2009 (CDC, 2011). Incidence rates among Black/African American men

and women were estimated to be 7 times as high as the incidence rate among whites (CDC, 2008).

The CDC estimated that in 2006, the incidence rate of HIV among individuals greater than 12 years old living in Puerto Rico was 45 per 100,000 habitants. During this same time period, 65% of these newly diagnosed cases were male, and 38% of all new cases occurred in individuals 30-39 years of age (CDC, 2009). The CDC also estimated that in Puerto Rico a total of 735 individuals were diagnosed with AIDS in the year 2008; at a rate of 18.5 new AIDS diagnoses per 100,000 habitants (CDC, 2011-Table 20).

Figure 6: HIV Incidence Rates for the US, 2008



Centers for Disease Control and Prevention (2010). Maps Based on Data from 2008 HIV Surveillance Report. Last accessed on 7/7/11 from http://www.cdc.gov/hiv/topics/surveillance/resources/slides/2008report_tables/index.htm

Infectious Agent

HIV is an enveloped double-stranded RNA retrovirus. There are two well-known types of HIV: HIV-1 and HIV-2. HIV-1 is predominately responsible for the majority of HIV cases reported in the world. HIV-2 is predominately found in Africa, with higher prevalence in West African Nations. The two types of HIV have similar replication mechanisms. The main difference in the two types is in how they attack the host's immune system, and in how HIV-2 progresses. It is believed that HIV-2 disease progression is slower than HIV-1; however, HIV-1 responds better to the drugs that were developed around its specific viral replication mechanisms (DDHS, 2011).

HIV needs to have a host cell in order to replicate and must go through a series of specific steps to accomplish viral replication. HIV must first bind and fuse with the host cell, using a CD4 immunological cell's receptors for entry. Then it inserts the entirety of its genetic composition into the CD4 cell, this step is called entry. Next the HIV viral RNA must go through reverse transcriptase to form a double-stranded DNA within the CD4's cells cytoplasm. Then the double-stranded viral DNA enters the CD4 cell's nucleus, where it splices the CD4 cell's DNA and then integrates through a set of processes called transcription and integrase, essentially re-writing how the original host cell operates. It is at this point that the CD4 cell has become fully infected and will no longer produce more CD4 generations through mitosis. Now the infected CD4 will go on to reproduce only new copies of HIV through the final enzymatic process of protease. It is in protease that new HIV viral molecules are created and repackaged into immature viruses. The replication rate of HIV is very high in comparison to other viruses; with the

capability of reproducing tens of billions of copies a day without the presence of antiretroviral medications (Coffin, 1997; Nelson & Masters, 2007).

HIV testing can either detect HIV genetic material or HIV antibodies. There are three main laboratory tests commonly performed for HIV testing. They are 1) the ELISA (enzyme-linked immunosorbent assay), also more recently known as EIA (rapid enzyme immunoassay), 2) the Western-Blot and 3) the PCR (polymerase chain reaction). The ELISA testing is used in a wide variety of settings, from doctor's offices to outreach vans. The test traditionally can be performed using serum; or rapidly through the use of saliva and EIA testing. Since the ELISA screening test checks for HIV antibodies, this test cannot be effectively used until there has been HIV antibody development, typically requiring 90 days post-exposure. If the ELISA test comes back negative, there were no HIV antibodies detected; however the test should be repeated in six months to confirm that the person is HIV negative. If the ELISA test is positive, the person will be re-tested. The Western-blot test is a more complicated and precise antibody detection lab, and is typically only used after two positive ELISA tests. HIV-PCR testing, unlike both the ELISA and Western-Blot test, detects HIV genetic materials, HIV antigens and HIV RNA. HIV PCR testing is what typically reports a person's HIV viral load. HIV-PCR testing can be performed within days of infection; however, it is typically not used in primary prevention efforts due to its high medical costs (CDC, 2010; Nelson & Masters, 2007; Coffin et al, 1997).

In today's health care settings, a person most likely will have a rapid oral HIV screening EIA which can produce results within 30 minutes; if positive then a serum ELISA test will be performed to verify proper HIV antibody detection. If a person has

two positive ELISA tests, then the person will need the confirmatory Western-Blot test. Once a person is entered into clinical care for their HIV infection, their health condition is followed by monitoring their CD4 counts and their HIV-PCR testing results in order to determine their HIV viral loads (CDC, 2010; Nelson & Masters, 2007; Coffin et al, 1997).

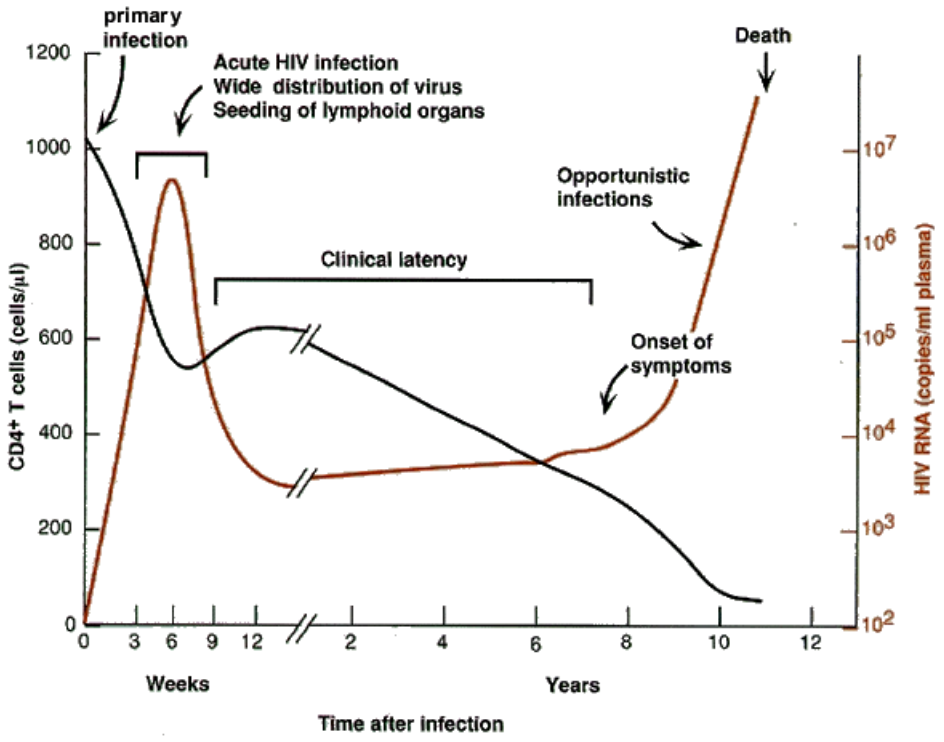
The Natural History of HIV

Within 7-10 days after infection, HIV viral RNA can be detected in the blood; within 1-3 weeks HIV antibodies can be detected in blood samples through the Western Blot test. Typically, during the acute HIV infection, the HIV infected individual's HIV viral load will dramatically increase until a proper adaptive immune response is developed among lymphocytes that directly interact with the virus, these are the CD3, CD4, CD8 t-cells. Once the adaptive immune response is fully established, the HIV infected individual's viral load will decline slightly; and then plateau within 3-4 months of initial infection (see Figure 7). In untreated HIV, this is commonly seen as a clinical latency period. An individual's onset of symptoms will most likely remain subclinical until the delicate balance of CD4 and HIV viral load levels flips, then the person will experience an increased susceptibility to common infection Coffin, 1997; Nelson & Masters, 2007).

In HIV infected individuals who do not begin Highly Active Antiretroviral Therapy (HAART), as the natural history of HIV infection progresses they will experience a loss of t-cell lymphocyte homeostasis, signifying that the host can no longer ward off the virus-induced immune system failures. This is the clinical onset of

symptomatic HIV, which increases the risk for occurrences of opportunistic infections, the hallmarks of the early onset of AIDS. A person is diagnosed with AIDS once their CD4 counts have gone below 200, or they have a history of an opportunistic infection (CDC, 2010; Nelson & Masters, 2007; Coffin et al, 1997).

Figure 7: Natural History of HIV Infection, without Antiretroviral Treatment



Coffin JM et al.,, editors (1997) Retroviruses. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.

Communicability and Modes of Transmission of HIV

The virulence and hence the transmissibility of HIV is directly linked to the individual's HIV viral load. Those with higher HIV viral load are believed to have higher odds of exposing their partners to the virus. Typically, it is during the acute HIV infection or in the late stages of AIDS, that a person is their most infectious because of

their high HIV viral load counts. Individuals with acute HIV infection often remain undiagnosed because they may not have established enough HIV antibodies to trigger an HIV positive ELISA test. This is why early detection of HIV is the key to proper disease management (CDC, 2010; Nelson & Masters, 2007).

HIV is transmitted through various bodily fluids, but mainly through blood, semen, vaginal secretions, and breast milk. Therefore, HIV may be transmitted through sexual intercourse (vaginal, anal, and oral), injection of drug use (IDU) or other parenteral exposures, transfusion of blood or blood products, organ transplantation, and by occupational exposure to HIV-contaminated bodily fluids or blood. The distribution of modes of HIV transmission varies by region and population. However, since major implementation of worldwide HIV primary prevention efforts, the proportion of persons exposed to HIV through blood transfusions, organ transplantations and occupational exposure has been radically reduced because of routine HIV screening of all blood and organ donations and the use of universal precautions. Mother-to-child transmission, in most countries, has also dramatically declined; due largely to public health measures that mandate HIV testing during pregnancy and the use of HAART during pregnancy to prevent infection. In the majority of the developed world, most primary prevention efforts still focus on sexual transmission and IDU (Nelson & Masters, 2007).

Clinical Management and Treatment

Once a person has been diagnosed as being HIV positive, it is crucial that they be connected with an HIV treatment provider to insure successful disease manageability.

The US Department of Health and Human Services recommends that the initial evaluation of a newly diagnosed individual includes a complete physical examination, a detailed medical history, and various laboratory tests. Typical labs associated with the initial evaluation of HIV are: CD4 T-cell count; plasma HIV RNA (viral load); complete blood count, chemistry profile, transaminase levels, blood urea nitrogen (BUN) and creatinine, urinalysis, and serologies for hepatitis A, B, and C viruses; fasting blood glucose and serum lipids; and genotypic resistance testing. The goal of the initial entry into treatment is to assess the person's disease stage, educate the patient on HIV and its transmissibility, and determine their course of care (DHHS, 2011).

To that end, there are two major classification systems used to monitor HIV disease progression, HIV tracking and monitoring; and finally to assist clinicians with guidelines for HIV clinical management. They are the CDC HIV Disease Staging System and the WHO Clinical Staging and Disease Classification System (see Table 1). The CDC system, mainly based on a person's nadir CD4 counts (the lowest recorded CD4 laboratory value in the medical record) and presence/absence of specific HIV-related conditions, was last revised in 1993. Its clinical relevance in guiding primary HIV care is limited, but when used in conjunction with treatment guidelines can be useful in helping clinicians and patients determine their appropriate course of treatment. The WHO system was designed to assist those serving HIV positive individuals in resource-limited settings, who may not have regular access to CD4 counts and HIV viral loads. The WHO system is based on clinical manifestations that can be recognized and treated by clinicians with varied professional backgrounds and in diverse settings (USDHHS, 2011; CDC, 2008)

Table 1: Comparison WHO and CDC stages of HIV infection,* by CD4+ T-lymphocyte count & percentage of total lymphocytes

WHO stage†	WHO T-lymphocyte count and percentage§	CDC stage¶	CDC T-lymphocyte count and percentage
Stage 1 (HIV infection)	CD4+ T-lymphocyte count of ≥500 cells/μL	Stage 1 (HIV infection)	CD4+ T-lymphocyte count of ≥500 cells/μL *or* CD4+ T-lymphocyte percentage of ≥29
Stage 2 (HIV infection)	CD4+ T-lymphocyte count of 350–499 cells/μL	Stage 2 (HIV infection)	CD4+ T-lymphocyte count of 200–499 cells/μL *or* CD4+ T-lymphocyte percentage of 14–28
Stage 3 (advanced HIV disease [AHD])	CD4+ T-lymphocyte count of 200–349 cells/μL	Stage 2 (HIV infection)	CD4+ T-lymphocyte count of 200–499 cells/μL *or* CD4+ T-lymphocyte percentage of 14–28
Stage 4 (acquired immunodeficiency syndrome [AIDS])	CD4+ T-lymphocyte count of <200 cells/μL *or* CD4+ T-lymphocyte percentage of <15	Stage 3 (AIDS)	CD4+ T-lymphocyte count of <200 cells/μL *or* CD4+ T-lymphocyte percentage of <14

* For reporting purposes only.
† Among adults and children aged ≥5 years.
§ Percentage applicable for stage 4 only.
¶ Among adults and adolescents (aged ≥13 years). CDC also includes a fourth stage, stage unknown: laboratory confirmation of HIV infection but no information on CD4+ T-lymphocyte count or percentage *and* no information on AIDS-defining conditions.

Centers for Disease Control and Prevention, (12/2008). Appendix B: Comparison of the Revised World Health Organization and CDC Surveillance Case Definitions and Staging Systems for HIV Infection. MMWR, 57 (RR-10).

Thirty years after HIV was initially identified, we now have a wide array of antiretroviral options for HIV infected individuals who are both *treatment naive* and those who are *treatment experienced*. For the main part, antiretrovirals focus on the enzymatic processes that occur during HIV viral replication within a CD4 lymphocyte. Available HAART medications try to prevent HIV viral replication through interference on viral entry and fusion into the CD4 cell, reverse transcriptase, integrase, and protease. Medications are grouped into classes based upon which step in HIV viral replication they inhibit: Entry Inhibitors, Fusion Inhibitors, Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Integrase Inhibitors, and Protease Inhibitors (FDA, 2011)

The FDA has currently approved 33 medications for use in managing HIV. There are also several ongoing clinical trials, by an assortment of pharmaceutical companies, involved in the various stages of new drug development; all seeking FDA approval. New antiretroviral development is a huge field in pharmaceuticals, mainly because so many of the older generations of antiretrovirals were deemed intolerable by patients, as they required a higher pill burden and were highly susceptible to resistance development. This is coupled with the fact that HIV frequently develops new mutations. In addition there is a growing population of HIV *treatment naive* individuals, who are diagnosed with multi-drug class antiretroviral resistance that was inherited during their exposure to HIV, For this newly diagnosed HIV positive patient, the initiation of HAART will be extremely difficult due to their inherited resistance; and will heavily rely on salvage HAART regimens (FDA, 2011; USDHHS, 2011; CDC, 2008).

The initiation of treatment for HIV is comprised of two main stakeholder's opinion: the client's desire/readiness to start HAART and the clinician's interpretation of current clinical treatment guidelines. Current US treatment guidelines are a bit ambivalent as to when exactly HAART should be initiated in individuals who have CD4 counts higher than 350. Half of the treatment guideline panel of experts thought that people with CD4 counts less than 500 but greater than 350 should initiate HAART treatment to maintain the integrity of the person's immune system. However the other half of the panel cautioned that early initiation of antiretrovirals means longer life time exposure to the medications, increased chance of developing HAART resistance, and high odds of developing long term side effects from long term use of antiretrovirals. Needless to say, the guidelines are clear that individuals with CD4 counts 350 or less

should initiate HAART treatment as soon as possible to prevent further disease progression and AIDS (DHHS, 2011).

CHAPTER 2: LITERATURE REVIEW

In this section we will review relevant information and studies about HPV/HIV co-infection. A large wealth of information regarding HPV prevalence in HIV positive women is readily available; nonetheless several gaps in our understanding of HPV/HIV co-infection remain. HPV/HIV co-infection research needs to explore risk factors or co-factors that lead to HPV infection and its persistence in the HIV positive population. The lack of specific guidelines for HPV management and monitoring in HIV positive women demands more epidemiological research to reduce the risk of cervical cancer in this population. In this chapter we discuss these ideas and justify the relevance of the current research for increasing our understanding of the prevalence of HPV infection in a sample of women attending a university Obstetrics/Gynecology (OB/GYN) clinic serving HIV positive women in Puerto Rico.

Prevalence of HPV among HIV positive women

It is believed that HPV infections, and their reoccurrence, are strongly linked to the immune system, so it is not surprising that the prevalence of HPV infection among HIV positive patients is very high. Recent immunological research into HPV has revealed that HPV is mainly regulated by CD4 cells. HPV is also very immunologically elusive; leading to longer subclinical infections before an abundance of innate immune responses trigger the adaptive regulation. This is demonstrated by the nonappearance of inflammation during HPV viral replication. In cases where HPV leads to persistent

infection and cancer, it is clear that HPV is able to outsmart both the innate and adaptive immune systems of the host (Palefsky J, 2006; Palefsky J, 2007; Stanley, 2009; WHO, 2011).

Literature suggests a wide range of estimated average prevalence for HPV in HIV positive women, 36.3% to 97.1%, (Anderson J et al., 2008; Banura C et al., 2008; Del Mistro A et al., 2001; Fakhry C et al., 2006; Gingelmaier A et al., 2007; Hernandez BY & Nguyen TV, 2008; Marais DJ et al., 2008; Richter KL, van Rensburg EJ, van Heerden WF, & Boy SC, 2008; Safaeian M et al., 2007; Tornesello ML et al., 2007; Yamada R et al., 2008). This large range in prevalence may be due to differences in study design, as well as to variations in geographic area and socioeconomic, clinical and lifestyle factors that interact with HPV positivity in these populations. In addition, there has yet to be HPV investigations among HIV positive women, with adequate sample sizes that produce significant statistical evidence that merit generalization at a population level. This discrepancy requires further epidemiological investigation into the variations in regional HPV type distribution and risk factors associated with HPV co-infections, to be conducted with adequate sample sizes and statistical power.

In Puerto Rico there is an estimated 14,546 women living with HIV/AIDS (PRDH, 2008). Despite this, it is currently unknown what the HPV prevalence is for HIV positive women living in Puerto Rico due to the lack of clinic- and population-based data.

Clearance of HPV in Co-Infected Women

learance of HPV in Co-Infected Women: In HIV positive women, the rate at which HPV co-infections self-clear is slower than in HIV negative women (Danso, Lyons, & Bradbeer, 2006). In HIV negative women, typically HPV lesions clear on their own within one to two years. There is some scientific evidence that HIV may directly interact with HPV. In cells similar to those found in the vaginal wall and cervix, HIV is known to alter local cytokine expression which regulates cell mediate immunity to pathogens like HPV (Danso, Lyons, & Bradbeer, 2006). This suggests that HPV/HIV co-infection in women may allow for frequent HPV reactivation when immunological function is reduced; thus permitting longer persistent states than are seen in HIV negative women. The associated factors surrounding HPV infection, as well as its frequent reoccurrences and persistence are currently the focus of much epidemiological research among HIV positive women.

The use of HAART in HPV/HIV co-infections

Research on the effects of HAART in relation to HPV co-infections have resulted in diverse conclusions (Minkoff, Ahdieh, Massad, et al, 2001; Sirera, Videla, Lopez-Blazquez, Llatjos, et al, 2007; Palefsky, Holly, 2003; Kojic, Cu-Uvin, 2007; Danso, Lyons, & Bradbeer, 2006). Some studies have suggested that women on HAART have a higher rate of regressing to normal cytology when they have higher CD4 counts (Danso, 2006; Sirera, 2007). Others have suggested that HAART may have no effect on HPV co-infection at all and may just improve immune system overall function (Palefsky, Holly

2003). Some have suggested that overall incidence of cervical cancer rates have remained unchanged or slightly increased in light of widespread HAART use (Kojic, Cu-Uvin, 2007). This may be due to the fact that women on HAART now live longer than prior to HAART use, largely because of reduced incidence of many AIDS-related malignancies.

Key Studies of HPV/HIV Co-Infection

Between the years, 1997-1999, Dr. Margherita Branca and her epidemiological team, as part of a larger multi-institutional DIANAIDS project, enrolled 142 women, aged 17-45, (89 HIV positive and 48 HIV negative) into a prospective follow-up study conducted in various clinics throughout Italy. The main objective was to analyze the clinical course of HPV infections and cervical Papanicolaou (Pap) smear abnormalities, as well as to identify factors associated with their persistence and clearance during the follow-up period (an average of 14 months, SD: 10.84). Women received regular HPV-PCR testing, Pap smears, and HIV testing at baseline and then were subsequently enrolled in either the HIV-positive or -negative arm of the study.

Branca et al., found that at time of entry into the study the HIV positive group of women, had a HPV prevalence of 38.6%; and at the time of follow-up had 42.9% HPV prevalence, with some 27.5% of these infections being newly diagnosed. The analysis of this study showed that women with HIV infections were more than eight times more likely to have the appearance of new HPV infections during the follow-up period than women who were HIV negative (OR= 8.8, 95% CI 1.199-64.61). The clearance of HPV infections in the HIV positive women (22.8%) was significantly less frequent than that

of their HIV negative counterparts (69.2%). It was also established that disease progression was more common among the HIV positive women, than in the HIV negative women (OR= 3.51; p=0.055), although the result was marginally significant.

