Published in final edited form as:
Sex Transm Infect. 2014 February ; 90(1): 64–69. doi:10.1136/sextrans-2012-050886.

DNA Detection and Seroprevalence of Human Papillomavirus in a Cohort of Adolescent Women

Aaron C. Ermel, MD[1] **[Fellow in Infectious Diseases]**, **Marcia L. Shew, MD, MPH**[2] **[Associate Professor of Clinical Pediatrics]**, **Bree A. Weaver, MD**[1,2] **[Assistant Research Professor of Medicine]**, **Brahim Qadadri, MS**[1] **[Laboratory Technitian]**, **Cheryl Denski**[3] **[Data management]**, **Wanzhu Tu, PhD**[3] **[Professor of Biostatistics]**, **Yan Tong, MS, PhD**[3] **[Biostatistician]**, **J. D. Fortenberry, MS, MD**[2] **[Professor of Pediatrics]**, and **Darron R. Brown, MD**[1,4] **[Professor of Medicine, Microbiology and Immunology]**

[1]Department of Internal Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

[2]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN 46202

[3]Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN 46202

[4]Department of Immunology and Microbiology, Indiana University School of Medicine, Indianapolis, IN 46202

Abstract

Background—Human papillomavirus (HPV infections are common in adolescent females, while the rare cancerous sequelae of HPV infections do not generally occur until the 4th or 5th decades of life. This prospective study of a cohort of adolescent women was performed to further our knowledge of the natural history of incident and prevalent HPV infections.

Method—Self-vaginal swabs collected from high-risk, unvaccinated adolescent women in a longitudinal study were analyzed for HPV DNA. Sera collected at enrollment and later were tested for HPV antibodies. Statistical analysis was performed to determine the HPV genotype

Corresponding Author: Darron R. Brown, MD, 635 Barnhill Drive, Room MS 224, Indianapolis, IN 46202, Phone: 317-274-1425, Fax: 317-274-1008, darbrow@iu.edu.

Conflicts of interest. Shew, ML: Investigator for Merck & Co., Inc. related HPV vaccine trials. Brown, DR: Received lecture fees, advisory board fees, and intellectual property fees from Merck and Co., Inc. Ermel, A., Tu, W., Weaver, B., Tong, Y., Qadadri, B., Fortenberry, J.D. report no conflicts of interest.

Information has been previously presented in part at the 2010 Society of Adolescent Health and Medicine Annual Meeting, April 7–10, 2010, Toronto, Canada and at the International Papillomavirus Society Meeting, December 6–9, 2012, San Juan Puerto Rico.

Ethics Approval. The study was approved by the local Institutional Review Board (IRB) at Indiana University School of Medicine, Indianapolis, IN, USA.

Contributors. AE, MS, and DB collaborated in the writing of this manuscript. JF, WT, YT, BW participated in the revision of the manuscript. MS and JD were involved in management of the cohort. DB was involved in project design and management as the Principal Investigator of the grant funding the project. BQ was involved in the testing of samples and preparation of data. CD was responsible for data management and assisted in data analysis. AE, DB MS, JF, WT, YT, BW were responsible for data interpretation and analysis.

distribution and duration of detection, and to determine rates of seropositivity and seroconversion for HPV types represented in the assays.

Results—146 subjects (mean age = 15.4 years at enrollment; mean duration of follow-up = 5.8 years) had samples adequate for analysis of HPV detection, and 95 of these subjects had paired sera available. The cumulative prevalence for high-risk and low-risk HPV types was 95.9% and 91.1%, respectively. HPV types 6, 11, 16, and 18 (HPV types represented in the quadrivalent vaccine) were found at some point in 40.4%, 6.2%, 48%, and 24% of participants, respectively. Serologic data confirmed exposure to these vaccine-covered types, as well as to other high-risk HPV types.

Conclusions—In this cohort of adolescent women, high-risk and low-risk HPV types were frequently detected, and serologic data confirmed exposure in most subjects. The high prevalence HPV types represented in the quadrivalent HPV vaccine further supports vaccination of women at an age well before sexual debut.