This study concluded that key factors for HPV infection among HIV positive women were low CD4 count and significant abnormal Pap smears. In addition, they concluded that younger age, non-use of barrier contraception and low CD4 counts were predictors of a persistent HPV infection in HIV positive women. Despite the light shed on to HPV/HIV co-infection by the Branca study, it had several limitations. A small sample size restricted the study's ability to demonstrate statistically significant associations between predictors of HPV and persistence in the study population. In addition, the inclusion criteria required the HIV positive women to be on HAART therapy, thereby limiting focus to only those with relatively well-controlled HIV infection.

Despite these limitations, this study by Branca et al. is important given their findings of key factors related to HPV/HIV co-infection and persistence. It demonstrated the strong association of CD4 counts to disease progression in this cohort, in terms of HPV infection and persistence. It was also a hallmark study by highlighting the differences in HPV clearance among HIV positive and HIV negative women. While the understanding of the relationship of HAART in HIV/HPV co-infection was not advanced, this study clearly demonstrated that despite the cohort being on stable HAART meds, HPV diseases persisted and progressed; leading the reader to question the impact of HARRT on HPV.

Denny et al., (2008) conducted a prospective study of 400 HIV-1 infected women who participated in frequent HPV DNA testing, cytology, colposcopy, histology, and CD4 count testing every 6 months for 36 months, between the years 2002-2006. The objective was to report the natural history of high risk HPV infection and cervical abnormalities in women infected with HIV-1 who lived in Cape Town, South Africa (Denny, L, Boa, R, Williamson, AL, Allan, B, Hardie, D, Stan, R, and Myer, L 2008). At baseline, 68% of the women in this study had HPV DNA detection. The majority of the sample had one HPV type (27%), however multiple types of HPV were more common in women with lower CD4 counts and higher viral loads ($p<0.001$). Multiple types of HPV were also associated with women having abnormal Pap smears with more advanced precancerous lesions (HSIL or CIN-3). In addition, this study encountered a very high occurrence of cytological abnormalities, with 55% of the women having abnormal Pap smears. Furthermore, investigators reported a wider variety of HPV types in the population than what was originally hypothesized. They concluded that this was attributed to greater regional variation of HPV type distribution than what was previously believed. This study is critical in our understanding of the dynamic relationship HPV and HIV share in co-infection population for three reasons; 1) its focus was on HPV genotyping, 2) focused on the role HPV genotypes played in disease progression, and 3) explored the relationship of HPV genotype persistence and virulence according to CD4 counts and HIV viral load.

During the period of 1996-1997, an interdisciplinary team under the direction of Dr. Maria Alice Goncalves conducted a cross-sectional study in HIV positive women who attend the Center of Reference in AIDS of Sao Paulo, Brazil. A total of 138 women

between the ages of 19-57 (mean age 31 years) were recruited. All participants completed a questionnaire to collect information on socio-demographics and lifestyle. In addition, the study evaluated CD4 counts of the women, performed HIV confirmatory tests, and performed HPV testing in three anogenital areas (vagina, cervix, and anus). Among study results, Goncalves et al, found the prevalence of HPV in the cervix to be 61.6%, with 36.6% considered to be high-risk HPV. Another major finding was that nearly half of all cervical samples (41.2%) were co-infected with multiple HPV types. Due to small sample size, OR values did not reach statistical significance causing one of the biggest limitations of this study. However, the authors did conclude that there was an increase in HPV infection in HIV positive women who were less than 25 years of age compared to those who were 35 years of age or older. Another major finding of this study was that the number of recent sexual partners of women was a stronger determinant of HPV infection than lifetime number of sexual partners. The Goncalves' study is important, in that it focused on HPV genotype distribution and HPV multiple-type infections in various body sites of HIV positive women. While this study lacked statistical power to further evaluate the associations between HPV and various risk factors, it did present great detail and analysis of the association between age and number of sexual partners and HPV infection.

In 2008, Sirera et al., published the results of their retrospective observational study on HAART and incidence of cervical squamous intraepithelial lesions (SIL) among HIV positive women with normal cytology and CD4 counts above 350. Their research investigated 127 women, who were evaluated at the University Hospital of Germans Trias I Pujol, in Barcelona Spain, between the years 1997 -2006. These women were

followed until SIL was diagnosed. They concluded that the use of HAART in women with CD4 counts over 350 did not lower their risk of developing precancerous lesions; and that the use of HAART may slightly increase a woman's odds of developing SIL (OR=1.84, 95% CI= 0.72-4.69). The research team suggested that HAART may alter the cervical immunology, producing an imbalance that cannot stabilize in the presence of HPV infection. The small sample size and retrospective observational-design of this study was the greatest limitation, and may account for these controversial results. Nonetheless, this study was important as it established research parameters for exploring CD4 counts and HPV related disease (SIL).

Between January 2007 and June 2009, Garbuglia et al., collected 533 cervical samples from HIV positive Italian women attending the gynecological service at "L. Spallanzani" Hospital. Using this data, Garbuglia and her team conducted a retrospective, observational study to measure the HPV prevalence, describe the HPV genotype distribution, and determine the relationship between multiple HPV infections with cervical abnormalities. Among study results, they found that 44.1% of the study subjects were HPV co-infected. The median age of the women was 40.3 years; 83.4% of them used HAART medications, and 86% had undetectable HIV viral loads. The median CD4 count was 501. Results also showed that 56% of the HPV infected women were infected with a single HPV genotype; multiple infections were significantly associated with lower CD4 counts. In fact, women with CD4 less than 200 were over 3 times more likely of having multiple infections than women with CD4 counts greater than or equal to 500 (OR=3.8, 95% CI=1.5 -9.6).

This study by Garbuglia and colleagues was able to isolate a total of 412 distinct HPV identifications belonging to 32 different genotypes and 12 distinct phylogenetic species. High-risk HPV genotypes were the most predominant and accounted for 54% of all HPV infections. HPV 16 was the most frequently found high-risk genotype followed by HPV 53, HPV 31, and HPV 66. HPV 16 was commonly found in multiple-type HPV infections, whereas HPV 53 was commonly found alone. Among low-risk HPV genotypes, the most frequently occurring was HPV 61, followed by HPV 62, 6, 84 and 83. Among the women of this study with normal cervical cytology, some 37% were found to have HPV DNA present in the sample; 69% of these normal pap smears had high-risk HPV genotypes present in the cervical sample. Overall, abnormal cervical cytology was associated with increasing number of HPV types ($p<0.0001$).

The Garbuglia et al., study is important when considering HPV infection in HIV positive women because of the degree to which it explored HPV genotype distribution. This study not only elaborated on the various specific genotypes found among the cohort; but it compared them to the phylogenetic species that are becoming more increasingly used. It was also able to demonstrate and quantify the relationship that HPV and lower CD4 counts maintain, which may shed more light on how to better manage gynecologic care in this population. One of its main limitations was that it was retrospective in nature, and as such they were unable to relate participant life style factors to the results, as this data had not been available for collection. Regardless, their findings and their ability to quantify the various genotypes found in this population highlight the need of including additional HPV genotypes in the next generation of HPV vaccines.

Justification

While the definitive connection between HPV and cervical cancer is well documented, our understanding of HPV factors that influence disease progression and persistence, in groups at high risk for HPV infection (like HIV positive women) still warrant further research. A great wealth of research exists that firmly establishes HPV as a powerful carcinogen, but clear treatment guidelines for specific high-risk groups, such as HIV positive women, for the prevention of cervical cancer, should be further developed. Furthermore, a recently published study by Ramirez-Marrero and colleagues in 2010, demonstrated a consistently high standardized incident ratio (SIR 28.7, 95% CI 14.8-50.2) of cervical cancer in Hispanic women with AIDS living in Puerto Rico when compared to the general population of Puerto Rico (Ramirez-Marreo, Smit, De La Torre-Feliciano et al., 2010).

The role of common place labs, like the CD4 counts and HIV viral load, don't have a common place in gynecological care among HIV positive women; yet research shows us that there is a link between these factors and HPV infection, and its persistence in this co-infected population. The link of HPV to these immunological biomarkers isn't fully understood, and further research into this area is needed to fully understand HPV infection among HIV positive women.

Despite scientific evidence of high rates of HPV infection among women with clinically normal cervical cytology; some clinicians are still recommending that women with normal pap smears forgo HPV testing. While most HPV infections remain sub-clinical for years, early detection is the key to preventing persistent infections and

disease progression. It is believed that while HPV is seemingly in a dormant state, that these infections are causing substantial cellular changes that later on will be detected through Pap smears and colposcopy. Such manifestations are then treated for the Pap smear abnormalities (ASC-US, LSIL, HSIL, and CIN) rather than for the underlying HPV infection (CDC, 2006; Danso, 2006). In order to move to prevention based medicine, as opposed to reactive medicine, we need a clearer picture of HPV infection before precancerous lesions develop.

In Puerto Rico, a clear picture of cervical cancer rates is routinely captured by the Puerto Rico Central Cancer Registry. This surveillance system documents that cervical cancer continues to be a leading cancer type among in this population (PR-DOH, 2008). In fact, cervical cancer still remains one of the top cancers found in women in Puerto Rico; despite wide spread access to gynecological services. Furthermore, in 2010, the Puerto Rico BRFSS estimated that 75% of surveyed women 18 and older reported having had a pap smear within the last three years; just slightly less than US average of 81% (CDC, 2010). Nevertheless, despite this relatively high coverage, cervical cancer screening rates in Puerto Rico fall short of the Healthy people 2010 and 2020 recommendations (Ortiz, Hebl, Serrano, Fernandez, Suarez and Tortolero-Luna, 2010). The Healthy People 2010 target for women 18 years of age or older to receive a pap smear within the last three years was 90%; the target of the Healthy People 2020's for this same group is 93% coverage (DHHS, 2000; DHHS, 2012).

Although surveillance data on cervical cancer and cancer screening is available for Puerto Rico, the prevalence of HPV infection in this population has yet to be fully explored. To date, there is no population based data available on the prevalence of HPV,

nor the genotypes found among the Puerto Rican population. The surveillance systems suffer from underreporting, are still in their early stages of utilization and generally focus on genital warts caused by low-risk HPV infections. As reporting increases, and hopefully includes high-risk HPV infections in the future; a greater understanding of HPV prevalence and incidence in the Puerto Rican population will be gained. Nonetheless, in the meantime, the lack of appropriate surveillance data calls for the development of epidemiologic research studies of HPV burden in Puerto Rico. To our knowledge, no epidemiological research has been conducted that measures the prevalence of cervical HPV infection and specific HPV types in HIV positive women. Information regarding the factors associated with HPV/HIV co-infections are unknown for women living with HIV infection in Puerto Rico. The HPV type distribution within HIV positive women in Puerto Rico is also unknown. This information is essential in determining the feasibility and efficacy of HPV vaccines in HIV positive Puerto Rican women. By furthering our knowledge of what HPV types are circulating within the Puerto Rican population, the appropriateness of current HPV vaccines among Puerto Rican young girls and women will be also better understood. Cervical cancer and HPV are totally preventable with safer sex measures, public health education, and open access to regular gynecological, cytology services like Pap smears, HPV testing, and vaccinations.

The justification for this current study is that in Puerto Rico, cervical cancer remains one of the leading cancers affecting women. The risk for cervical cancer is greater in HIV positive women who are also infected with HPV than among their HIV negative counterparts. However, there are no known population-based studies related to

HPV infection in the Island in this population. High levels of standardized incident ratios for HPV related cancers, such as vaginal and vulvar cancer, anal cancer, penile cancer and oropharyngeal cancer, have also been reported among Hispanics living with AIDS in Puerto Rico when compared to the general population of Puerto Rico (Ramirez-Marrero et al., 2010). This high burden of HPV –related cancers clearly asserts a greater need for HPV investigation among people living HIV/AIDS in Puerto Rico. Finally, our scientific knowledge of the HIV co-factors and the relationship CD4s and HIV viral load have with HPV infections still remains elusive. These items are crucial for effective gynecological management of HPV-related diseases in HIV infected women. Without understanding how to better manage and prevent HPV infections among these women, they will continue to be at high risk for HPV related malignancies and continue to add to the rising costs of health care management.

CHAPTER 3: METHODOLOGY

In this chapter we describe the study's research questions, objectives, and hypotheses, and the methods used to collect the data. Finally, we describe the statistical methods used for evaluating the data and testing the hypotheses. In the appendix of this document, we include the questionnaires used for data collection, the informed consent form and the IRB approval letter. The goal of this cross-sectional study is to understand the relationship of immunological function with HPV infection among HIV positive women.

Research Questions

The study has the following research questions: (1) What is the prevalence of HPV among HIV positive women, 21 years of age and older, who attend an investigational clinic at the MSC, UPR? (2) What types of HPV infections are present in the study population? (3) What is the relationship of clinical HIV factors, such as levels of CD4, HIV viral load counts and use of HAART, with HPV infection in this study population?

General Objective

Describe the prevalence (overall and type-specific) of HPV infection and its association with CD4 counts, HIV viral load, and HAART among a clinic-based sample of adult HIV positive women in Puerto Rico.

Specific Objectives

1. Describe the study population in terms of demographic, lifestyle and clinical characteristics.
2. Estimate the prevalence of HPV infections in HIV positive women (overall and by risk types).
3. Describe HPV genotype distribution present in the HPV infected population.
4. Describe women who are HPV infected, and those who are not infected with HPV in terms of: socio-demographics, lifestyles, and clinical characteristics.
5. Estimate the magnitude of the association between HPV infection and the independent variables CD4 counts, HIV viral load, and HAART within the study population.
6. Estimate the magnitude of the association between HPV infection and the independent variables CD4 counts, HIV viral load, and HAART within the study population, while adjusting for potential confounders.

The Principal Hypotheses

- The prevalence of HPV infection among HIV positive women attending the clinic will be approximately 60%, with the majority of women having multiple HPV type infections.
- HPV genotypes 6, 11, 16, and 18 will be the most commonly found genotypes among the study population.
- Women with low CD4 counts (less than 350) will have higher prevalence rates of HPV infections than women with higher CD4 counts (higher or equal to 350).

- Among women with undetectable HIV viral loads, the prevalence rates of HPV infection will be lower.
- Women who are not on HAART will have higher prevalence of HPV infections than those women who were currently using HAART.

Methodology

The Maternal Infant Studies Center (CEMI, for its Spanish acronym), is a longitudinal investigational OB/GYN clinic at the University of Puerto Rico Medical Sciences Campus, dedicated to working with HIV positive women. It has been in operation since the early days of the HIV epidemic in Puerto Rico, and has served nearly 2000 women in over twenty years. It began as a place where women who were HIV positive could go to receive treatment during their pregnancies, as a way to reduce vertical transmission.

Today CEMI offers a wide array of gynecological services for HIV positive women, including empowerment workshops and access to social workers; and it is also involved in the HIV Vaccine Trial Network and several privately funded clinical trials. In order for women to receive services at CEMI, they must have laboratory documentation of their HIV infection, i.e. western blot or Elisa tests, as well as provide written consent to have their medical records used in investigational research.

Study Population

CEMI's database of patients at the time of the study included over 1800 women who were considered currently active; meaning that they were neither lost to follow-up

or deceased. The participants of this study consisted of women, 21 years of age and older, who had *a documented diagnosis of HIV infection, with no prior history of cervical cancer,* and who attend CEMI for clinical services. All study materials, including the informed consent form, questionnaires and source documents (See appendix I-VI), were reviewed and approved by the Office for Human Subject Protection (IRB) of the Medical Sciences Campus prior to study initiation.

Sample Selection

Consecutive sampling of women attending CEMI from September 2009 to February 2010 was used to select the study population, for a final sample size of 130 women who consented to participate in the study. Given that it is common for patients of CEMI to frequently change their contact information without prior notification, the hard-to-reach nature of this population required this form of sampling, in order to achieve an adequate sample size, while keeping investigational costs to a minimum.

Inclusion criteria included: (a) that the participant had a documented diagnosis of HIV; (b) that participant had no prior history of cervical cancer; (c) and had come to CEMI for a routine clinical, including pregnant women. Women meeting these criteria were offered participation in the study. Those participants who came to CEMI in order to receive results from a previously taken Pap smear or for abnormal cytological treatment were excluded from the study.

Data Collection: As patients enter CEMI for clinical check-up, they are required to take a number; trained study staff met with potential participants in the order that they arrived, in order to adhere to the consecutive sampling methods. At this time, study staff

determined the eligibility of the subjects for study participation. Participants who met all eligibility requirements (i.e., no prior history of cervical cancer and who had come to CEMI for a routine clinical visit) were offered participation in the present study. At this time, participants had the opportunity to review the informed consent form and ask any questions about the study. If the participant agreed to participate, a copy of their signed informed consent form was provided for their records, and the original form was kept in their study file (see appendix II)

Once the informed consent form was signed, participants met with a member of the study staff who explained the study procedures to them. All study participants were seen at CEMI for all protocol procedures. Participants were asked to complete two self-administered questionnaires to capture information related to their previous exposures or risk factors associated with HPV infections, as well as socio-demographics. The first questionnaire used was the *Common Questionnaire*, a standardized data collection tool used by all studies conducted as a part of the Puerto Rican Comprehensive Center for the Study of HIV Disparities (PR-CCHD) (see Appendix IV). In addition, all women completed The *HPV Cross-Sectional Study Questionnaire*. This was the primary data collection tool used for our analyses (see Appendix V), as it captured information specifically related to HPV, and factors related to HPV/HIV co-infection.

Study staff also used a source document to collect and verify participant reported clinical data (see Appendix VI). There are portions of the source document that were completed specifically by the attending physician/gynecologist during the study visit, as well as portions completed by study staff once laboratory data was returned to CEMI. All study staff who worked with these questionnaires and source documents, were

extensively trained in how to administer and complete these materials to insure consistency among the study sample.

After completion of the study questionnaires, participants met with a clinician/gynecologist for the collection of the HPV cervicovaginal samples. The collection of the HPV samples was conducted in a uniform and consistent manner to ensure proper data collection and handling of samples. The clinician asked the participant to prepare for her routine clinical evaluation. After the participant was positioned and draped for the routine pelvic examination, the study clinician/gynecologist collected the cervical specimens for HPV-DNA using the following procedure: Immediately following the routine pap-smear as part of their scheduled clinical visit, each woman received a standard pelvic examination to collect cells for HPV. Testing was obtained using one Digene hc2 HPV DNA Collection Device (Qiagen). A vaginal swab (Qiagen), similar to a cotton q-tip, was used for those women who were pregnant at time of HPV sample collection; in those women who were not pregnant, a cytobrush was used to collect the sample. Those participants who had a history of radical hysterectomy had samples taken from the vaginal canal, in place of cervical samples.

In order to collect the cervical HPV sample, the swab or cytobrush was inserted by the clinician in the endocervical/ectocervical canal and turned for two full rotations to maximize cell collection. Each swab or cytobrush was placed in an individual 5-mL vial, identified with their unique study identifier, that contained 1 mL Digene specimen sample transport medium (Digene Corp./Qiagen) All samples were stored in a 5 °C refrigerator until the messenger was able to deliver the samples to Dr. Yamamura's

laboratory, of the Ponce School of Medicine, for HPV typing. Dr. Yamamura's lab provided the HPV genotyping analysis after PCR linear array genotyping was completed.

Next the participants met with a study nurse, who collected the routine clinical visit blood samples to measure CD4 and HIV viral load counts. These lab results were later collected from the participant's clinical record at CEMI and included in the study's data collection forms. After all samples were collected, participants met with study staff to answer any questions that the participant might have had about the visit, to check questionnaires for completion, and provide a $15 stipend for any expenses related to study participation (i.e., transportations costs). Participant's contact information was also verified at this time, so that once laboratory results were returned to CEMI; the woman could be contacted by CEMI staff if any gynecological follow-up was needed given their results.

All data was compiled and entered into SPSS 18.0 for data analysis. Data entry was verified through review of clinical file, source documents, and laboratory findings provided by the Ponce School of Medicine. Any missing data fields were revised, rechecked with the original study file, and if needed re-entered.

Study Variables

Main outcome variable. The main dependent variable of this study was HPV positivity (yes/no) (See table 4 for a detailed overview of all study variables). All HPV testing was done locally at the Ponce School of Medicine, under the direction of Dr. Yamamura. His team performed PCR-DNA typing using a Roche Linear Array Genotyping Test that

provided the specific genotypes of HPV present in the tissue. This method is able to capture the 37 types of HPV commonly found in the genital tract.

If a sample tested positive, it was then amplified to capture the following 37 HPV genotypes: -6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -45, -51, -52, -53, -54, -55, -56, -58, -59, -61, -62, -64, -66, -67, -68, -69, -70, -71, -72, -73, -81, -82, -83, -84, -IS39, -CP6108. The women were divided into two categorical groups, based upon their HPV laboratory test results. Those with positive HPV tests were assigned to the HPV infected group. According to the Roche package insert for the linear array HPV genotyping, one cannot rule out the presence of additional HPV genotypes from those tested above. If a woman was to test positive, yet does not have a single genotype of those detected from the Roche linear array genotype kit, she would be positive and would have undetermined genotypes. While this did not occur in our study, we cannot rule out any additional genotypes present in the study population that were not included in Roche's 37 genotyping linear array (Roche, 2006).

A sample was determined HPV negative if there was no evidence of HPV DNA, and both the low and high β-globin results were positive; if either of the β-globin results were negative the sample run was deemed invalid and a new aliquot from the original sample would be newly amplified. For quality control assurance each linear array HPV genotyping kit comes with one HPV negative control and one HPV positive control. These controls must be processed with each HPV test samples, and should be amplified in the same run in order for the results to be deemed valid. (Roche, 2006)

The assignment of cervical cancer risk for each HPV genotype was assigned by the Ponce School of Medicine PR-CCHD laboratory (Appendix VII). These risk assignments match the literature set for by the IARC (IARC, 2007; IARC, 2012). The following HPV genotypes are classified as high-risk: HPV-16, -18, -31, -33, -35, -39, -45, -52, -56, -58, -59, and -68. Those classified as low- risk are HPV genotypes were: -6, -11, -26, -40, -42, -53, -54, -55, -61, -62, -64, -66, -67, -69, -70, -71, -72, -73, -81, -82, -83, -84, HPV IS39, and HPV CP6108.

Main independent variables- HIV Co-factors: The main HIV co-factors included: CD4 and HIV viral load counts, and use of HAART; and data was captured from laboratory analysis and verified through medical record review. Such immunological biomarkers like CD4 counts and HIV viral load were measured and analyzed for the study to see how HIV and the immune system interact with HPV. The study participants CD4 counts and HIV viral load were taken by blood samples and then were sent to the local Puerto Rican Health Department for analysis by flow cytometry.

Initially, the CD4 variable was categorized according to the CDC's HIV categorical scheme. The CDC for purposes of tracking HIV disease progression has established the following classification system: Stage 1: CD4 count is $\geq$ 500; Stage 2: CD4 count is <500 but > 200; and Stage 3: CD4 count is $\leq$ 200. When examining the efficacy of the CD4 CDC-categorical variable, it became apparent that this variable would be unstable in multivariate analysis because of its small size when sub-categorized (Stage 3, n=2). Therefore, we examined the frequency of CD4 counts as classified under the World Health Organization (WHO) system (CDC, 2008). In the WHO classification system there are four clinical stages for HIV-associated immunodeficiency, they are: Clinical

stage 1, described as asymptomatic with CD4 $\geq$ 500; Clinical stage 2, mild, with CD4 between 350-499; Clinical stage 3, advanced, with CD4 between 200-349; and Clinical stage 4, severe, with CD4<200.

Other independent variables. The other independent variables under investigation for this study fall into three categories: socio-demographics and lifestyle factors associated to HPV infection, and clinical characteristic possibly associated to HPV/HIV infections (See table 2). Those variables that were categorized as socio-demographic included age, marital status, educational attainment, monthly income, among others. Lifestyle factors like age of sexual debut, history of STI, number of current and lifetime sexual partners, use of oral contraception, and smoking habits have all been strongly associated with HPV infection and cervical cancer development in HIV negative women. Also, clinical data collected included the results of the Pap smear that women received at the time of study recruitment. The cervical cytological samples were sent to the Puerto Rican Health Department for analysis. Given that many of these variables have been associated to HPV infection in previous studies, the data collection of this information was essential as some of these variables may act as confounders in our current study. Therefore, information was thoroughly collected to be able to consider these variables in the analyses.

Table 2: Description of Study Variables			
Variable Conceptual Definition	Variable Operational Definition		Variable Measurement
	Nominal, Ordinal, or Interval	Question	
A. Socio-demographics			
Age (years)	Interval	What is your age? When is your birthday?	Data obtained from questionnaire and verified in chart review.
Marital Status	Nominal	Which of the following best describes your marital status, never married, married, living with significant other, divorced, separated, or widow?	Data obtained from questionnaire responses.
Educational Attainment	Interval	What is the highest level of grade or degree you have completed?	Data obtained from questionnaire responses.
Monthly Income	Ordinal	Which best describes your monthly income at this time (including all salaries or other types of economic help that you may receive): < than $300, $301-$600, $601-$900, $901-$1,200, or ≥ $1,201?	Data obtained from questionnaire responses.
B. Lifestyle and clinical characteristics			
Sexual debut (coitarche)	Nominal and Interval	Have you ever had vaginal intercourse? Yes or no? At what age was your first vaginal intercourse?	Data obtained from questionnaire responses.
Sexual partners	Interval	In the last 12 months how many partners did you have vaginal intercourse? In your lifetime?	Data obtained from questionnaire responses.
Harm Risk Reduction	Nominal	In the last 12 months how often did you use protection (like condoms) during sexual intercourse? Never, Sometimes, Frequently, Almost Always, Always.	Data obtained from questionnaire responses.
Contraception	Nominal	Which have you used in your lifetime? Abstinence, condoms, birth control pills, the ring, diaphragm, deprovera, IUD, surgically sterilized. For how long? What do you currently use?	Data obtained from questionnaire responses and verified with medical record
Age of Conception (years)	Interval	At what age did you first get pregnant? did you first give birth?	Data obtained from questionnaire responses.

Gravidity/Parity	Nominal and Interval	Have you ever been pregnant? Yes or no? How many pregnancies have you had? How many deliveries?	Data obtained from questionnaire responses and verified with medical record
Hx of cytology	Nominal	Have you ever had an abnormal Pap smear result? Yes/No	Data obtained from questionnaire responses and verified with medical records
Cytology Results	Ordinal	What was the result of the participant's Pap smear at time of study visit? Normal, ASCUS, LSIL, HSIL, CIS, or Carcinoma? Normal or Abnormal	Data obtained from medical record and lab results.
C. Human Papillomavirus			
HPV Infection	Ordinal and Interval	Does the participant currently have an HPV infection? Yes or no? How many types of HPV is the participant infected with? What type of HPV does the participant have? High-risk, low-risk, or both? What HPV types were found in the PCR analysis done during the study visit?	Data obtained from medical record and lab results.
D. HIV Co-factors			
Viral Load	Nominal and Interval	What was participant's last viral load count? What was participant's viral load count day of study visit?	Data obtained from medical record and lab results.
CD4 count	Nominal and Interval	What was participant's last CD4 count? What was participant's CD4 count day of study visit?	Data obtained from medical record and lab results.
HAART	Nominal	Do you take medication for HIV? Yes or no? Which HIV medications does participant currently take? Which of the following sentences best describes your treatment adherence since the last study visit? Good, I take my pills every day when I'm supposed to; Fair, I take my pills every day, but only when I remember them; Poor, I occasionally forget to take my daily dosage, but typically take them; or Bad, I rarely remember to take my pills?	Data obtained from questionnaire responses and verified with medical record

Data Analysis

Univariate Analysis: Descriptive analysis was conducted in order to describe the overall study population; and the two separate groups of women (HPV infected and HPV negative) according to their socio-demographic/epidemiological profiles. Measures of central tendency such as mean and median, and measures of variability such as standard deviation and interquartile range (IQR), were considered to summarize continuous variables. Absolute frequency and proportions measures were used to summarize categorical variables. Continuous variables were assessed for normal distribution using quantile-quantile plots. Both HIV viral load and CD4 count variables, did not have normal distribution, and were thus recoded into categorical variables.

The prevalence of overall HPV positivity was estimated by point prevalence and through the construction of its 95% confidence interval. In order to describe the distribution of HPV types among this population of women, we used frequencies for each individual HPV genotype, and then determined the prevalence for HPV infection based upon their risk type (high-risk vs. low-risk) for cervical cancer, and constructed their respective 95% confidence intervals.

Bivariate Analysis: Bivariate analyses were conducted in order to compare those women who were HPV infected to those who where HPV negative. Two-sided two-independent samples *t*-tests were used to determine differences in terms of age, age of sexual debut, and other continuous variables with normal distribution, between HPV infected and HPV negative women. Contingency tables and chi-

square tests for independence between characteristics were tabulated to identify associations between socio-demographical, lifestyle, clinical characteristics and HIV co-factors (including CD4 counts, HIV viral load and HAART) and HPV positivity. The magnitude of association, measured by the prevalence odds ratio (OR), between the dependent variable, HPV, and the above mentioned independent variables, was analyzed to estimate the association between HPV status and the relevant variables.

Multivariate Analysis: Multiple logistic regression models were developed to estimate the magnitude of association between HPV infection and selected HIV-cofactors, while adjusting for potential confounders. We first evaluated statistical interaction using the likelihood ratio test; however, due to issues of collinearity the statistical model was unstable and unable to estimate the parameters with precision. In order to observe the effect of HIV-cofactors we used stratified analysis instead. The Mantel Haenzsel method was used to compare those variables associated with HPV in order to identify and evaluate those that are acting as potential confounders. Adjusted odds ratio were compared to crude odds ratio values, and those variables whose adjusted OR estimate had a change greater than 10% were then considered to be confounding variables. Finally, appropriate adjusted and stratified ORs with 95% confidence intervals were estimated.

The final logistical regression model was utilized on two different strata: HIV viral load detectable and HIV viral load undetectable. The following is the

final logistical regression model used in the aforementioned strata, *where p= prevalence of HPV*:

$$\ln(\frac{p}{1-p}) = \beta_0 + \beta_1(CD4) + \beta_2(\text{cytology}) + \beta_3(\text{age}) + \beta_4(\text{partners})$$

CHAPTER 4: RESULTS

In this chapter we present the results of our data analysis as outlined in the previous chapter's methodology. Here we describe the characteristics of the study population, and the HPV prevalence and genotype distribution among the women in the study. We also present the results of the bivariate and multivariate analyses regarding the association of the independent study variables with HPV positivity.

HPV Prevalence and Genotype Distribution

The final study population consisted of 130 HIV positive women, consecutively seen at CEMI during the time period of September 2009 and February 2010. In the study population, the estimated point prevalence of women testing positive for HPV, by way of PCR linear array laboratory analysis, was 57.7% (95% confidence interval as =49.1, 66.0). The true prevalence of HPV infections within this clinic-based population lies somewhere in between the abovementioned interval. The prevalence of high-risk HPV genotypes among women HPV positive was 58.7% (95% CI: 47.3, 69.4), compared to the 85.3% (95% CI: 76.0, 92.0) who tested positive for low-risk HPV genotypes (See tables 3).

The most common HPV genotypes in the study were HPV 61 (10.8%) and HPV 66 (10.8%); each with 14 women infected with these low-risk HPV

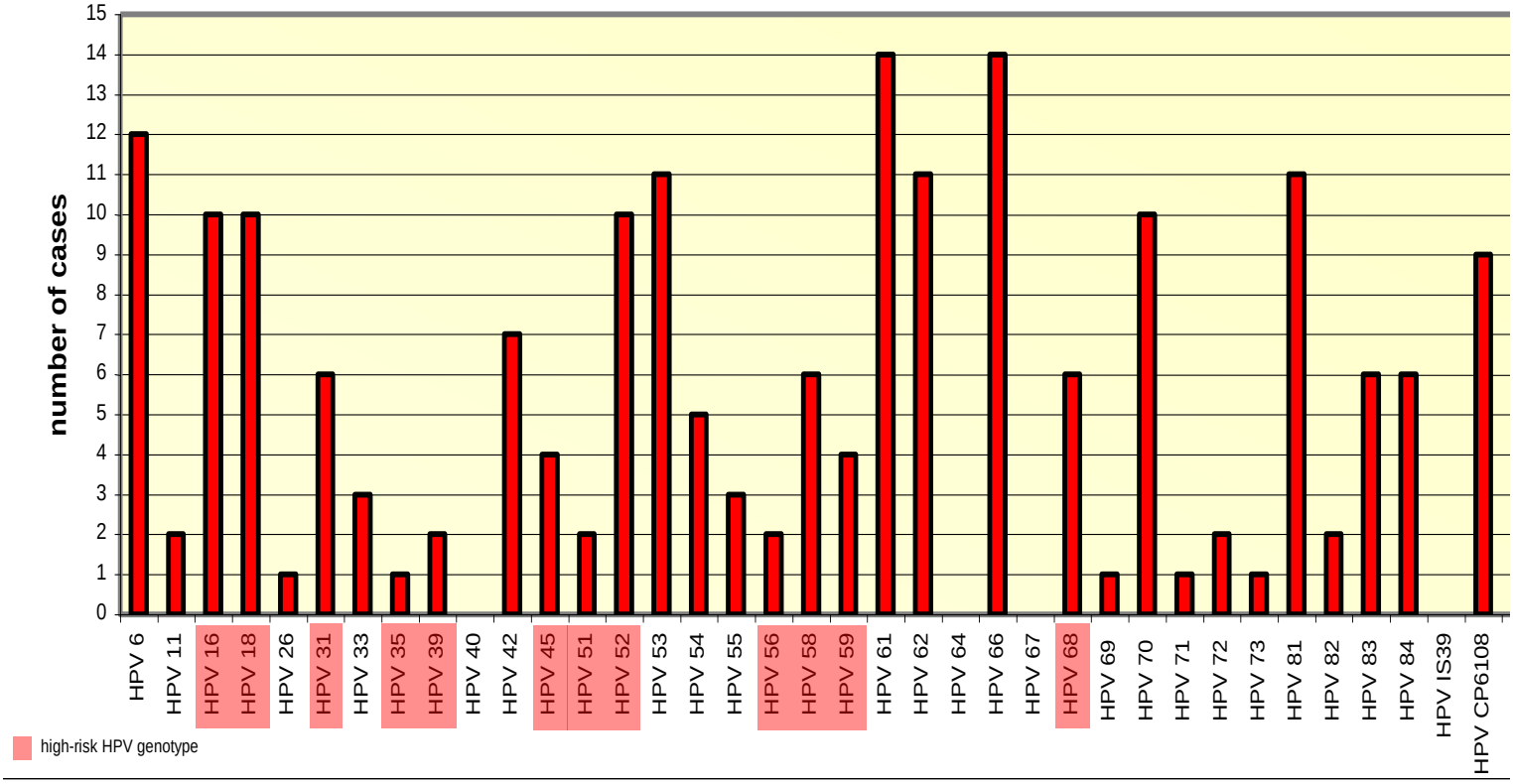
genotypes (See figure 8). The most frequently seen high-risk HPV genotypes were: HPV16 (7.7%), HPV 18 (7.7%), and HPV 52 (7.7%); all registering 10 women for each genotype. These high risk genotypes accounted for almost 23% of all HPV infections in the study population. The most common LR-HPV genotypes found in the study (with 10 or more women each), in order of frequency were: HPV 70 (7.7%), HPV 81 (8.5%), HPV 62 (8.5%), HPV 53 (8.5%), HPV 6 (9.2%), HPV 61 (10.8%), and HPV 66 (10.8%).

Table 3: Prevalence of Human Papillomavirus within the Study Population (n=130)

	n	% (95% CI)
Overall HPV Prevalence	75	57.7 (49.1, 66.0)
High Risk HPV Prevalence	44	58.7 (47.29, 69.4)
Low Risk HPV Prevalence	64	85.3 (75.94, 92.04)
Prevalence of Multiple-Type Infections among HPV infected Women	50	66.7 (55.5, 76.6)
One HPV genotype	25	33.3 (23.4, 44.5)
Two HPV genotypes	20	26.7 (17.6, 37.5)
Three HPV genotypes	13	17.3 (10.0, 27.2)
Four or more HPV genotypes	17	22.7 (14.3, 33.2)

Among the women HPV positive, two thirds of these women had multiple HPV genotype infections, ranging from two to nine genotypes. Almost a third of all HPV positive women had only one HPV genotype, 25% had two types of HPV, 17% had three genotypes, and 22% had four or more genotypes (see table 3). There were 28 women (21% of the study populations) who tested positive for at least one of the four genotypes (Types 6, 11, 16, and 18) covered under the quadrivalent recombinant HPV vaccine, GARDASIL®.

Figure 8: HPV Genotype Distribution Among Study Participants



Description of the Study Population:

Demographics. The mean age among the study participants was 39 years of age (SD $\pm$ 10.8). The majority of the women (63.2%) were either high school graduates or had gone on to college; 36% graduated high school and more than a quarter (27.2%) obtained a higher degree, while only 36.8% had not graduated from high school. In addition, 38.5% of participants had a monthly income <$300 (38.5%). Among the study participants, 43.1 % of the women were either married or living with their consensual partner at the time of study recruitment, 33.1 % reported themselves being divorced, separated, or widowed, and 23.8% of the women had never been married. The majority of the participants (83%) reported having "La Reforma" (government based insurance plan) as their health insurance coverage (See table 4).

Table 4: Description of Socio-Demographic and Lifestyle Characteristics of the Study Population Overall and by HPV status

Study Variables	Overall N=130 (%/SD)	HPV Negative N=55 (%/SD)	HPV Positive N=74 (%/SD)	X^2 p-value
SOCIO-DEMOGRAPHIC VARIABLES				
Mean Age (years)	39 (10.8)	40 (11.2)	39 (10.6)	0.704†
Educational Attainment				
Did Not Graduated High School	46 (36.8)	20 (37)	26 (36.6)	
High School Graduates	45 (36)	18 (33.3)	27 (38)	0.820
College Graduates	34 (27.2)	16 (29.6)	18 (25.4)	
Civil Status				
Married or Consensual Union	56 (43.1)	24 (43.6)	32 (42.7)	
Separated, Divorced, or Widowed	43 (33.1)	15 (27.3)	28 (37.3)	0.354
Never Married	31 (23.8)	16 (29.1)	15 (20)	
Insurance				
Medicare	4 (3.1)	2 (3.6)	2 (2.7)	
"La Reforma"	108 (83.1)	45 (81.8)	63 (84)	0.549
Private Insurance	14 (10.8)	5 (9.1)	9 (12)	
Uninsured	4 (3.1)	3 (5.5)	1 (1.3)	
Monthly Income				
<$300	50 (38.5)	22 (40)	28 (37.3)	
$300-$600	38 (29.2)	17 (30.9)	21 (28)	0.796
>$600	42 (32.3)	16 (29.1)	26 (34.7)	
LIFESTYLE VARIABLES				
Tobacco Use				
Non-Smoker	51 (39.2)	21 (38.2)	30 (40)	
Former Smoker	43 (33.1)	21 (38.2)	22 (29.3)	0.511
Smoker	36 (27.7)	13 (23.6)	23 (30.7)	
Mean Age of Coitarche	16 (4.05)	17 (4.0)	15 (4.05)	0.053† *
Mean Lifetime Sexual Partners	6 (6.5)	5 (3.1)	7 (8.0)	0.057† *
Mean Partners in last 12 Months	1 (.99)	1 (0.96)	1 (0.99)	0.065†
Harm Risk Reduction Methods Used in last 12 Months				
Abstinence	22 (17.2)	15 (27.3)	7 (9.5)	
Always Uses Condoms	36 (28.1)	12 (21.8)	24 (32.4)	0.048*
Inconsistently Uses Condoms	44 (34.4)	18 (32.7)	26 (35.1)	
Never Uses Condoms	26 (20.3)	9 (16.4)	17 (23)	

† Independent t-test to compare means between groups

Lifestyles. Close to one third (27.7 %) of the women identified themselves as smokers, while 39.2% reported never having smoked. The mean age of coitarche, or sexual debut, for the study was age 16 (SD $\pm$ 4.1 years). The mean number of lifetime sexual partners for the study population was 6 partners (SD $\pm$ 6.5), and on average the women had only one partner in the last 12 months. Close to half of the study population (45%) reported using safer sex by either way of abstinence or by always using condoms, and it was only 20% of the women in the study who reported never using condoms (See table 4).

Clinical characteristics. In terms of the gynecological characteristics, the average age of menarche (first menses) of the study was 12 years of age (SD +2.1). On average, women in the study had, had 3 pregnancies (SD $\pm$ 1.8) and 2 live births (SD $\pm$ 1.5) at the time of their participation in the study. Also the mean age of the women when their first child was born was 20 years (SD $\pm$ 5.0) of age. Only 35% of the women reported ever having used oral contraception, and for on average 1 year (see table 5).