Keywords

Human Papillomavirus; adolescents; antibodies; seroprevalence

INTRODUCTION

Certain oncogenic or high risk human papillomavirus (HR-HPV) types are associated with malignancies of the cervix, vagina, vulva, anus, and oropharynx[1–4]. An initial HPV infection of the genital tract occurs in most young women within a few years of first vaginal sex, and subsequent infections by other types often occur for several years[5–7]. Malignant sequelae (specifically carcinoma in situ and invasive cervical cancers) of HPV infection occur in the 4th or 5th decades of life, primarily in a subset of individuals with “persistent” infection, meaning that the same HR-HPV type can be detected over varying time intervals before the clinical diagnosis of malignancy[8]. Cohorts of women have been followed for HPV infections and cytologic abnormalities in longitudinal studies that utilized various periods of follow-up[9]. Few studies of adolescent women include sufficient follow-up to define incident and prevalent HPV infection, describe HPV detection within the context of detailed assessment of behavioral risk factors, and assess HPV seroprevalence during longitudinal observation.

Though it is known that administration of L1 VLP based vaccines produces a vigorous antibody response, the knowledge of the immune response in natural infection is incomplete[10, 11]. For example, Carter et al. determined in a cohort of University aged women (unvaccinated against HPV) that the time to seroconversion after detection of HPV types 6, 16, or 18 was approximately 12 months. They also noted that 54.1–68.8% of these women with incident infections seroconverted, and that transient infections were associated with a failure to seroconvert[12]. Thus, there is still much to be learned about HPV infection in adolescent women. In an era where safe and effective vaccines are available, answering such questions about the natural immune response to HPV infection maybe challenging as the presence of antibody would reflect prior vaccination.

MATERIALS AND METHODS

Subjects

This analysis consists of 150 women, none of whom received HPV vaccination because it was not available in the clinics until 2007 and was initially limited at that time to women less than 19 years of age. Subjects were enrolled under the Young Women's Project (YWP) protocol, which was a cohort of young adolescent women (n=386) was enrolled starting in 1998 to study behaviors related to sexually transmitted infections (STIs) and was approved by the local Institutional Review Board at the Indiana University School of Medicine[13] Adolescent women attending one of three primary care clinics for routine visits in Indianapolis were eligible for enrollment. Inclusion criteria for the study were as follows: age of 14 to 17 years, able to understand English and provide written consent, no serious psychiatric problems, and have parental permission for participation in the study. Adolescents could be enrolled regardless of past sexual experience, although pregnant women were not enrolled. Informed consent and parental permission were obtained at enrollment. All subjects received financial compensation for their time and effort.

Subjects provided self-obtained vaginal samples approximately every three months that were tested for STIs, including HPV. Self-obtained vaginal swabs were used for this study as they are easily collected, patient compliance with collection and follow-up is generally higher, recovery of HPV DNA is sufficient for testing, and results correlate well with cervical swab samples[14, 15]. Specifically, *Chlamydia trachomatis* and *Neisseria gonorrhea* were tested using a nucleic acid amplification test (NAAT)[16, 17]. Testing for Trichomonas vaginalis was performed by NAAT as described previously[18]. Sera were collected from subjects at or near enrollment and at a second point near end of the study period. At enrollment and annually, a written questionnaire assessed vaginal intercourse and other sexual behaviors, numbers of sexual partners, and history of STI. Every three months, face to face interviews were conducted to assess contraceptive methods used (oral contraceptive pills, depo-medroxyprogesterone, and condoms), number of sexual partners, frequency of coital events, and the number condom-protected coital events over the preceding three months.

DNA isolation and HPV testing

DNA was extracted from self-obtained vaginal cotton swabs ("samples") using QIAamp MinElute Media Kit (Qiagen, Valencia, California)as previously described[19]. The Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Indianapolis, Indiana) (LA-HPV) was used for HPV detection and genotyping[20]. Reactions were amplified in a PerkinElmer TC9600 Thermal Cycler (PerkinElmer), and positive and negative controls (included in the LA-HPV) were performed with every PCR run. The GH20/PC04 human β-globin target was co-amplified to determine sample adequacy, and detection of specific HPV types was performed as previously described[21, 22]. The 37 individual HPV types detected in the LA-HPV are comprised of HR-HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, and Subtype 82 W13B) and low-risk (LR-HPV) HPV types (6, 11, 40, 42, 54, 55, 61, 62, 64, 72, 81, 83, 84, and 89).