The majority of the women (60%) had a normal Pap smear result at the time of the clinical visit. Women found to have an abnormal cervical cytology, that would require medical follow-up, accounted for 40% of the study population. Women with atypical squamous cells, unspecified (ASC-US) accounted for 22.3% of the study population; low-grade squamous intraepithelial lesion (LSIL) was found in 11.5% of the women; and high-grade squamous intraepithelial lesions (HSIL) accounted for 6.2% of the women. The majority of the women in this study (56.2%) reported having a history of abnormal pap smears (see table 5).

Table 5: Description of Clinical Characteristic and HIV Co-Factors of Study Population Overall and by HPV status

Study Variables	Overall N=130 (%/SD)	HPV Negative N=55 (%/SD)	HPV Positive N=74 (%/SD)	X^2 p-value
CLINICAL CHARACTERISTIC VARIABLES				
Mean Age of Menarche	12 (2.1)	12 (2.1)	12 (2.04)	0.387†
Mean Gravity	3 (1.8)	3 (1.8)	3 (1.8)	0.703†
Mean Parity	2 (1.5)	2 (1.5)	2 (1.5)	0.610†
Mean Age at 1st Birth	20 (5.0)	20 (4.7)	20 (5.2)	0.861†
Used/Uses Oral Contraception	46 (35.4)	16 (29.1)	30 (40)	0.199
Mean years of Oral Contraception Use	1 (2.5)	1 (2.0)	1 (2.8)	0.419
History of Abnormal Cervical Cytology	73 (56.2)	25 (45.5)	48 (64)	0.035*
Cervical Cytology at time of Study				
Normal	78 (60)	40 (72.7)	38 (50.7)	0.011*
Abnormal	52 (40)	15 (27.3)	37 (49.3)	
HIV CO-FACTOR VARIABLES				
CD4 Counts				
Mean CD4 Count	570 (535.1)	597 (233.4)	550 (674.1)	0.630†
CD4 ≥ 350	92 (70.8)	48 (90.6)	44 (59.5)	<0.0001*
CD4 < 350	35 (29.2)	5 (9.4)	30 (40.5)	
HIV Viral Load				
Mean HIV Viral Load (VL)	13,606 (46,759)	2,434 (8,748)	21,830 (60,006)	0.008† *
Undetectable HIV VL	67 (51.5)	34 (64.2)	33 (44)	0.045*
Detectable HIV VL	63 (48.5)	19 (35.8)	42 (56)	
Use of Antiretroviral Therapies				
Currently Using HAART	98 (75.4)	40 (72.7)	58 (77.3)	0.712
Adherence to HAART				
Adherent	68 (52.3)	30 (54.5)	38 (49.3)	0.270
Non-Adherent	30 (23.1)	10 (18.2)	20 (26.7)	
Not on HAART	32 (24.6)	15 (27.3)	17 (24)	

† Independent t-test to compare means between groups

* p-value ≤ 0.05, showing a statistical significance

HIV co-factors. The following HIV co-factors were also evaluated: mean CD4 count, CD4 counts (<350/≥350), HIV viral load, HIV viral load detectability, actual use of highly active antiretroviral therapies (HAART), and adherence to HAART (See table 6). Three study participants did not have their either their

CD4 or their HIV viral load test results returned from the laboratory at the time of final data collection, so they were eliminated from the following analysis (N=127).

The estimated average CD4 count for the entire study population was 570 (SD=535.1). The majority of the study participants (51%) were in CDC HIV Stage 1; an estimated 36% of the women were in Stage 2; and 16% were in Stage 3. The study population had a mean CD4 count of 570, according to the CDC classification system, based on CD4 counts taken at the time of HPV testing. Meanwhile, the estimated mean viral load of the study population was 13,606 copies (SD= 14,759). Nevertheless, the majority of the women (51%) in the study were found to have undetectable HIV viral loads; which is not surprising given that over 75% of the women in the study were on HAART, and 52% of these women reported to be adherent to their therapies (See table 6).

Bivariate Analysis

Demographics. The bivariate analyses comparing the sociodemographic characteristics of HPV positive and HPV negative women revealed that there were no statistical difference between groups based on these variables (See tables 4).

Lifestyles. The body of HPV investigational literature suggests that the most common lifestyle factors statistically associated with HPV positivity are: smoking, age of menarche, gravity (number of pregnancies), parity (number of live births), use of oral contraception, history of abnormal Pap Smears, age at coitarche,

number of lifetime partners, partners within the last year, and harm risk reduction methods. This analysis examined such factors, and their association to HPV; the results are displayed in tables 4 and 6.

Comparison of means by way of independent t-test analysis for continuous variables, revealed a slight difference between the HPV groups for the following variables: age of coitarche and total number of lifetime partners. The HPV positive group began sexual activity nearly a year and half earlier than the HPV negative group (p-value = 0.05), and had more lifetime partners than their HPV negative counterparts (p-value =0.05) (See Table 4). Bivariate analysis of categorical and ordinal variables was completed to determine the strength of the relationship between the previously mentioned lifestyle factors and HPV by way of Chi-square tests (See Tables 6). Of the lifestyle variables, harm risk reduction practice was the only risk factor significantly associated with HPV positivity (p-value = 0.048). Women who reported always using condoms had over 4 times the odds of having HPV than women who reported being abstinent (OR=4.3; 95% CI= 1.2, 15.8); similarly women who inconsistently used condoms had also over 4 times the odds of having HPV when compared to the women who reported being abstinent (OR=4.6; 95% CI: 1.3, 17.0); nonetheless there wasn't a statistically significant finding when comparing women who never use condoms and women who were abstinent (OR=4.1; 95% CI= 1.0, 16.4) (see table 6).

Clinical characteristics. Bivariate analysis of those clinical characteristics of interest in this present study was also conducted (See table 5). We are able to conclude with a degree of 95% confidence, that HIV positive women reporting a

history of abnormal Pap smears were more than two times more likely to have HPV than HIV positive women without a prior history of abnormal cervical cytology (OR= 2.1, 95% CI: 1.1, 4.3) (See Table 6).

Of the 78 women with normal Pap smear results, 48.7% were HPV positive; 35% with multiple HPV genotype infections. The majority (44%) of the women with normal cervical cytology were found to have HPV infections from low-risk genotypes. Among the women with normal cytology, an astonishing 23% had cervical HPV infections with high-risk genotypes, with 8% being multiple genotype high-risk HPV infections (data not shown).

HIV co-factors. Bivariate analysis of HIV co-factors revealed differences between HPV groups for the following variables: CD4 count with 350 as a cut off, actual HIV viral load count, and detection of HIV viral load. There was no statistical association of HPV positivity to HAART use or to adherence to antiretroviral therapies. See Table 6 for complete details. Meanwhile, there was a strong relationship between WHO clinical stages and HIV positivity (p-value <0.001). Using WHO stage 1 as the reference group, women with CD4 counts less than 350 were 5.49 times more likely to have HPV infections than women with CD4 counts greater than or equal to 500 (95% CI : 1.78, 20.18). There was no association of HPV infection between WHO stages 1 and 2. See Table 6 for detailed bivariate analysis. The women with CD4 counts less than 350 were then compared to those with CD4 counts greater or equal to 350, and found to have a likelihood of HPV infection six times greater than those with CD4 > 350 (OR=6.55, 95% CI : 2.21,23.21).

Table 6: Unadjusted Bivariate Analysis of Statistically Significant Factors Associated with HPV Positivity

Characteristic	HPV Prevalence			
	Yes, n=74	No, n=53	OR_c	95% CI
Coitarche				
Coitarche ≥ 16 years of age	39	32	---	---
Coitarche < 16 years of age	36	23	1.28	0.64, 2.59
Lifetime Sexual Partners				
Lifetime partners < 7	52	42	---	---
Lifetime partners ≥ 7	16	9	1.44	0.58, 3.58
CD4 count of 350 as cut off point*				
CD4 > 350	44	48	---	---
CD4 ≤ 350	30	5	6.55	2.21 23.21
HIV Viral Load*				
Undetectable	33	34	---	---
Detectable	41	19	2.22	1.02, 4.91
WHO Clinical Stage of HIV-associated Immunodeficiency				
Not Significant	35	33	---	---
Mild	9	17	0.48	0.17, 1.35
Advanced or Severe	30	5	5.49	1.78, 20.18
Reported History of Abnormal Pap				
No	27	29	---	---
Yes	47	24	2.10	0.96, 4.59
Cervical Cytology Results*				
Normal	38	38	---	---
Abnormal	36	15	2.22	1.07, 5.50
Condom Usage*				
Abstinence	7	15	---	---
Always uses Condoms	24	12	4.29	1.21, 15.75
Inconsistent Condom Usage	26	18	4.64	1.32, 16.95
Never Uses Condoms	17	9	4.05	1.04, 16.44

* p-value ≤ 0.05, showing a statistical significance

The independent *t*-test for equality of means, showed differences in number of HPV genotypes based upon whether a woman had CD4 counts above/below the 350 marker. Women with CD4 below 350 on average had nearly two more HPV genotypes than women with CD4 counts greater than or equal to 350 (p-value <0.001) (data not shown).

There was also an association between HPV positivity and the detection of HIV viral load (Pearson's Chi-square = 4.03, p-value = 0.045). Women with detectable HIV viral load were two times more likely to have HPV than women with undetectable HIV viral load (OR= 2.22; CI95%: 1.02, 4.91) (See table 6). There was no statistical association between HIV viral load detection and risk specific HPV genotypes, i.e. high-risk HPV and low-risk HPV (respective p-values= 0.321; 0.380). Nor was there any relationship with HIV viral load detection and number of HPV genotypes found.

Evaluation of Confounding

The following variables were statistically associated to HPV positivity in bivariate analyses: abnormal cervical cytology at time of HPV testing; harm risk reduction methods, CD4 counts; and HIV viral load detection. Given the lack of association between HAART use and HPV infection in bivariate analysis, we focused further analysis on the variables CD4 counts and HIV viral load. The strongest statistical relationship established through the bivariate analysis was between HPV (dependent variable) and CD4 counts < 350 (independent variable), thus, we focused evaluation of confounding considering CD4 counts as our main independent variable (See Table 7).

Thus, to further explore the potential confounding effects of these variables, we proceeded to evaluate their relationship to CD4 counts <350 for both statistically and clinically significance. Analysis confirmed that only the variables, lifetime sexual partners, HIV viral load detection and actual abnormal cervical cytology were associated to both HPV and CD4 counts, and could be influencing their relationship by acting as confounders. These crude OR associations were then verified by using Mantel Haenszel method and found these confounders as all having relative difference between their crude OR and adjusted OR greater than 10%. The variable age was also considered to be included in the list of covariates because of its strength in HPV positivity literature.

Table 7: Evaluation of Statistical Association of HPV Risk Factors and CD4 <350

Characteristics	CD4 counts		X^2 p-value
	< 350, n=35	≥ 350, n=93	
HIV Viral Load			
Undetectable	10	57	0.001*
Detectable	25	35	
Cervical Cytology Results			
Normal	14	62	0.005*
Abnormal (ASC-US, LSIL, HSIL)	21	30	
Life Style Variable			
Mean age at time of HPV testing	39.49	39.74	0.907
Mean age of Coitarche	16.23	16.36	0.871
Mean Number of Lifetime Partners	8.24	4.40	< 0.001*
Harm Risk Reduction Practices			
Abstinence	4	17	0.552
Always uses Condoms	12	23	
Inconsistent Condom Usage	10	33	
Never Uses Condoms	8	18	

Multivariate Analysis

As previously mentioned, given the lack of association between HAART use and HPV infection in bivariate analysis, we focused our multivariate analysis on the variables CD4 counts and HIV viral load. The covariates lifetime sexual partners and cervical cytology were selected due to their strong relationship to CD4 count and HPV positivity in this present study; the covariate age was selected due to its effect found in the literature.

Using multivariate analysis, we developed two different models to explore the relationship between CD4 counts and HPV positivity. The first model was a logistic regression model between CD4 counts (<350 vs. ≥ 350) and HPV infection, adjusted for HIV viral load, cervical cytology, lifetime partners, and age. The first model proved to be inadequate due to issues of collinearity. This model was not able to evaluate the interaction between CD4 counts and HIV viral load; therefore we were unable to estimate the parameters with precision. In order to observe the effect of HIV co-factors we used stratified analysis instead. Simple stratified analysis revealed that HIV viral load acted as an effect modifier in the CD4 count-HPV infection relationship under study. Therefore, further multivariable analyses were conducted stratified by HIV viral load.

The second analytical approach consisted of a stratified analysis, were two logistic regression models were constructed, based on the participant's HIV viral load detectability. These final models were developed to estimate the association between HPV positivity and CD4 count, stratified by HIV viral load detection

levels and adjusted for age, lifetime number of sexual partners, and study cervical cytology. These variables were selected, due to their strength of association found in the bivariate analysis (lifetime number of sexual partners and study cervical cytology); and their relevance to the literature (age). Finally, appropriate adjusted and stratified ORs with 95% confidence intervals were then estimated.

With marginal 95% confidence, we were able to conclude that HIV positive women, who have detectable HIV viral loads and CD4 counts below 350, had almost 4 times the odds of having cervical HPV infection than women with detectable HIV viral loads with CD4 counts above or equal to 350 (OR=3.90, 95% CI=0.91, 16.78, p-value=0.068). This result was marginally significant ($p<0.10$). Nonetheless, among women with undetectable viral load, there was no association between CD4 counts and HPV infection (OR=1.81; 95% CI=0.24, 13.49; p-value 0.563) (See table 8).

Table 8: Final Model: Stratified Logistical Regression of CD4 Counts' Relationship to HPV positivity, by HIV Viral Load

Characteristic		HPV Positivity +	HPV Positivity -	OR	95% CI	p-value
HIV Viral Load	CD4 cells per uL					
Undetectable	CD4 ≤ 350	8	2	OR_a = 1.81	(0.24, 13.49)	0.563
	CD4 > 350	25	32			
Detectable	CD4 ≤ 350	22	3	OR_a = 3.90	(0.91, 16.78)	0.068
	CD4 > 350	19	16			
Global	CD4 ≤ 350	30	5	OR_c = 6.55	(2.21, 23.25)	< 0.0001
	CD4 > 350	44	48			

OR_a = Odds Ratio adjusted for age, cervical cytology, and lifetime number of sexual partners

CHAPTER 5:

DISCUSSION AND CONCLUSIONS

In this final chapter we will discuss and summarize the key findings of this investigation in the context of their epidemiological and clinical importance. We will demonstrate how the results of our study support general concepts and theories related to HPV and HIV co-infection. We will also address the limitations of this current study and highlight its strengths. Finally, we will outline how some of the key findings of this research that warrant further investigation into HPV infections in HIV positive women.

This was an important study in HPV epidemiology among HIV positive women living in Puerto Rico because for the first time it identified the HPV genotypes present within a sub-group of this population, those that attend a clinic at the University of Puerto Rico Medical Sciences Campus; and quantified the odds of HPV infection based upon immunological markers like CD4 counts and HIV viral load.

HPV Prevalence and Type Distribution

The prevalence of HPV in our study population was 58%, which is higher than the prevalence of HPV documented in the US general population (28.6 %, Dunne, et al., 2007; 43%, Hariri et al., 2011), although slightly lower than other studies examining the prevalence of HPV in HIV positive women. Denny et al (2008) in Cape Town, South Africa documented a prevalence of HPV of 68% among a sample of adult HIV positive; while Goncalves (2008) et al documented

a prevalence of 62% among HIV positive women who attended Center of Reference in AIDS of Sao Paulo, Brazil. Nevertheless, our findings showed higher prevalence than a similar study conducted in Italy; where they found HPV prevalence of 44.1% among HIV positive Italian women attending the gynecological service at "L. Spallanzani" Hospital (Garbuglia, 2012).

In terms of HPV genotype distribution, an interesting finding was related to the high frequency of HPV genotypes present in this population not covered by any of the vaccinations currently available to the general public. This was unexpected given our hypothesis that those genotypes covered by current vaccines would have the highest frequency. In fact some 36% of the HPV infections were among HPV genotypes -16, -18, -6, and -11. This was surprisingly some four times higher than the prevalence of these genotypes found in the US general population. Between the years, 2003-2006 the National Health and Nutrition Examination Survey (NHANES) found the prevalence for those HPV genotypes covered by the quadrivalent HPV vaccine to be 8.8 % (95% CI 7.8, 10.0%) (Dunne et al., 2011). While studies of HPV genotype prevalence among HIV positive women fall short, the comparison of data does shed light on the possible benefit this quadrivalent vaccine will have in Puerto Rico.

The most predominant HPV genotype found in our study was HPV 61 and 66, both low-risk genotypes. Of the high-risk genotypes, HPV 16, 18, 52, were also very commonly found genotypes. In comparison to Garbuglia's study of adult HIV positive Italian women, our results are somewhat similar; except HPV 53 was more frequent over HPV 18 (Garbuglia, 2012). These variations in HPV genotype distribution may be attributed to geographical differences.

The IARC published in 2011 that the most commonly found HPV type identified in European studies was HPV 16, with a prevalence range of 8-66% in HPV positive women (depending on geographic and age distribution) (IARC, 2011). In our study, HPV 16 was found in 13.3% of HPV infected women. The IARC further states that HPV 6, 11, 59, 68, 73, and 82 are consistently rare in HPV genotypes distribution studies (IARC, 2011). Our study showed similar results, with the exception for HPV 6 (9.2%), and HPV 68 (4.6%); furthermore, HPV 40, 64, 67, and HPV IS39 all failed to have a single positive case in our study.

As described in Chapter 2, there is a relatively new movement of IARC to measure HPV species rather than individual HPV genotyping, and to group HPV types into those that cause squamous cell carcinomas (SCC) and adenocarcinomas, genital warts, and commensal infections. Those HPV species believed to cause SCC and adenocarcinomas belong to HPV alpha -7, -9, -11, -5, and -6 (IARC, 2007; 2012). In this study we found that 45.5% (95% CI : 45.4%, 45.5%) of the women tested positive for having at least one HPV genotype among these HPV species.

The IARC has also identified the eight most important HPV genotypes for their prevalence in cervical cancer tissue as: HPV 16, 18, 31, 33, 35, 45, 52, and HPV 58 (IARC, 2007; 2012). In this study, 41.3% of the HPV positive women in our study had at least one of these HPV genotypes. Forty of the forty-four women with high-risk HPV genotypes had a genotype that belonged to either HPV alpha 9 or HPV alpha 7. While women in Puerto Rico have greater access to gynecological services than any other neighboring Caribbean nation, cervical

cancer remains one of the leading cancers among women living in Puerto Rico. A shift in research that focuses on the oncogenic species of HPV, and their proper treatment, will be crucial in lowering this and other cancer trends that burden this population. Development of public health programs that target HIV positive women and focus on HPV and cervical cancer prevention is sorely needed for this population, as seen in the results of our study's genotype distribution.

HPV infection and HIV Co-Factors

Within the field of HIV, a patient with a CD4 count over 500 cells per uL and with undetectable HIV status, by CDC definition, is asymptomatic and immunocompetent (CDC, 2008). Interestingly, the majority of the women (52.3%) who participated in this study are considered to be healthy as they typically had high CD4 counts (> than 500) and had undetectable HIV viral loads. Perhaps the high prevalence of HPV found in our study, supports the theory that there may be a synergistic relationship between HIV and HPV; suggesting HPV to be more persistent despite healthy immunological function in HIV infected individuals (Palefsky J, 2006; Palefsky J, 2007; Stanley, 2009; WHO, 2011). CD4 counts of 350 proved to be the better suited statistical biomarker, compared to the other HIV co-factors in our study. This may lend support to those clinicians who choose to initiate HAART at this point, rather than when counts drop below 500 cells per uL.

Our study also showed that there was no statistical association between HIV viral load detection and risk specific HPV genotypes, i.e. high-risk HPV and low-risk HPV (respective p-values= 0.321; 0.380). Nor was there any relationship

with HIV viral load detection and number of HPV genotypes found. Interestingly, we observed that HAART, and adherence to HAART, had no statistical relationship with HPV positivity (respective p-vales: 0.712, 0.270). This was unexpected, since most literature suggests it acting as either a risk or protective factor. Perhaps it was the cross-sectional design of the investigation that limited our ability to detect such an association, given that it only captures a moment in time as opposed to prospective studies that can capture causal relationships. Another explanation for this short-coming could be that the large majority of the women were on HAART, and fairly adherent. Similar to the Branca study discussed in Chapter 2, perhaps this finding adds more uproar to the question: why do women, who are successfully taking HAART, still have high rates of HPV?

The actual relationship that HPV and HIV share still remains unclear. In the literature we find the general hypothesis that when HIV in uncontrolled within the body it readily destroys the same immunological cells that regulate HPV infection, the CD4 t-cell; the findings from this study support this theory (Palefsky J, 2006; Palefsky J, 2007; Stanley, 2009; WHO, 2011). The final statistical model of our study lends support to this hypothesis. Although our results were marginally significant (p value= 0.068), we found that women with a detectable HIV viral load, with CD4 counts below 350 were almost four times more likely of having cervical HPV infection than women with CD4 counts above or equal to 350 (OR=3.90, 95% CI 0.91, 16.78). Whereas the odds of HPV infection among women in the study with undetectable HIV viral loads were not statistically different between the two groups of women based on CD4 counts of above or below 350 (OR=1.81, 95% CI=0.24, 13.49). Perhaps if the sample size of

the study had been larger, the power of our study to detect these associations would have been stronger. Nonetheless, these study findings still suggest that clinicians should really educate and advocate for women who are within the former category to regularly access gynecological services given that these women are found to be more likely to have cervical HPV infection.

Gynecological Management of HPV/HIV Co-infected Women:

Our findings do highlight some key concepts as they relate to the gynecological management of HIV positive women. The role of HPV testing in clinical settings is still unclear, and even more illusive in gynecological settings that treat women who are HIV positive. Perhaps this is mostly attributed to insurance and what health management organizations are willing to pay for, given that HPV testing, especially HPV genotyping are expensive. Currently gynecological guidelines recommend that newly diagnosed HIV positive women have a Papanicolaou test performed within the first year of HIV diagnosis, and then every six months if showing any abnormality in the cytology. However, for HIV positive women with a normal pap smear, they recommend that no further follow-up be required for another a year. These guidelines recommend that for HIV positive women, with cervical abnormalities, treatment should be aggressive, regardless of CD4 count or use of HAART, by using invasive procedures such as a colposcopy and direct biopsies to reduce infection persistency (ACOG, 2010).

Our study suggests that the standard protocol for managing HIV positive cervical cytology may not be as effective as HPV testing, in terms of early detection of abnormalities. An alarming finding of this study is that despite the

majority of the women having normal cervical cytology, almost half of these women tested positive for a HPV genotype; and almost a quarter of them with high-risk HPV genotypes. This finding was substantial different to Garbuglia et al., who found that among women with HPV infections and normal cervical cytology, 69% were attributed to high-risk genotypes (Garbuglia, 2012). For most women, the initial activation and then subsequent reactivations, of HPV infections begin to occur at a subclinical level that if checked by conventional measures would be undetected. Scientific evidence now clearly shows that HPV testing has the capability of detecting the viruses that cause cervical abnormalities before they have a chance to progress to precancerous lesions. These findings strongly support the guidelines that women who are HIV infected may need more aggressive gynecological care than healthy HIV negative women

Finally, we were surprised to find that the relationship between HPV positivity and smoking was not found to be statistically significant in bivariate analysis (p-value =0.511). The literature review found that this variable is frequently associated to HPV and cervical cancer; yet the science behind the specific relationship between HPV, cervical cancer, and smoking remains vague. Some suggest that smoking increases the risk of having high-risk HPV; while others believe smoking has little to do with the initial infection but rather increases the risk of developing cervical carcinogenesis; and still some have stated that it is the nicotine in cigarettes that increases risk of high-risk HPV infection, and the likelihood of persistent infections which lead to an increasing risk for cervical cancer (Fonseca-Moutinho, 2011). Perhaps one explanation for no statistical association is related to the study design itself; as a cross-sectional

study there isn't any ability to infer a causal relationship between dependent and independent variables. As such, our study isn't able to see the long term outcomes of smoking in this study sample.

Investigational Limitations and Strengths

Perhaps the biggest limitation of the current investigation was a small sample size, which limited the statistical power of final results. Another limitation of this study was that the study participants offered participation in this study were limited to a single clinic, thus preventing generalizations of study results to the HIV positive female population at large on Puerto Rico. CEMI is a very particular location that offers a level of clinical care and patient follow-up equal to none. The clinical staff at CEMI have an outstanding rapport with their patients, and the clinic sees unusually high levels of treatment adherence. The women of CEMI are unique in that they have very high levels of HIV management and may not be representative of HIV positive women without access to this clinic. Women who participate in this clinic have regular access to HIV antiretroviral therapies regardless of insurance, as this clinic frequently participates in clinical trials that provide free medication to participants. They also have an outstanding social work and follow-up team. Finally, the study design is a limitation, as it only shows HPV as a snap shot among these women, not allowing us to establish any causal relationship between the independent variables and HPV infection.

The strengths of this investigation include that the clinical staff at this clinic are highly trained in clinical research, so the potential for information bias

is expected to be low. Staff at this clinic are routinely trained in good clinical practices, regularly participate in private and public clinical trials, and have excellent regulatory experience that insure study protocols are strictly followed through a series of checks and balances. This is a highly sought out clinic within the pharmacology industry to do clinical trial research because of its long outstanding track record of protocol integrity and compliance.

Among other strengths, the cross-sectional design of the study also lead to high rates of participation among women who were offered enrollment. Furthermore, this study was fully funded through a grant of the PR-CCHD, allowing us to the PCR-linear array HPV genotype analysis. Finally, the collective expertise of PR-CCHD laboratory at Ponce School of Medicine added strength to the laboratory analyses performed.

Potential Sources of Bias and their Minimization

While every epidemiological study suffers from the effects of bias, the potential for bias in this study was considered to be minimal. There is always the potential for recall bias when participants are asked to complete self-administered questionnaires. Nonetheless, with the majority of data collected via self-administered structured questionnaires, we were able to minimize the effect of interviewer bias.

Conclusion

This present study presents the first glimpse into the prevalence of human papillomavirus in HIV positive women living in Puerto Rico. Although our study

results cannot be generalized to the general population; we were able to demonstrate that the prevalence of HPV in this clinic-based sample of HIV positive women was high (58%), despite excellent care and follow up by clinical staff, excellent HIV treatment adherence of the patients, and their relative good health status. The HPV genotype distribution observed in this study clearly shows that HPV vaccines may prove very useful for HIV positive women, as the prevalence of the HPV genotypes currently covered by the quadrivalent FDA approved vaccine was found in 36% of the women who tested HPV positive. Nonetheless, the high observed prevalence of other HPV types not included in current available vaccines highlights the need for the development of vaccines that cover additional HPV types.

Our study showed no association between use of HAART and HPV infection. Nonetheless, our study showed marginally statistically significant evidence that women with CD4 counts below 350 and with detectable HIV viral load are more likely to be HPV infected. This shows the relevance of these two variables as strong biomarkers that should be considered and monitored in terms of the clinical care of people living with HIV.

Clearly, there is a great deal more to be explored in terms of the immunological relationship between HPV and HIV, as our population was very healthy and was found to have a high prevalence of cervical HPV infection. Given the aggressive and persistent nature of HPV in HIV positive women, these results support the debate that gynecological clinicians need to incorporate HPV testing into their standard of care for this population. Public health efforts that target

HIV positive women in Puerto Rico should be developed in order to have an impact on the prevention of HPV infection and related malignancies in this population. These efforts should include patient education, HPV vaccination and continued promotion of cervical cancer screening.

Future Research

One area of particular interest for future research would be to expand our collective knowledge of the HPV genotype distribution not only within HIV positive population, but also the general population living in Puerto Rico. This is extremely important when considering HPV vaccine efficacy and vaccination guidelines for adolescents living in Puerto Rico. The high prevalence of HPV infection with HPV types 6, 11, 16 and 18 supports the importance of HPV vaccination in this population. Nonetheless, further research is needed into the cross-immunization of the vaccines currently available that prevent other HPV. This is of particular relevance given that a large proportion (64%) of HPV infected women were infected with HPV types not included in current vaccines. Our study clearly demonstrates high frequencies of HPV genotypes of other high-risk and low-risk HPV genotypes that the IARC has clearly stated as being highly carcinogenic. These comments are not meant to overshadow the public health measure of HPV prevention currently offered through these two vaccines, however new vaccine research is needed in order to develop preventive and/or therapeutic treatments for those genotypes that are prevalent in high risk populations like HIV positive women.

Epidemiologist and other researchers also need to develop more prospective studies of HPV infection among HIV positive women. The literature available for review was largely focused on men who have sex with men, in terms of prospective design. But clearly, HPV research in HIV positive women is a must, given its high prevalence, persistency and relationship to cervical cancer and other malignancies.

Another area within the field of HIV that requires further exploration is the role of HAART on HPV. The literature is very mixed and our study offers no new information, except that HAART is not related to HPV. Theoretically this does not settle well, given that we know that HAART aims to restore immune function; so perhaps prospective studies, relating HIV treatment adherence to HPV infection and persistence would yield more interesting information.

Finally, research is sorely needed in the field of HPV treatment. No antiretroviral medications exist for HPV to our knowledge and STI treatment guidelines still only focus on treating wart manifestations and do not adequately address squamous cell intraepithelial lesions caused by HPV. Furthermore, recent changes in the guidelines even suggest that Pap smears be forgone in women less than 21 years of age. Yet, this is the very same group of women who are experiencing higher rates of HPV infection.

Current treatment of HPV related infections and disease resorts to invasive procedures that aim to cut out infected tissue, and is based on cervical cytology rather than HPV testing. HPV testing is still not regularly accessed, despite being included in treatment guidelines; most gynecologists still take a reactive approach in treating resulting lesions and disease, rather than a

proactive approach that could eradicate HPV infections in the earliest of stages of disease progression.

HIV positive women deserve care and treatment specific to their needs, and not those based on women who don't have HIV. Women who are HIV positive, are at a higher risk for HPV related infections and diseases; and as such, warrant being incorporated into more gynecological research. By readily incorporating HPV testing and genotyping into clinical guidelines and standards of care, we would not only reduce the rates in which these women suffer from advanced squamous intraepithelial lesions; but may also reduce the related costs to treating HPV disease progression. HPV testing and screening makes good public health sense; financially, clinically, and in terms of disease prevention.

Appendix I: IRB Letters of Approval

UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MÉDICAS
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS

OFICINA DEL RECTOR
OFFICE OF THE CHANCELLOR

COMITÉ DE DERECHOS HUMANOS (IRB)
INSTITUTIONAL REVIEW BOARD

Date: June 2, 2009

Protocol Number: 1350509

Principal Investigator: Carmen Zorrilla

Department / Division: CEMI

Sponsor: NIH

Title: ***Cross-sectional Study of HPV and HIV co-infection among women who attend the Maternal Infant Studies Center - (CEMI)***

Thank you for your response to requests from a prior review of your application. This type of response qualifies for expedite review under FDA and OHRP regulations. This is to confirm that your application is now fully approved.

This action involves:

- ☒ New proposal/project
- ☐ Waiver of Consents
- ☐ Continuing Review of Previously Approved Protocol
- ☐ Protocol Amendment
- ☐ Amendment
- ☐ Revised Informed Consent Form
- ☐ Adverse Events
- ☐ Serious Adverse Events

The following documents were reviewed under this submission:

- ☒ Protocol Version 1.0 8 sept 08
- ☐ Assent Document English and Spanish Version
- ☒ Informed Consent Document English and Spanish Version .0 Main 30 Jan 09, Specimens 09 Dec 08
- ☐ Letter of Amendment
- ☐ Survey Instrument
- ☐ Package Insert
- ☐ Advertisement
- ☒ Human Subject Certified
- ☐ Investigator Brochure
- ☐ Authorization Letter
- ☐ Informative Sheet
- ☒ Curriculum Vitae
- ☒ HIPAA Certified
- ☐ FDA #1572
- ☒ Others: "HIPAA Identifiers"

In compliance with federal regulations the approval for this study is valid through:**April 29, 2010**.

This project includes children or adolescents IRB determined the risk/ benefit category as:

☒ 45 CFR §46.405 Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects

For additional information please contact Human Research Subjects Protection Office at 787-282-0010 or 787-282-0018; e-mail opphi.rcm@upr.edu.

Cordially,

Margarita Irizarry, PhD
Chairperson IRB 2

rnco

1. Research must be conducted according to the proposal that was approved by the IRB.
2. Changes to the protocol or its related consent document must be approved by the IRB prior to implementation.
3. All serious or unexpected adverse events/drug reactions should be reported.
4. Each subject should receive a copy of the consent document, if appropriate.
5. Records must be retained for at least three years.
6. Any future correspondence should include the IRB identification number provided and the study title.

UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MÉDICAS
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS

OFICINA DEL RECTOR
OFFICE THE CHANCELLOR

COMITÉ DE DERECHOS HUMANOS (IRB)
INSTITUTIONAL REVIEW BOARD

Date: April 28, 2010

Protocol Number: 1350509

Principal Investigator: Carmen Zorrilla

Department / Division: School of Medicine

Sponsor: NIH

Title: ***Cross-sectional Study of HPV and HIV co-infection among women who attend the Maternal Infant Studies Center - (CEMI)***

Thank you for your response to requests from a prior review of your application. This type of response qualifies for expedite review under FDA and OHRP regulations. This is to confirm that your application is now fully approved.

This action involves:
- ☐ New proposal/project
- ☐ Waiver of Consents
- ☒ Continuing Review #1 of Previously Approved Protocol

The following documents were reviewed under this submission:
- ☐ Protocol
- ☐ Assent Document English and Spanish Version
- ☒ Informed Consent Document English and Spanish Version .April 2010
- ☐ Letter of Amendment
- ☐ Survey Instrument
- ☐ Package Insert
- ☐ Advertisement
- ☐ Human Subject Certified
- ☐ Investigator Brochure
- ☐ Authorization Letter
- ☐ Informative Sheet
- ☐ Curriculum Vitae
- ☐ HIPAA Certified
- ☐ FDA #1572
- ☐ Others:

In compliance with federal regulations the approval for this study is valid through:**April 21, 2011**.

This project includes children or adolescents IRB determined the risk/ benefit category as:

☒ 45 CFR §46.405 Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects

For additional information please contact Human Research Subjects Protection Office at 787-282-0010 or 787-282-0018; e-mail opphi.rcm@upr.edu.

Cordially,

Margarita Irizarry, PhD
Chairperson IRB 2

rnco

1. Research must be conducted according to the proposal that was approved by the IRB.
2. Changes to the protocol or its related consent document must be approved by the IRB prior to implementation.
3. All serious or unexpected adverse events/drug reactions should be reported.
4. Each subject should receive a copy of the consent document, if appropriate.
5. Records must be retained for at least three years.
6. Any future correspondence should include the IRB identification number provided and the study title.

Appendix II: Informed Consent Form

UNIVERSITY OF PUERTO RICO
MEDICAL SCIENCES CAMPUS
INFORMED CONSENT FOR TO PARTICIPATE
IN RESEARCH STUDY

TITLE: **Cross-Sectional Study of HPV and HIV co-infection among women who attend the Maternal Infant Studies Center**

INVESTIGATORS: **Carmen D. Zorrilla, MD (PI)**
Vivian Tamayo, MD (Co-PI)
Ana P. Ortíz, PhD (Co-PI)
Heidi Venegas, MS, DrPH (Co-PI)
Ann Marie Scorsone, MS(c) (Co-PI)

SITE: **University of Puerto Rico- Medical Sciences Campus**
Obstetrics and Gynecology Department
Maternal Infant Studies Center – (CEMI, Spanish acronym)
Tel. (787) 771-4740 or (787) 753-5913
Fax. (787) 771-4739

This consent form may contain words that you do not understand. Please ask the study investigator or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss it with family or friends before making your decision.

I- INTRODUTION:

You have been invited to participate in an investigational research. Before you decide to participate in this study, please read this informed consent form carefully. Ask all and any questions you may have to assure that you understand the procedures of this study, including the risks and benefits.

The human papillomavirus (HPV) is a virus that is acquired mainly from sexual contact. The infections of this virus can cause different manifestations in women. Some of the types (or

strains) of this virus cause genital warts and other types can cause changes in the cells of the cervix that can result in cancerous growths. It is known that this virus is very aggressive and very persistent in those patients who are immunologically compromised, like for example patients who are living with HIV. Despite this, the majority of the information that is currently available about manifestations, complications and therapy for this disease is in HIV negative patients. It is necessary to obtain more information about how this virus affects women who live with HIV, to be able, in the future, develop adequate therapies to reduce the associated complications of this disease in this population.

II- PURPOSE OF STUDY:

The purpose of this study is to evaluate how common human papillomavirus (HPV) infections occur in the mouth and anal-genital areas of women who are living with HIV, that attend the clinic at CEMI. We will also analyze the factors associated with HPV infections; and identify the types or strain of HPV that are present in this population.

III- STUDY PARTICIPANTS:

Taking part in this study is completely voluntary. You do not have to participate if you don't want to. You may also leave the study at any time. If you leave the study before it is finished, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled.

To be able to participate, you must be a woman who attending the clinic at CEMI for a routine visit and require a pap smear, or whose last Pap smear was done more than six month ago. We will be recruiting 320 HIV positive women for this study. In order to participate, you must be able to give consent and be 21 years of age, or older.

IV- PROCEDURES:

If you decide to participate in this study, you will begin by signing this informed consent form. As a part of this study, the following procedures will occur:

1. This study consists of only one visit, and consists of two self-administered questionnaires, gynecological examination, and collection of samples, to detect HPV, in the cervix or vagina, anus, and mouth.

2. If required, as part of your routine evaluation for your visit to CEMI, a blood sample will be collected to detect your levels of CD4s and HIV viral load. This procedure requires two tube of blood to be taken (14mL or almost 2 ½ teaspoons). An evaluation gynecological will also be done, that includes a pelvic exam to take cervical cytology sample otherwise known as the Pap smear. If these laboratories are not required for your routine clinical visit, then this information will be collected from your medical record.

3. We will take a sample to detect HPV in the cervical/vaginal areas. This sample consists of passing a small brush and spatula for the cervical area or vagina, and then place the sample in its container. In addition, we will take a sample from the anal area by introducing a swap or Q-tip that will also be placed in a separate container. An oral sample will also be taken, and you will gargle with mouthwash and then spit it out into a special container.

4. If the cervicovaginal HPV test is positive, we will contact you so that you can return to CEMI for follow-up care as part of your medical care. If the results of this test are negative we will not contact you. We will not notify you of the laboratory results of either the anal or oral HPV tests, as currently there is no existing information available in medical literature that indicates how to interpret these results or of their relevance in your medical care.

5. Your medical record will be reviewed to obtain information regarding you HIV diagnosis, stages of your treatment, clinical laboratories (CD4, HIV viral load, Pap smear) and other pertinent information for this study. By signing this consent form you are authorizing study staff to obtain this information from your medical record.

V- RISKS AND DISCOMFORTS:

It is possible that during your participation in this study that you may feel a little uncomfortable, worried, anxious, or upset by answering some of the questions in the questionnaires. If you feel any of these emotions, please motion to the person administering these questionnaires. They will provide you with emotional support from staff that have been trained to manage these situations. Among those staff are Dr. Vivian Tamayo, Dr. Carmen D. Zorrilla and CEMI's psychologist.

The collection of the cervical (or vaginal), anal or oral sample can cause minimal physical discomfort or embarrassment for the nature of the sample collection. You understand that if any health problems are detected you will be referred to the appropriate doctor or clinic for follow-up.

VI- BENEFITS:

It is probable that you will not receive any personal benefit from participating in this study. The information from this investigational research may improve the future treatment of women who are living with HIV.

VII- COSTS

There is no cost for the visit of this study.

VIII- COMPENSATION FOR PARTICIPATION

You will receive $15.00 for completing the study visit. This amount is to cover the costs of meals and transportation.

IX- PRIVACY AND CONFIDENTIALITY

If you choose to be in this study, the investigator will get personal information about you. This may include information that might identify you. The investigator may also get information about your health including:

- Current or previous medical records
- Research records
- Records about phone calls made as part of this research
- Information obtained during this research about:
  - Infectious disease like HIV/AIDS, hepatitis or other sexually transmitted diseases
  - Physical Examine
  - Results from laboratories or physical exams
  - questionnaires

The investigators of this study may have to give information about you or your health that may identify you to:

- The UPR Medical Sciences Campus Institutional Review Board (UPR MSC IRB)
- The Health Department of the Commonwealth of Puerto Rico

Information about you and your health that might identify you may be given to others to carry out the research study. The investigators will analyze and evaluate the results of the study. The records will be safeguarded as HIPAA regulations.
Your personal health information will be kept as confidential as possible under the law. However, your personal health information may no longer be protected by the privacy rule once it is disclosed to our associates, and may be shared with others. The information may be reviewed

by The UPR Medical Sciences Campus Institutional Review Board (UPR MSC IRB). UPR MSC IRB is a group of people who perform independent review of research as required by regulations.

The results of this research may be published in scientific journals or presented at medical meetings, but your identity will not be disclosed.

Your permission expires at the end of the study, unless you cancel it sooner. You may cancel this authorization at any time by sending a written notice to the principal investigator at the following address:

Carmen D. Zorrilla, MD
Maternal Infant Studies Center (CEMI)
UPR-RCM-School of Medicine OB-GYN
PO Box 365067
San Juan, PR 00936-5067
Tel. (787) 771-4740 or (787) 753-5913 Fax. (787) 771-4739

If you cancel this authorization, the principal investigator will no longer use or disclose your personal health information under the authorization for this study, unless it is needed to use or disclose some of your personal health information to preserve the scientific integrity of the study. Information submitted before you cancel this authorization can still be used by the associates.

The Authorization for Use and Disclosure of Protected Health Information for research purposes is completely voluntary. However, if you do not sign this document you will not be able to participate in this study. If in the future you cancel this authorization, you will not be able to continue participating in this study.

X- COMPENSATION FOR INJURY

In the event of any physical and/or mental injury resulting from this research study, you will receive medical treatment free of charge at the University Hospital or any other hospital designated by the Chancellor or the Medical Sciences Campus of the University of Puerto Rico. The University of Puerto Rico has no plans to provide any form of compensation directly to you. However, by signing this consent form, you do not give up any legal rights.

XI- VOLUNTARY PARTICIPATION AND WITHDRAWL

This consent form gives you information about the study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign this consent

form. You will be given a copy to keep.

Before you learn about the study, it is important that you know the following:

- Your participation in the research is entirely voluntary.
- You may decide not to take part or to withdraw from the study at any time without losing any benefits that you might otherwise be entitled to have. Your decision will not result in any penalty or loss of benefits to which you are entitled.

If it is necessary, your participation in this study may be ended in any moment by the investigator, without your consent.

XII- Questions

If you have any questions about this study or your participation in this study or if at any time you feel you have experienced a research related injury contact Dr. Carmen D. Zorrilla, or Ann Marie Scorsone, at (787)753-5913/ (787) 771-4740.

If you have questions about your rights as a participant in the study, you should contact:

Human Research Subjects Protection Office
University of Puerto Rico
Medical Sciences Campus
PO Box 365067
San Juan, PR 00936-5067
Phone: (787) 282-0018 or (787)-282-0010
E-mail: opphi.rcm@upr.edu

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

If you agree to be in this study, you will receive a signed and dated copy of this consent form with the stamp of IRB approval for your records.

XIII-CONSENT

I have read the information in this consent form (or it has been read to me). All my questions about the study and my participation in it have been answered. I freely consent to be in this research study.

I authorize the use and disclosure of my health information to the parties listed in the authorization section of this consent for purposes described above.

By signing this consent form, I have not given up any of my legal rights.

Participant Name	Participant Signature	Date

Name of the person conducting the informed consent discussion	Signature person conducting the consent discussion	Date

---

IF REQUIRED:
An impartial witness will sign this form if the participant is unable to read the consent.

By signing this form, you confirm that the information in the consent form was accurately explained to, and apparently understood by the subject. The subject freely consents to be in the research study.

Impartial Witness Name (printed)	Impartial Witness Signature	Date

Appendix III: Letter to Participants

Dear Participant,

We would like to thank you for your participation in the study about Human Papillomavirus. We will be asking you to complete two questionnaires that will collect the following information: demographic (ex., where you were born, your civil status, age, income), about your current state of health (ex., dental hygiene, use of medications, and gynecological information) as well as information regarding your sexual behaviors (ex., use of birth control, sexual practices, diagnosis) and finally lifestyle (ex., use of tobacco, alcohol, drugs).

It is really important that you respond to all the questions in the questionnaires even though some of them might make you to feel uncomfortable or anxious. Should this happen, we ask that you please let the person who is administering the questionnaire know. We thank you for your honesty in answering these questions. Remember that there are no right or wrong answers. This information will be used to analyze the impact that HPV has in the study group. Your identity will be protected, and your participation is confidential, or in other words your answers to these questions will be kept separate from your medical chart in another record. If at any moment while you are completing these questionnaires should you have any questions or doubts, please feel free to ask the person administering them for help.

Carmen D. Zorrilla, MD

Ann Marie Scorsone, MS(c)

Appendix IV: PR-CCHD Common Questionnaire

Favor de no marcar nada en esta página es para el uso de la investigación.

1. ID del proyecto ☐☐☐
2. ID de la institución ☐☐
3. ID del participante ☐☐☐☐☐
4. Visita inicial (dejar en blanco si es seguimiento) ☐
5. Fecha de primera visita ___ / ___ / ___ (mes / día / año)
6. Número de seguimiento (dejar en blanco si es inicial) ☐
7. Fecha de seguimiento (dejar en blanco si es inicial) ___ / ___ / ___ (mes / día / año)
8. Tipo de visita
   1. regular 2. no planificada ☐
9. Lugar de la visita
   1. Clínica de Planificación Familiar RCM
   2. RCM CLETS
   3. RCM CEMI
   4. Clínica de Inmunología de Bayamón
   5. Sala de Emergencia Bayamón
   6. Hospital Regional de Bayamón
   7. Playa de Ponce
   8. Escuela de Medicina Ponce
   9. Otro _____ ☐☐
10. Persona que administró el cuestionario ☐☐☐
11. Status de seguimiento (si es inicial no aplica)
    1. vivo
    2. muerto
    3. perdido
    4. no se sabe
    5. confinado ☐

6/20/2007

Favor contestar las preguntas en los cuadros marcando la alternativa que corresponde con una X o llenando las líneas. De tener alguna duda relacionada con el cuestionario, favor consultar con el entravistador/a o Investigador/a del caso.

DATOS SOCIODEMOGRAFICOS

1. Indica tu sexo:
   - ☐ 1. Masculino
   - ☐ 2. Femenino
   - ☐ 3. Otro _____ ____

2. ¿Cuál es tu edad? _____ ____

3. ¿Cuál es tu fecha de nacimiento?
   ___ día ____ mes _____ año

4. ¿Dónde Naciste?
   - ☐ 1. Puerto Rico
   - ☐ 2. Estados Unidos
   - ☐ 3. República Dominicana
   - ☐ 4. Otra isla del Caribe (específica)_____
   - ☐ 5. América Latina
   - ☐ 6. Otro _____ ____

5. ¿Con quién vives en estos momentos?
   - ☐ 1. Solo/a
   - ☐ 2. Con Familiares (mamá, papá, hermano/a, pareja/o)
   - ☐ 3. En una institución
   - ☐ 4. Deambulante
   - ☐ 5. Otro: _____ ____

6. En estos momentos estás:
   - ☐ 1. Empleado/a
   - ☐ 2. Trabajas por cuenta propia (chivos, chiripas)
   - ☐ 3. Incapacitado/a
   - ☐ 4. Desempleado/a
   - ☐ 5. Retirado/a
   - ☐ 6. Otro _____ ____

7. ¿Durante el último año; en qué pueblo(s) has vivido?

1 _____	3 _____
2 _____	4 _____

8. ¿Durante el último; año en qué país(es) has vivido?

1 _____	3 _____
2 _____	4 _____

9. ¿Cuál es tu último año de escuela completado?

_____ ____

10. ¿Cuál de los siguientes describe mejor tu estado civil?

- ☐ 0. Nunca casado/a
- ☐ 1. Casado/a
- ☐ 2. Unión Consensual (Conviviendo)
- ☐ 3 .Divorciado/a
- ☐ 4. Separado/a
- ☐ 5. Viudo/a

____

11. ¿Qué tipo de seguro médico tienes en estos momentos?

(Puedes marcar más de una alternativa)

1. Seguro de Medicare	☐ sí	☐ no	____
2. Seguro Privado	☐ sí	☐ no	____
3. Seguro de Reforma (Plan del Gobierno)	☐ sí	☐ no	____
4. No tengo	☐ sí	☐ no	____

12. ¿Eres de origen hispano o latino? ☐ sí ☐ no ____

13. Te consideras:

- ☐ 1. Blanco
- ☐ 2. Negro
- ☐ 3. Indio americano
- ☐ 4. Asiático
- ☐ 5. Hawaiano Nativo
- ☐ 6. Otro _____
- ☐ 7. No sé.

____

14. En estos momentos tu ingreso mensual es:(Incluyendo sueldo o cualquier tipo de ayuda ecónomica que reciba).

- ☐ 1. menos de $300
- ☐ 2. de $301 a $600
- ☐ 3. de $601 a $900
- ☐ 4. de $901 a $1,200
- ☐ 5. de $1,201 o más

____

ESTILOS DE VIDA

15. Alguna vez, ¿has fumado cigarrillos o tabaco? ☐ sí ☐ no ____

16. En los **últimos 6 meses,** ¿has fumado cigarrillos o tabaco? ☐ sí ☐ no ____

17. Alguna vez, ¿has consumido bebidas alcohólicas como cervezas, vino, cidra, whisky, ron, etc? ☐ sí ☐ no ____

18. En los **últimos 6 meses,** ¿has consumido bebidas alcohólicas como cervezas, vino, cidra, whisky, ron, etc? ☐ sí ☐ no ____

19. ¿Haces ejercicios o practicas alguna actividad física semanalmente?(ej. como: caminar, correr,etc.) ☐ sí ☐ no ____

20. En los **últimos 6 meses,** ¿haces ejercicios o practicas alguna actividad física semanalmente?(ej. como:caminar, correr,etc.) ☐ sí ☐ no ____

21. Alguna vez, ¿has consumido algunos de los siguientes?
- a. suplementos vitamínicos ☐ sí ☐ no ____
- b. productos naturales ☐ sí ☐ no ____
- c. medicamentos que han sido recetados para otras personas ☐ sí ☐ no ____
- d. medicamentos que no necesitan receta (over the counter) ☐ sí ☐ no ____

22. En los **últimos 6 meses,** ¿has consumido algunos de los siguientes?
- a. suplementos vitamínicos ☐ sí ☐ no ____
- b. productos naturales ☐ sí ☐ no ____
- c. medicamentos que han sido recetados para otras personas ☐ sí ☐ no ____
- d. medicamentos que no necesitan receta (over the counter) ☐ sí ☐ no ____

23. ¿Cuándo fue la última vez que te hiciste un examen médico o chequeo de rutina (sin estar enfermo)?

☐ menos de 3 meses ☐ de 4 a 6 meses ☐ 6 a 12 meses ☐ 12 meses o más ____

24. ¿Cómo describes tu estado de salud?

☐ Excelente ☐ Bueno ☐ Regular ☐ Malo ____

USO DE SUBSTANCIAS

25. Alguna vez, ¿has usado alguna de estas substancias o drogas?

a. heroína	☐ sí	☐ no	____
b. cocaína	☐ sí	☐ no	____
c. heroína y cocaína	☐ sí	☐ no	____
d. anfetaminas	☐ sí	☐ no	____
e. crack	☐ sí	☐ no	____
f. marihuana	☐ sí	☐ no	____
g. éxtasis	☐ sí	☐ no	____
h. otras substancias:_____	☐ sí	☐ no	____

26. En los **últimos 6 meses,** ¿has usado alguna de estas substancias o drogas?

a. heroína	☐ sí	☐ no	____
b. cocaína	☐ sí	☐ no	____
c. heroína y cocaína	☐ sí	☐ no	____
d. anfetaminas	☐ sí	☐ no	____
e. crack	☐ sí	☐ no	____
f. marihuana	☐ sí	☐ no	____
g. éxtasis	☐ sí	☐ no	____
h. otras substancias:_____	☐ sí	☐ no	____

27. Alguna vez, ¿has usado substancias o drogas de las siguientes maneras?

a. oralmente (por boca)	☐ sí	☐ no	____
b. fumándola	☐ sí	☐ no	____
c inhalándola	☐ sí	☐ no	____
d. intravenosa (inyectándola por vena)	☐ sí	☐ no	____

28. En los **últimos 6 meses,** ¿has usado substancias o drogas de las siguientes maneras?

a. oralmente (por boca)	☐ sí	☐ no	____
b. fumándola	☐ sí	☐ no	____
c inhalándola	☐ sí	☐ no	____
d. intravenosa (inyectándola por vena)	☐ sí	☐ no	____

29. Alguna vez, ¿has utilizado jeringuillas que otras personas han usado para inyectarte drogas? ☐ sí ☐ no ____

30. En los **últimos 6 meses,** ¿has utilizado jeringuillas que otras personas han usado para inyectarte drogas? ☐ sí ☐ no ____

COMPORTAMIENTO SEXUAL

31. ¿Has tenido relaciones sexuales alguna vez en tu vida? ☐ sí ☐ no ____
(si contesta **No, pase a la pregunta 44.**)

32. ¿A qué edad fue tu primera relación sexual? _____años ____

33. ¿Qué edad aproximadamente tenía la persona con la cual tuviste tu primera relación sexual? _____años ____

34. Actualmente ¿tienes pareja sexual principal? ☐ si ☐ no ____
(si contesta **No, continúe con la pregunta 36**)

35. ¿Qué edad aproximadamente tiene tu pareja sexual actual? _____años ____

36. Alguna vez, ¿has tenido relaciones sexuales,
a. con personas del sexo opuesto?..... ☐ sí ☐ no ____
b. con personas del mismo sexo?..... ☐ sí ☐ no ____

37. En los **últimos 6 meses,** ¿has tenido relaciones sexuales
a. con personas del sexo opuesto?..... ☐ sí ☐ no ____
b. con personas del mismo sexo?..... ☐ sí ☐ no ____

38. Alguna vez, ¿has tenido sexo a cambio de dinero, comida, drogas o alguna otra cosa?..... ☐ sí ☐ no ____

39. En los **últimos 6 meses** ¿Has tenido sexo a cambio de dinero, comida, drogas o alguna otra cosa?..... ☐ sí ☐ no ____

40. Alguna vez, ¿has sido obligado(a) tener relaciones sexuales?..... ☐ sí ☐ no ____

41. En los **últimos 6 meses,** ¿has sido obligado(a) a tener relaciones sexuales?..... ☐ sí ☐ no ____

42. Si te obligaron a tener relaciones sexuales, ¿qué edad tenias en ese momento? _____años ____

43. ¿Cuántas parejas sexuales has tenido aproximadamente?
_____ en total ____
_____ en los últimos **tres** meses ____

VIOLENCIA Y ABUSO

44. Alguna vez, ¿has sido golpeado/a por:

a. tu mamá	☐ sí	☐ no	____
b. tu papá	☐ sí	☐ no	____
c. tu pareja sexual	☐ sí	☐ no	____
d. otro familiar	☐ sí	☐ no	____

45. En los **últimos 6 meses,** ¿has sido golpeado/a por:

a. tu mamá	☐ sí	☐ no	____
b. tu papá	☐ sí	☐ no	____
c. tu pareja sexual	☐ sí	☐ no	____
d. otro familiar	☐ sí	☐ no	____

46. Alguna vez, ¿has sido:

a. castigado emocionalmente	☐ sí	☐ no	____
b. humillado/a	☐ sí	☐ no	____
c. amenazado/a	☐ sí	☐ no	____
d. gritado/a	☐ sí	☐ no	____
e. insultado/a?	☐ sí	☐ no	____

47. En los **últimos 6 meses,** ¿has sido:

a. castigado emocionalmente	☐ sí	☐ no	____
b. humillado/a	☐ sí	☐ no	____
c. amenazado/a	☐ sí	☐ no	____
d. gritado/a	☐ sí	☐ no	____
e. insultado/a?	☐ sí	☐ no	____

Subject ID: _____
Subject Initials: _____
Visit Date: _____

Appendix V: HPV Questionnaire

Section A: Medical History

First we are going to ask you questions about yourself:

A1. How would you describe your current health?

01 Poor
02 okay
03 good
04 very good
05 Excellent

A2. How frequently do you brush your teeth?

00 never
01 less than once a day (some days out of the week)
02 once a day
03 twice a day
04 three times a day
05 four times a day or more

A3. How often do you use dental floss?

00 never
01 less than once a day (some days out of the week)
02 once a day
03 twice a day
04 three times a day
05 four times a day or more

A4. How often do you go to the dentist for a teeth cleaning?

00 I don't remember
01 Every two or three years
02 Once a year
03 Once every 6 months
04 More than every 6 months

A5. When was the last time you went to your dentista?

_____ - _____
month - year

☐ I don't know

A6. Are you currently taking medications for HIV?

00 No (**Please go to question C1)**

01 Yes

A7. How many of your pills for HIV medications did you NOT take in the **last three days**?

_____

A8. What were the reasons (if any) for why **<u>you didn't take</u>** your medications at some moment during **the last 3 days? Number the 5 reasons that apply, where 1= "the most frequent reason" and 5= "the least frequent reason."**

___ a. I forgot
___ b. I was very tired
___ c. They make me feel ill
___ d. I was sick
___ e. I was depressed
___ f. I am afraid of the medication side effects
___ g. I was nauseous and vomiting from my pregnancy ("morning sickness")
___ h. I ran out of meds
___ i. other: _____

A9. Have you followed the indicated dosage timing for taking your pills during the **last 3 days?**

___ a. I always follow take my meds at the time indicated (**Please go to question B1)**
___ b. Most of the time

___ c. sometimes

___ d. never

___ e. I don't know what specific time I'm support to take them

A10. What are the different reasons for not following a scheduled time for taking your medications? *Please mark all that apply:*

___ a. I was sick

___ b. the schedule conflicted with eating my meals

___ c. difficulties coordinating my diet with the medications

___ d. I was very tired

___ e. I overslept

___ f. they make me feel ill

___ g. I forgot

___ h. my baby was born

___ i. I was depressed

___ j. other: _____

Section B: Reproductive Health

Now we would like to ask you a few questions about your reproductive health history (gynecological and obstetrics).

B1. What age were you when you had your first menses? ____ ____ años

B2. When was your last menstruation? ___ ___ -- ___ ___ -- ___ ___
Día Mes Año

B3. Have you ever been pregnant?

00 No (**Please go to question B8**)

01 Yes

B4. How many pregnancies have you had? *(Please include all lost pregnancies and abortions)*

_____pregnancies

B5. How many times have you gone into delivery? *(Include vaginal deliveries and cesareans)*

_____ deliveries **(if you have never delivered, please go to question B8)**

B6. What types of deliveries have you had?

☐ Vaginal 00 ☐ Cesaerians01 ☐ Both 02

B7. How old were you during your first pregnancy?

_____ years old

B8. In the last six (6) months have you had any of the following occur?

	No	**Yes**
a. Warts in the genital area, that may itch or bleed	☐0	☐1
b. Sores in the vagina that don't heal	☐0	☐1
c. Genital discharge that are watery, yellow, or white	☐0	☐1
d. Bleeding after sexual relations	☐0	☐1
e. Rectal bleeding	☐0	☐1
f. Itchiness in the vulva area	☐0	☐1
g. Rectal itching	☐0	☐1

B9. Have you ever had a Pap Smear?

00 No
01 Yes

B10. How frequently do you get a Pap Smear?

00 I have never has a pap smear
01 every 6 months
02 every year (annually)
03 every 2 years
04 every three years
05 every 4 years or more

B11. When was the last time you had a pap smear? ____ ____ -- ____ ____
Month **Year**

B12. Have you ever had an abnormal Pap smear?

00 No **(Please go to question B17**)

01 Yes

B13. How many times in your life have you had an ***abnormal*** pap smear?

_____ times

B14. How long has it been since your last abnormal Pap smear result?

____ _____years _____ _____months

B15. Have you ever received treatment for the abnormal Pap smear results?

00 No (**Please go to question B17)**

01 Yes

B16. What type of treatment(s) have you had? *(Please mark all that apply)*

☐ Vaginal Cream (5-FU o Effudex)

☐ Cryosurgery (freezing)

☐ Cone Biopsy

☐ LEEP

☐ Hysterectomy

☐ Other: _____(specify)

Subject ID: _____
Subject Initials: _____
Visit Date: _____

B 17. Have you ever in your lifetime, used any of the following methods of birth control (to avoid pregnancy)?

00 No

01 Yes

¿Have you used…..?	For how long did you use this method?	What do you currently use? *(marc "X" to all that apply)*
☐ Rhythm Method a	____ years b	☐ No ☐ Yes c
☐ Oral birth control (pills) d	____ years e	☐ No ☐ Yes f
☐ Intrauterine Deposit (IUD) g	____ years h	☐ No ☐ Yes i
☐ Diaphragm j	____ years k	☐ No ☐ Yes L
☐ Condom (Rubber) m	____ years n	☐ No ☐ Yes o
☐ Patch p	__ __ years q	☐ No ☐ Yes r
☐ Vaginal Ring s	____ years t	☐ No ☐ Yes u
☐ Depo Provera (injections) v	____ years w	☐ No ☐ Yes x
☐ Spermicides y	____ years z	☐ No ☐ Yes aa

B18. Have you ever been diagnosed with a sexually transmitted infection/disease (STI or STD)?

00 No (**Please go to question C1**)

01 Yes

If your answer was "Yes" please answer the following section:

Has a doctor ever told you had the following ...	At what age were you diagnosis with the following……	Did you receive medical treatment ...? *(mark "X" to all that apply)*	Did the doctor say that you were cured of …? *(mark "X" to all that apply)*
☐ Hepatitis C? B19	__ __ years B19a	☐ No ☐ Yes B19b	☐ No ☐ Yes B19c
☐ Hepatitis B? B20	__ __ years B20a	☐ No ☐ Yes B20b	☐ No ☐ Yes B20c
☐ Gonorrhea? B21	__ __ years B21a	☐ No ☐ Yes B21b	☐ No ☐ Yes B21c
☐ Syphilis B22	__ __ years B22a	☐ No ☐ Yes B22b	☐ No ☐ Yes B22c
☐ Genital Warts? B23	__ __ years B23a	☐ No ☐ Yes B23b	☐ No ☐ Yes B23c
☐ Genital Herpes? B24	__ __ years B24a	☐ No ☐ Yes B24b	☐ No ☐ Yes B24c
☐ Genital Chlamydia? B25	__ __ years B25a	☐ No ☐ Yes B25b	☐ No ☐ Yes B25c
☐ Trichinosis? B26	__ __ years B26a	☐ No ☐ Yes B26b	☐ No ☐ Yes B26c
☐ Bacterial Vaginosis? B27	__ __ years B27a	☐ No ☐ Yes B27b	☐ No ☐ Yes B27c

Section C: Sexual Practices

Now we will ask you about your sexual relationships. ***When we talk about sexual relationships we are referring to any type of sexual act: oral sex (mouth-penis, mouth vagina, mouth anus), vaginal sex (penis-vagina, vagina-finger) or anal sex) anus-penis, anus-finger).*** *We know this information is very personal, and we appreciate your honesty in answering these questions. Remember that* ***all your answers are completely confidential.***

C1. At what age did you have your first sexual experience?

_____ years

C2. At what age did you have your first vaginal sexual relationship?

_____ years

C3. With how many persons (including both men and women) have you had sexual relations:

_____ persons in **lifetime?** _____ persons **in the last 12 months?**

Subject ID: _____
Subject Initials: _____
Visit Date: _____

Section: **SEX with Men**

C4. With how many **men** have you had sexual relations

_____ During your **lifetime** , and _____ During the **last 12 months**

C5. With the men you have had sexual relationships, **during your lifetime**, which of the following were your sexual practices? *(Please mark all that apply)*

- ☐ vaginal sex (penis-vagina)00
- ☐ vaginal sex (finger-vagina)01
- ☐ anal sex (penis-anus) 02
- ☐ anal sex (finger-anus) 03
- ☐ oral sex (mouth-penis) 04
- ☐ oral sex (mouth-anus) 05
- ☐ oral sex (mouth-vagina) 06

C6. With the men you have had sexual relationships, **during the last 12 months**, which of the following were your sexual practices? *(Please mark all that apply)*

- ☐ vaginal sex (penis-vagina)00
- ☐ vaginal sex (finger-vagina)01
- ☐ anal sex (penis-anus) 02
- ☐ anal sex (finger-anus) 03
- ☐ oral sex (mouth-penis) 04
- ☐ oral sex (mouth-anus) 05
- ☐ oral sex (mouth-vagina) 06

C7. During the last 12 months, how often have you used protection (like dental dams or condoms) during your sexual activities with men? *(please circle the answer that most applies):*

00 Never
01 Sometimes
02 Frequently
03 Almost always
04 Always

Section: **SEX with WOMEN**

C8. Have you ever in your lifetime ever had sexual relations with a woman? By sexual relations we mean **any kind of sexual act including: oral sex, vaginal sex, or anal sex**?

00 No **(Please go to question C15)**

01 Yes

C9. With how many **women** have you had sexual relations?

During **your lifetime**, and During the **last 12 months:**

_____women _____ women

C10. Of these women, how many of them have had sexual relations with men?

00 I don't know

01 a few

02 some

03 the majority them

04 All of them

C11. With the **women** you have had sexual relationships, **during your lifetime**, which of the following were your sexual practices? *(Please mark all that apply)*

☐ sex oral (mouth-vagina) 00 ☐ oral sex (mouth-anus) 01

☐ vaginal sex (finger-vagina)02 ☐ anal sex (finger-anus) 03

C12. With the **women** you have had sexual relationships, **during the last 12 months**, which of the following were your sexual practices? *(Please mark all that apply)*

☐ sex oral (mouth-vagina) 00 ☐ oral sex (mouth-anus) 01

☐ vaginal sex (finger-vagina)02 ☐ anal sex (finger-anus) 03

C13. During the last 12 months, how often have you used protection (like dental dams or condoms) during your sexual activities with these **women**? *(Please circle the answer that most applies):*

00 Never
01 Sometimes
02 Frequently
03 Almost always
04 Always

Section: **Sex Toy Use**

C14. Have you ever in your lifetime used a sex toy (or an object to stimulate your genital zone or anus?

00 No (**Please go to question C19**)
01 Yes

C15. Have you ever used any of the following *(please mark all that apply):*

☐ dildos?
☐ vibrators?
☐ bottles?
☐ other objects, Which?_____

C16. How frequently do you use sex toys?

00 Never
01 Sometimes
02 Frequently
03 Almost always
04 Always

C17. Have you ever shared your sex toys with your sex partners?

00 No

01 Yes (if, Yes with whom: ☐ men 00, ☐ women 01, o ☐ both 02)

C18. How frequently do you share your sex toys?

00 Never

01 Sometimes

02 Frequently

03 Almost always

04 Always

C19. How do you clean your sex toys? (Please explain) _____

_____

_____

_____

C20. Have you ever been forced to have sexual relations or sexual acts in which you didn't want to participate?

0 No **(Please go to question D1)**

1 Yes

C21. How old were you the first time that this happened?

_____ Years old

C22. How old were you the last time that you were forced to have sexual relations?

_____ Years old

C23. What was the nature of your relationships with the person who forced you to have sexual relations? *(mark all those that apply)*

☐$_0$ I prefer not to answer ☐$_1$ a stranger ☐$_2$ someone from my family

☐$_3$ a friend ☐$_4$ a significant other ☐$_5$ a client

☐$_6$ other: _____

Section D. Knowledge about HPV

D1. Have you ever heard of the Human Papillomavirus (HPV)?

0 No (**If you respond No, you have completed your participation. THANKS!)**

1 Yes

D2. Which of the following sources of information did you learn about the Human Papillomavirus (HPV)? **(Mark all those that apply)**

	No	**Yes**
a. Mother	☐$_0$	☐$_1$
b. Female Friend	☐$_0$	☐$_1$
c. Male Friend	☐$_0$	☐$_1$
d. Family members	☐$_0$	☐$_1$
e. Health Professional (such as doctor, nurse, health educator, etc…)	☐$_0$	☐$_1$
f. School or university		
g. Television, movies, or radio	☐$_0$	☐$_1$
h. Magazines, newspapers, pamphlets, flyers, or brochures	☐$_0$	☐$_1$
i. Internet	☐$_0$	☐$_1$
j. Other sources (Please specify) _____	☐$_0$	☐$_1$

D3. Which of the following increases the risk of getting Human Papillomavirus?

Subject ID: _____
Subject Initials: _____
Visit Date: _____

	No	Yes
a. To begin to have sexual relations before the age of 16 years old.	☐0	☐1
b. To have many sexual partners	☐0	☐1
c. Your sexual partners have had many sexual partners	☐0	☐1
d. To take birthcontrol pills	☐0	☐1
e. To smoke	☐0	☐1
f. Excessive stress	☐0	☐1
g. Poor nutrition	☐0	☐1
h. To not use condoms during sexual encounters	☐0	☐1

Subject ID: _____
Subject Initials: _____
Visit Date: _____

If you agree with the statement please respond, TRUE; if you don't agree please mark FALSE.

False	True	
☐$_0$	☐$_1$	**D4.** Human Papillomavirus (HPV) causes herpes.
☐$_0$	☐$_1$	**D5.** Genital warts are caused by HPV.
☐$_0$	☐$_1$	**D6.** HPV is a virus that can cause cervical cancer.
☐$_0$	☐$_1$	**D7.** The best way to prevent HPV complications is too regularly to have Pap Smear.
☐$_0$	☐$_1$	**D8.** A normal Pap Smear result indicates that the woman is not infected with HPV.
☐$_0$	☐$_1$	**D9.** Changes in Pap Smear results can indicate that a woman has HPV.
☐$_0$	☐$_1$	**D10.** Genital warts are caused by Herpes Virus.
☐$_0$	☐$_1$	**D11.** The pap smear almost always can detect HPV.
☐$_0$	☐$_1$	**D12.** Currently, there is no existing treatment for people infected with HPV.
☐$_0$	☐$_1$	**D13.** HPV is sexually transmitted.
☐$_0$	☐$_1$	**D14.** There is a vaccine that exists that can prevent certain types of HPV.

Thank You for your Cooperation!

Appendix VI: Source Document

INFORMED CONSENT:

Subject 4-digit ID: ____ ____ ____ ____ **Subject's**

Initials:_____

Visit Date: _____
(dd-mmm-yy)

Date Informed Consent was signed: _____**Copy provided to subject?** ☐ Yes ☐No
(dd-mmm-yy)

DEMOGRAPHIC DATA:

Date of Birth: _____ **Age:_____**
(dd-mmm-yy)

Race/Ethnicity: ☐ Caucasian/White ☐ Hispanic ☐ Black

☐ Asian ☐ Other: _____

Type of health insurance of participant: ☐ None ☐ Medicare ☐ Reforma

☐ Private:

_____

Is this female of childbearing potential?

☐ Yes **or** ☐ No (specify): ☐Post-Menopausal ☐Bilateral Oophorectomy

☐ Surgical sterilization ☐ Hysterectomy

Type of birth control method(s) used: *(please mark all that apply)*

☐ Condom ☐ Birth control pills ☐ Depo-Provera ☐ Rhythm method

_____

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

☐ IUD ☐ Lesbian Relationship ☐ Abstinence ☐ None

☐ Other: _____

HIV CLINICAL HISTORY:

Date of first confirmed, positive HIV-1 test: _____
(dd-mmm-yy)

Current clinical stage of HIV-infection, at screening, according to the CDC classification system for HIV infection and AIDS:

☐ **Stage 1:** *(HIV infection, and no AIDS-defining condition, and either CD4 ≥ 500 or CD4% ≥ 29)*

☐ **Stage 2:** *(HIV infection, and no AIDS-defining condition, and either 200 ≥ CD4 ≥ 499 or 14 ≥ CD4% ≥ 28)*

☐ **Stage 3:** *(HIV infection, or documented AIDS-defining condition, and either CD4 < 200 or CD4% < 14)*

Mode of HIV-infection (check all that apply):

☐ Heterosexual contact ☐ Blood transfusion ☐ Mother to Child Transmission

☐ Injection Drug Use ☐ Occupational Exposure

Absolute CD4 lab value from baseline or ± 3 months: _____ (____%)

Date of this value (mm/yyyy):_____

Average absolute CD4 value for the last 12 months, including baseline: _____

(_____ %)

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

(see CD4 and Viral Load log to calculate this value)

Lowest CD4 count ever: _____ (_____%)

Date of lowest CD4 count (mm/yyyy):_____

HIV Viral Load value from baseline ± 3 months: _____Date (mm/yyyy):_____

Average HIV Viral Load value for the last 12 months, including baseline: _____

(see CD4 and Viral Load log to calculate this value)

Highest HIV Viral Load ever: _____ Date (mm/yyyy):_____

GENERAL HISTORY:

Has subject ever smoked? ☐ Yes ☐ No

Is the subject a current smoker? ☐ Yes ☐ No

☐ Mild (no more than 10 cigarettes or 2 cigars or 2 pipes per day)

☐ Moderate (no more than 11-25 cigarettes, or 3-5 cigars or 3-5 pipes per day)

☐ Heavy (more than 25 cigarettes or 5 cigars or 5 pipes per day)

Does the subject drink alcohol? ☐ No ☐ Yes *If yes, please specify (check all that applies):*

☐ Beer, ☐ Wine, ☐ Distilled Spirits, and/or ☐ Other: _____

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

Subject ID: _____
Subject Initials: _____
Visit Date: _____

Source Document

How many days a week? _____

Does the subject use recreational drugs? ☐ No ☐ Yes, *(if yes, please check all that apply):*

☐ Amphetamines (e.g. Speed, Meth)
☐ Barbiturates (e.g. Barbs, Sleepers)
☐ Benzodiazepines (e.g. Benzos, Tranx)
☐ Cocaine (e.g. Coke, Crack)
☐ Cannabinoids (e.g. Marijuana, Weed)
☐ Opioids (e.g. Morphine, Oxycodone, Heroin)
☐ Methadone
☐ Other:_____

Medical and/or Surgical History	Condition	Start Date	Stop/Continuing Date

GYNECOLOGICAL HISTORY:

Date of last menses: _____
(dd-mmm-yy)

Is participant pregnant: ☐ Yes ☐ No ☐ N/A

Was Pap smear sample taken today? ☐ Yes ☐ No

Results of <u>baseline</u> Pap smear: ☐ Normal ☐ ASC-US ☐ ASC-H

☐ Atypical Endo-cervical Cells ☐ CIS ☐ Carcinoma ☐ LSIL ☐ HSIL

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

HPV Cross-Sectional Study

Subject ID: _____
Subject Initials: _____
Visit Date: _____

Source Document

Date of last PAP: _____ (mm/yyyy)

Results of last Pap smear: ☐ Normal ☐ ASC-US ☐ ASC-H ☐ Atypical Endo-cervical Cells

☐ CIS ☐ Carcinoma ☐ LSIL ☐ HSIL

Has participant ever been treated for cervical (or vaginal) dysplasia? ☐ Yes ☐ No

If yes, what treatment was used (select all that apply):

☐ Cryosurgery ☐ LEEP ☐ Cone Biopsy

☐ Hysterectomy (rate :_____)

Was HPV Testing done today: ☐ Yes ☐ No, *if no why:* _____

What areas were tested for HPV: ☐ Cervix (or Vagina) ☐ Oral Cavity ☐ Anus

Area Specific HPV Infections

HPV infections in CERVIX (or VAGINA): ☐ Yes ☐ No

_____ # of types ☐ high-risk HPV types ☐ LR-HPV types

Please list all TYPES of HPV found in the CERVIX (or VAGINA): _____

_____

_____

_____

_____

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

HPV Cross-Sectional Study

Subject ID: _____
Subject Initials: _____
Visit Date: _____

Source Document

HPV infections in ANUS: ☐ Yes ☐ No

_____ # of types ☐ high-risk HPV types ☐ LR-HPV types

Please list all TYPES of HPV found in the ANUS:

_____

HPV infections in ORAL CAVITY: ☐ Yes ☐ No

_____ # of types ☐ high-risk HPV types ☐ LR-HPV types

Please list all TYPES of HPV found in the ORAL CAVITY:

_____

Is there HPV-DNA present in participant? ☐ Yes ☐ No

How many types of HPV, *in total*, does participant have? _____

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

Subject ID: _____
Subject Initials: _____
Visit Date: _____

Source Document

Please list all TYPES of HPV found:

_____

_____

_____

_____

USE OF HIV MEDICATIONS

Has participant ever taken antiretroviral? ☐ Yes ☐ No **Date of very first antiretroviral intake:** _____
(mm-yyyy)

Is participant currently on antiretroviral? ☐ Yes ☐ No

Do labs indicate treatment compliance? ☐ Yes ☐ No

Does participant report treatment compliance? ☐ Yes ☐ No

Please mark all applicable antiretrovirals that are *currently being taken* (check all that apply):

Site Staff Signature: _____ **Date** *(dd-mmm-yy)*: _____

☐ Abacavir/Ziagen

☐ Amprenavir/ Agenerase/ APV

☐ Atazanavir/ Reyataz/ ATV

☐ Combivir (AZT + 3TC)

☐ Darunavir/ Prezista/ DRV/ TMC114

☐ Didanosine/ Videx/ Videx EC/ dideoxyinosine/ ddl

☐ Efavirenz/ Sustiva/ Stocrin/ EFV

☐ Emtricitabine/ Emtriva/ FTC

☐ Enfuvirtide/ Fuzeon/ T-20

☐ Epzicom (ABC + 3TC)

☐ Etravirine, Intelence

☐ Fosamprenavir/ Lexiva/ Telzir/ FPV

☐ Indinavir/ Crixivan/ IND

☐ Lamivudine/ Epivir/ 3TC

☐ Lopinavir + Ritonavir/ Kaletra/ LPV/r

☐ Nelfinavir/ Viracept/ NFV

☐ Nevirapine/ Viramune/ NVP

☐ Raltegravir/Isentress

☐ Ritonavir/ Norvir/ RTV (≤800mg/day)

☐ Saquinavir/ Invitase/ Fotovase/ SQV/ INV/ FTV

☐ Selzentry, Maravirok

☐ Stavudine/ Zerit/ Zerit ER/ d4t

☐ Tenofovir DF/ Viread/ TDF

☐ Tipranavir/ TPV/ Aptivus

☐ Trizivir (AZT + 3TC + ABC)

☐ Truvada (FTC + TDF)

☐ Zalcitabine/ Hivid/ dideoxycytidine/ ddC

☐ Zidovudine/ Retrovir/ AZT

☐ Other/ Investigational antiretrovirals (please specify): _____

Length of time on *this* regimen: _____

Site Staff Signature: _____ **Date** *(dd-mmm-yy)***:** _____

COMMENTS:

Site Staff Signature: _____ **Date** *(dd-mmm-yy)***:** _____

Appendix VII: Sample Ponce School of Medicine HPV Laboratory Report

Linear Array HPV Genotyping Test
Patient Record

Patient Information:

Sample ID: 0001
Date: 1/12/2009
Location: Puerto Rico

High Risk:

☐ HPV 16	☐ HPV 39	☐ HPV 58
☑ HPV 18	☐ HPV 45	☐ HPV 59
☐ HPV 31	☑ HPV 51	☐ HPV 68
☑ HPV 33	☑ HPV 52 *Can't be ruled out*	
☐ HPV 35	☐ HPV 56	

Low Risk:

☐ HPV 6	☐ HPV 53	☐ HPV 64	☐ HPV 71	☐ HPV 83
☐ HPV 11	☐ HPV 54	☐ HPV 66	☐ HPV 72	☐ HPV 84
☐ HPV 26	☐ HPV 55	☐ HPV 67	☐ HPV 73	☐ HPV IS39
☐ HPV40	☐ HPV 61	☐ HPV 69	☐ HPV 81	☐ HPV CP6108
☐ HPV 42	☐ HPV 62	☐ HPV 70	☐ HPV 82	☐ B-Globin low
				☐ B-Globing high

Sequencing Results: HPV 33 **Notes This result is obtain in Sequencing process; Its not a Patient Band Result.**

The LINEAR ARRAY HPV Genotyping Test uses biotinylated primers to define a sequence of nucleotides within the polymorphic L1 region of the HPV genome that is approximately 450 base pairs long. A pool of HPV primers present in the Master Mix is Designed to amplify HPV DNA from 37 HPV genotypes including 13 high risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Capture probe sequences are located in polymorphic regions of L1 bound by these primers.

REFERENCES

American College of Obstetricians and Gynecologists (2010). Practice Bulletin: Gynecologic Care for Women with Human Immunodeficiency Virus. *Obstetrics and Gynecology,* 116(6); 1492-1509.

Anderson J, Hoy J, Hillman R, Gittleson C, Hartel G, Medley G, et al. (2008). Abnormal anal cytology in high-risk human papillomavirus infection in HIV-infected Australian. *Sexually Transmitted Infections,* 84(2), 94-6.

Banura C, Franceschi S, Doorn LJ, Arslan A, Wabwire-Mangen F, Mbidde EK, et al. (2008). Infection with human papillomavirus and HIV among young women in Kampala, Uganda. *Journal of Infectious Diseases,* 197(4), 555-62.

Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, and Munoz N (2008). Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine* 26 (S10): K1-16.

Branca, M., Garbuglia, AR., Benedetto,A., Cappiello, T., Leoncini, L., Migliore, G., Agarossi, A., Syrianen, K., and DIANAIDS Collaborative Study group (2003). Factors predicting the persistence of genital human papillomavirus infections and PAP smear abnormality in HIV-positive and HIV-negative women during prospective follow-up. *International Journal of STD and AIDS*, 14(6); 417-425.

Burchell AN, Winer RL, de Sanjosé S, Franco EL (2006). Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. *Vaccine* 24S3, S3/52-S3/61.

Castellsagué X., Díaz M., de Sanjosé S., Muñoz N., Herrero R., Franceschi S., Peeling R.W., Ashley R., Smith J.S., Snijders P.J.F., Meijer C.J.L., and Bosch F.X., (2006). Worldwide Human Papillomavirus Etiology of Cervical Adenocarcinoma and Its Cofactors: Implications for Screening and Prevention. *Journal of the National Cancer Institute* 98(5), 303-315.

Centers for Disease Control and Prevention, (12/2008). Appendix B: Comparison of the Revised World Health Organization and CDC Surveillance Case Definitions and Staging Systems for HIV Infection. *MMWR,* 57 (RR-10). Last accessed on 7/7/11 from http://www.cdc.gov/mmwr/PDF/rr/rr5710.pdf

Centers for Disease Control and Prevention, Division of Sexually Transmitted Disease, Surveillance and Statistics. Tracking the hidden epidemics: Trends in STDs in the United States, 2000.

Centers for Disease Control and Prevention (2008). HIV Prevalence Estimates—United States, 2006. *MMWR*;57(39):1073-76.

Center for Disease Control and Prevention, (8/2006). HPV and HPV Vaccine: Information for Healthcare Providers. Last accessed electronically December 27, 2006 from http://www.cdc.gov/std/hpv/STDFact-HPV-vaccine-hcp.htm

Centers for Disease Control and Prevention, (11/2006). Human Papillomavirus: HPV Information for Clinicians: Transmission, Prevention, Detection, and Clinical Management. Last accessed electronically December 27, 2006 from http://www.cdc.gov/std/hpv/hpv-clinicians-brochure

Centers for Disease Control and Prevention, (2009). Incidence and Diagnoses of HIV Infection --- Puerto Rico, 2006. MMWR, 58(21);589-591. Last accessed on 7/7/11 from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5821a3.htm

Centers for Disease Control and Prevention (2010). Maps Based on Data from 2008 HIV Surveillance Report. Last accessed on 7/7/11 from http://www.cdc.gov/hiv/topics/surveillance/resources/slides/2008report_tables/index.htm

Centers for Disease Control and Prevention, (12/2010). Sexually Transmitted Disease Treatment Guidelines, 2010. *MMWR,* 59 (RR-12). Last accessed on 7/7/11 from http://www.cdc.gov/std/treatment/2010/STD-Treatment-2010-RR5912.pdf

Centers for Disease Control and Prevention. *HIV Surveillance Report, 2009*; Table 23 vol. 21.. Published February 2011. Last accessed on 7/7/11 from http://www.cdc.gov/hiv/topics/surveillance/resources/reports/

Centers for Disease Control and Prevention (2010). FDA licensure of bivalent human papillomavirus vaccine (HPV2, Cervarix®) for use in females and updated HPV vaccination recommendations from the Advisory Committee on Immunization Practices (ACIP). *MMWR* **59** (20): 626–629

Clifford, GM, Goncalves, AG, and Franceschi, S., (2006). Human papillomavirus types among women infected with HIV: a meta-analysis. *AIDS,* 20(18); 2337-2344

Coffin JM, Hughes SH, Varmus HE, editors (1997) *Retroviruses*. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.

Cohn JA, Gagnon S, Spence MR, Harrison DD, Kluzak TR, Langenberg P, Brinson C, Stein A, Hellinger J; Cervical Disease Study Group of the American Foundation for AIDS Research Community Based Clinical Trials Network, 2001. The role of human papillomavirus deoxyribonucleic acid assay and repeated cervical cytological examination in the detection of cervical intraepithelial neoplasia among human immunodeficiency virus-infected women. Cervical Disease Study Group of the American Foundation for AIDS Research Community Based Clinical Trials Network. *American Journal of Obstetrics and Gynecology*;184(3):322-30.

Coissard CJ, Besson G, Polette MC, Monteau M, Birembaut PL, and Clavel CE, (2005). Prevalence of human papillomaviruses in lung carcinomas: a study of 218 cases. *Modern Pathology* 18, 1606-1609.

Colón-López V, Ortiz AP, Palefsky J (2010). Burden of human papillomavirus infection and related comorbidities in men: implications for research, disease prevention and health promotion among Hispanic men. Puerto Rican Health Science Journal. 2010 Sep;29(3):232-40.

Crum C.P., (2000). Contemporary Theories of Cervical Carcinogenesis: The Virus, the host, and the Stem Cell. *Modern Pathology,* 13(3), 243-251.

Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH, Dillner J, Meijer CJ (2008). Overview of human papillomavirus-based and other novel options of cervical cancer screening in developed and developing countries. *Vaccine* 26(S10); K29-41.

Danso D, Lyons F, Bradbeer C, 2006. Cervical screening and management of cervical intraepithelial neoplasia in HIV-positive women. *AIDS*, 17(9); 579-587.

Del Mistro A, Bonaldi L, Bertorelle R, Minucci D, Franzetti M, Cattelan A, et al. (2001). Genital Human Papillomavirus Types in Immunocompetent and Immunodepressed Women in Northeast Italy: Prevalence and Cytomorphological Correlations. *Journal of Lower Genital Tract Infections*; 5(1), 12-20.

Denny, L, Boa, R, Williamson, AL, Allan, B, Hardie, D, Stan, R, and Myer, L, (2008). Human Papillomavirus infection and cervical disease in HIV-1 infected women. *Obstetrics and Gynecology*, 111(6), 1380-1387.

Department of Health and Human Services, Panel on Antiretroviral Guidelines for Adults and Adolescents (1/2011). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Last accessed on 7/711 from http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf

Departamento de Salud de Puerto Rico. (1/10/07). Epidemiologiá de las Enfermendades de Transmisión Sexual (ETS). Oral presentation presented at The University of Puerto Rico, Graduate School of Public Health, Department of Biostatistics and Epidemiology by Rodriguez Bidot M.,. San Juan.

Departamento de Salud de Puerto Rico (August, 2008). Puerto Rico Central Cancer Registry Stat Fact Sheet: Cáncer of the Cerxi Uteri. Last accessed on June 10, 2010 from http://www.salud.gov.pr/RCancer/Reports/Documents/Hojas%20informativas/Cuello%20Uterino.pdf

Departamento de Salud de Puerto Rico, División de Prevención de ETS/VIH/SIDA, Oficina de Vigilancia de ETS (2011). Datos Generales de ETS 2000-2010. Last accessed on June 21, 2011 from: http://www.salud.gov.pr/Programas/DivisiondePrevencionETSVIH/Documents/Datos_Generales_de_las_ETS%202000-2010.pdf

de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, and Bosch FX; (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *The Lancet Infectious Diseases,* 7(7): 453-459.

Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. (2007). Prevalence of HPV infection among females in the United States. *JAMA,* 297 (8); 813-819.

Dunne EF, Sternberg M, Markowitz LE, McQuillan G, Swan D, Patel S, Unger ER (2011). Human papillomavirus (HPV) 6, 11, 16, and 18 prevalence among females in the United State- National Health and Nutrition Examination Survey, 2003-2006: opportunity to measure HPV vaccine impact? *Journal of Infectious Disease*, 204 (4); 562-565.

Fakhry C, D'souza G, Sugar E, Weber K, Goshu E, Minkoff H, et al. (2006). Relationship between prevalent oral and cervical human papillomavirus infections in human immunodeficiency virus-positive and -negative women. *Journal of Clinical Microbiology;* 44 (12), 4479-4485.

Fonseco-Moutinho JA (2011). Smoking and Cervical Cancer: Review Article. *International Scholarly research Network- Obstetrics and Gynecology,* 2011: 847684.

Food and Drug Administration (FDA) (2009). FDA approves new vaccine for the prevention of cervical cancer. Last accessed on 5/1/12 from http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm187048.htm?utm_campaign=Google2&utm_source=fdaSearch&utm_medium=website&utm_term=Cervarix&utm_content=5

Food and Drug Administration (FDA) (2009). FDA approves new indication for Gardasil to prevent genital warts in men and boys. Last accessed on 5/1/12 from http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm187003.htm

Franco E.L., Duarte-Franco E., and Ferenczy A., (2001). Cervical Cancer: epidemiology prevention and the role of human Papillomavirus infection. *CMAJ,* 164 (7), 1017-1025.

Franco EL, Bosch FX, Cuzick J, Schiller JT, Garnett GP, Meheus A, and Wright TC (2006). Chapter 29: Knowledge gaps and priorities for research on prevention of HPV infection and cervical cancer. *Vaccine* 24S3, S3/242-S3/249.

Garbuglia AR, Piselli P, Lapa D, Sias C, Del Nonno F, Baiocchini A, Ciamaglia C, Agresta A, Capobianchi MR (2012). Frequency and multiplicity of human papillomavirus infection in HIV-1 positive women in Italy. *Journal of Clinical Virology,* 54(2); 141-146.

Gingelmaier A, Grubert T, Kaestner R, Mylonas I, Weissenbacher T, Bergauer F, et al. (2007). High recurrence rate of cervical dysplasia and persistence of HPV infection in HIV-1-infected women. *Anticancer Research,* 27 (4A); 1795-1798.

GlaxoSmithKline, 2011. Cervarix® Package Insert. GlaxoSmithKline Biologicals. Research Triangle Park, NC.

Goncalves, MAG, Randi, G., Arslan, A., Villa, LL, Buranttini, MN, Franceschi, S., Donadi EA and Massad, E., (2008). HPV type infection in different anogenital site among HIV-positive Brazilian women. *infectious Agents and Cancer*, 3(5).

Hernandez BY, & Nguyen TV. (2008). Cervical human papillomavirus infection among female sex workers in southern Vietnam. *Infectious Agents and Cancer,* 3 (1); 7.

International Agency For Research On Cancer of The World Health Organization (2005). *IARC Handbook of Cancer Prevention Volume, 10*. Last accessed on 06/11/2012 from http://www.iarc.fr/en/publications/pdfs-online/prev/handbook10/HANDBOOK10.pdf

International Agency For Research On Cancer of The World Health Organization (2007). *IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans* Volume 90: Human Papillomaviruses. Last accessed on 12/15/11 from http://monographs.iarc.fr/ENG/Monographs/vol90/index.php

International Agency For Research On Cancer of The World Health Organization (2012). *IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans* Volume 100b: Human Papillomaviruses. Last accessed on 05/01/12 http://monographs.iarc.fr/ENG/Monographs/vol100B/mono100B-11.pdf

International AIDS Vaccine Initiative & PATH (2007): *HPV Vaccine Adoption in Developing Countries: Cost and Financing Issues*. Last accessed on 6/4/12 from http://www.iavi.org/HPVfinancing or at www.path.org/publications.

Joint United Nations Programme on HIV/AIDS (UNAIDS) (2010). *Global report: UNAIDS report on the global AIDS epidemic 2010.* Last accessed on 7/7/11 from http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf

Kan CY, Iacopetta BJ, Lawson JS, and Whitaker NJ, (July, 2005). Identification of human papillomavirus DNA gene sequences in human breast cancer. British Journal of Cancer, 93, 946-948.

Karagas MR, Nelson HH, Sehr P, Waterboer T, Strukel TA, Andrew A, Green AC, Bouwes Bavinck JN, Perry A, Spencer S, Rees JR, Mott LA, and Pawlita M, (2006). Human Papillomavirus Infection and Incidence of Squamous Cell and Basal Cell Carcinomas of the Skin. Journal of the National Cancer Institute, 98 (6), 389-395.

Kojic EM, and Cu-Uvin S, (2007). Special Care Issues of Women Living with HIV-AIDS. *Infectious Disease Clinics of North America*, 21; 133-148.

Marais DJ, Passmore JA, Denny L, Sampson C, Allan BR, & Williamson AL. (2008). Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. *Journal of Medical Virology*, 80(6); 953-959.

Minkoff H, Ahdieh L, Massad LS, Anastos K, Watts DH, Melnick S, Muderspach L, Burk R, and Palefsky J, (2001) The effect of highly active antiretroviral therapy on cervical cytological changes associated with oncogenic HPV among HIV-infected women. *AIDS*, 15; 2157-2164.

Moscicki A.B., Schiffman M., Kjaer S., and Villa L.L., (2006). Chapter 5: Updating the natural history of HPV and Anogenital cancer. *Vaccine* 24S3, S3/42-S3/51.

Muñoz N., Castellsagué X., Berrington de González A., Gissmann L., (2006). Chapter 1: HPV in the etiology of human cancer. *Vaccine* 24S3, S3/1-S3/10.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, et al., (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England Journal of Medicine, 348 (6), 518-528.

Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, Shah KV, Meijer CJLM, Bosch FX, (2002). Role of parity and human papillomavirus in cervical cancer: the IARC multicentre case-control study. *The Lancet,* 359 (9312); 1093-1101.

National Cancer Institute (2012). SEER Cervical Cancer Statistics. Last accessed on July 7, 2012 from http://seer.cancer.gov/statfacts/html/cervix.html

National Cancer Institute (2008). Human Papillomavirus and Cancer: Questions and Answers. Last accessed on June 11, 2010 from http://www.cancer.gov/cancertopics/factsheet/Risk/HPV

Nelson KE, & Masters CF (2007). *Infectious Diseases Epidemiology: Theory and Practice, (2nd Edition)*. Sudbury, Massachusetts: Jones and Bartlett Publishers.

Ortiz AP, Soto-Salgado M, Calo WA, Tortolero-Luna G, Pérez CM, Romero CJ, Pérez J, Figueroa-Vallés N, Suárez E. (2010). Incidence and mortality rates of selected infection-related cancers in Puerto Rico and in the United States. Infect Agent Cancer. 2010 14(5); 10.

Ortiz AP, Hebl S, Serrano R, Fernandez ME, Suarez E, Tortolero-Luna G (2010). Factors associated with cervical cancer screening in Puerto Rico. *Preventing Chronic Disease,* 7(3): A58.

Ortiz A.P., (12/15/2006). Infectious disease and cancer. Oral presentation presented at The University of Puerto Rico, Graduate School of Public Health, Department of Biostatistics and Epidemiology. San Juan.

Palefsky J. (2006). Biology of HPV in HIV infection. *Advances in Dental Research,* 19(1); 99-105.

Palefsky J. (2007). Human papillomavirus infection in HIV-infected persons. *Topics in HIV Medicine,* 15(4): 130-3.

Palefsky JM, Holly EA (2003). Chapter 6: Immunosuppression and Co-Infection with HIV. *Journal of the National Cancer Institute Monographs,* 31; 41-46.

Pan American Health Organization, (2004). A Situational Analysis of Cervical Cancer in Latin American and the Caribbean. Washington, DC.

Parkin DM and Bray F (2006). Chapter 2: The burden of HPV-related cancers. *Vaccine* 24SW3, S3/11-25.

Ramírez-Marrero FA, Smit E, De La Torre-Feliciano T, Pérez-Irizarry J, Miranda S, Cruz M, Figueroa-Vallés NR, Crespo CJ, Nazario CM (2010). Risk of cancer among Hispanics with AIDS compared with the general population in Puerto Rico: 1987-2003. *Puerto Rico Health Science Journal;* 29(3):256-64.

Registro Central de Cáncer de Puerto Rico, Archivo de Incidencia de Cáncer en Puerto Rico (May, 2007), División de Epidemiología, Departamento de Salud Puerto Rico. Last accessed on June 21, 2011 from http://www.salud.gov.pr/Datos/InfoSalud/Cancer/Incidence/Pages/Age%20Adjusted%20Incidence%20Rates%20For%20Top%20Sites,%20All%20Ages,%20Females.aspx

Richter KL, van Rensburg EJ, van Heerden WF, & Boy SC. (2008). Human papillomavirus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*.

Roche Molecular Systems, Inc. (2003), Linear Array Detection Kit. *Package Insert*. Roche Group: Branchburg, NJ.

Roche Molecular Systems, Inc. (2006), Linear Array HPV Genotyping Test. *Package Insert*. Roche Group: Branchburg, NJ.

Safaeian M, Kiddugavu M, Gravitt PE, Ssekasanvu J, Murokora D, Sklar M, et al. (2007). Comparability of self-collected vaginal swabs and physician-collected cervical swabs for detection of human papillomavirus infections in Rakai, Uganda. *Sexually Transmitted Diseases,* 34(7); 429-436.

Scheurer ME, Tortolero Luna G, Alder-Storthz K, (2005). Human papillomavirus infection: biology, epidemiology, and prevention. *International Journal of Gynological Cancer*, 15: 727-746.

Schiffman M, Clifford G, Buonaguro FM, (2009). Classification of weakly carcinogenic human papillomavirus types addressing the limits of epidemiology at the borderline. *Infectious Agents and Cancer*, 4(8): 1-8. Last access on June 10, 2010 from http://www.infectagentscancer.com/content/4/1/8#refs

Sirera G, Videla S, Lopez-Blazquez R, Llatjos M, Tarrats A, Castella E, Grane N, Rey-Joly C, and Clotet B, (2008). Highly active antiretroviral therapy and incidence of cervical squamous intraepithelial lesions among HIV-infected women with normal cytology and CD4 counts about 350 cells/mm3. *Journal of Antimicrobial Chemotherapy,* 61; 191-194.

Shapiro S., Hoffman M., Constant D., Rosenberg L., Carrara H., Allan BR., Marais DJ., Passmore JAS., and Williamson AL, (2007). Papanicolaou Smears Induce Partial Immunity Against Sexually Transmitted Viral Infections. *Epidemiology,*18(6); 709-715.

Smith JF, Brownlow M, Brown M, Kowalski R, Esser MT, Ruiz W, Barr E, Brown DR, Bryan JT (2007). Antibodies from women immunized with Gardasil cross-neutralize HPV 45 pseudovirions. *Human Vaccine,* 3(4); 109-115.

Stanley MA (2006). Immune responses to human papillomaviruses. *Vaccine*, 24 Suppl 1:S16-22.

Suárez E, Calo WA, Hernández EY, Diaz EC, Figueroa NR, Ortiz AP (2009). Age-standardized incidence and mortality rates of oral and pharyngeal cancer in Puerto Rico and among Non-Hispanics Whites, Non-Hispanic Blacks, and Hispanics in the USA. BMC Cancer. 2009 28(9); 129.

Tornesello ML, Duraturo ML, Buonaguro L, Vallefuoco G, Piccoli R, Palmieri S, et al. (2007). Prevalence of human papillomavirus genotypes and their variants in high risk West Africa women immigrants in South Italy. *Infectious Agents and Cancer*, 2:1-9.

U.S. Department of Health and Human Services (2000). Healthy People 2010: Understanding and Improving Health. 2nd ed. Washington, DC: U.S. Government Printing Office.

U.S. Department of Health and Human Services (2012). Healthy People 2020: Objectives. Washington, DC: U.S. Government Printing Office. Last accessed on 6/6/12 from http://healthypeople.gov/2020/topicsobjectives2020/default.aspx

U.S. Department of Health and Human Services, Health Resources and Services Administration (1/2011). The Guide for HIV/AIDS Clinical Care. Last accessed on 7/7/11 from http://www.aids-ed.org/pdf/p07-cg/CM_Jan2011.pdf

U.S. Food and Drug Administration (5/2011). Antiretroviral drugs used in the treatment of HIV infection. Last accessed on 7/7/11 from http://www.fda.gov/ForConsumers/ByAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm

Wright TC, Bosch FX, Franco EL, Cuzick J, Schiller JT, Garnett GP, Meheus A. Chapter 30: HPV vaccines and screening in the prevention of cervical cancer; conclusions from a 2006 workshop of international experts. Vaccine. 2006 Aug 31;24 Suppl 3:S3/251-61

World Health Organization: The Department of Immunization, Vaccines and Biologicals (2011). Immunological Basis for Immunization Series. Module 19: Human Papillomavirus. WHO Press, Geneva. Last retrieved on 7/7/11 from http://whqlibdoc.who.int/publications/2011/9789241501590_eng.pdf

World Health Organization: The Department of Immunization, Vaccines and Biologicals (2010). Human Papillomavirus Laboratory Manuel, 1st Edition. WHO Press, Geneva. Last retrieved on 7/7/11 from http://whqlibdoc.who.int/hq/2010/WHO_IVB_10.12_eng.pdf

WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre) (11/15/2010). Human Papillomavirus and Related Cancers in World. Summary Report 2010. Last accessed on 6/22/11 from www. who. int/ hpvcentre

World Health Organization, (2006). Preparing for the introduction of HPV vaccines: policy and programme guidance for countries. WHO Press, Geneva.

Wright TC Jr. (2007). Cervical cancer screening in the 21st century: Is it time to retire the Pap smear? *Clinical Obstetrics and Gynecology* 50(2); 313-323.

Yamada R, Sasagawa T, Kirumbi LW, Kingoro A, Karanja DK, Kiptoo M, et al. (2008). Human papillomavirus infection and cervical abnormalities in Nairobi, Kenya, an area with a high prevalence of human immunodeficiency virus infection. *Journal of Medical Virology,* 80(5); 847-855.

INDEX

I

J

L

M

N

O

P

Q

R

S

T

U

V

W