Serology Testing

Two different assays were used to determine antibody titers to several specific HPV types. The Total IgG LIA is a nine-valent Luminex-based immunoassay, developed utilizing yeast-derived L1 VLPs of HPV types 6, 11,16, 18, 31, 33, 45, 52 and 58 coupled to a set of nine distinct fluorescent Luminex microspheres. The Total IgG LIA measures all antibody binding to HPV L1 VLPs and does not distinguish between neutralizing and non-neutralizing antibodies. The defined serostatus cutoffs were set at a level that delineated HPV-negative samples above VLP adsorption-depleted serum background[23].

The Competitive Luminex Immunoassay (cLIA) is a type-specific assay that measures antibody binding to a single neutralizing epitope for each HPV-type L1 VLP (HPV 6, 11, 16 and18), and therefore does not measure complete antibody binding. Instead, the cLIA measures a type-specific, conformational, neutralizing response which is a subset of the total immune response[24, 25]. As a unique reference standard curve is generated for each HPV type, and because each HPV type employs a type-specific monoclonal antibody (mAb) with a unique binding affinity, the recorded cLIA mMU/mL titers cannot be directly compared between HPV types. The serostatus cutoffs employed for this study were those used for the cLIA in the quadrivalent HPV vaccine clinical trials[26, 27]. The results were expressed as either less than the cutoff value or the absolute number. For both serologic assays, values less than the cutoff were assigned a value of zero for calculating the mean values for each HPV type.

Statistical Analysis

Demographic and clinical characteristics of the study subjects were summarized by descriptive statistics. The duration of a period of detection of a specific HPV type was defined as the time between the initial detection and the last detection of that HPV type or until the end of the observation period. Median durations (in days) for type-specific infections were obtained from Kaplan-Meier estimates of the survival functions. Type-specific HPV detection ended when there were no further positive samples for that type and at least two or more samples were negative to the end of observation. However, if a period of detection lasted until the end of observation period, i.e., the last or the next to last sample remained positive for that type, the duration of the detection was considered as right censored, meaning that the infection lasted at least until the end of observation.

Point and cumulative prevalence rates of HPV (per DNA test) for high risk, low risk, and vaccine-protected types were provided at study entry, closeout, as well as for the entire observational time period. A subject was considered to have a type-specific HPV period of detection if two or more quarterly samples tested positive for that HPV type during the study. However, these quarterly samples did not have to be consecutive. For an individual subject, all samples positive for an HPV type previously detected were considered part of one type-specific period of detection. Any HPV type detected in only one sample during a subject's period of enrollment was dropped from the analysis.

Results

Description of Subjects

Data from 150 subjects were analyzed. Subjects with ≥ 3 consecutive β-globin negative samples or ≤75% β-globin positive samples during the entire study period were excluded as this indicated specimen inadequacy. Specimens from 146 subjects (97.3%) were adequate based on β-globin positivity. For these 146 subjects, the mean age at enrollment was 15.4 years (range, 14–17 years old). The study group was 94.5% African American and the remaining 5.5% were white. Mean duration of follow-up was 5.8 years (range, 3.9–9.2 years). At enrollment, 124 of 146 (84.9%) reported a history of vaginal sex, while the remaining 22 of 146 (15.1%) reported no vaginal sex prior to enrollment. The number of subjects who reported vaginal sex increased during the study (Table 1). The mean age at first vaginal sex for those subjects who reported vaginal sex prior to enrollment was 14.4 years old. The mean cumulative numbers of lifetime sexual partners were at enrollment and as of the last visit were 2.9 (SD, 3.7) and 10.6 (SD, 6.8), respectively. The prevalence of other STIs (*Chlamydia trachomatis, Neisseriagonorrhea*, and Trichomonas vaginalis) increased during the study period confirming that this population was at higher risk for HPV infections (Table 1).

Analysis of Self-Collected Vaginal Samples

At total of 3165 samples were collected from the 146 adolescent women and 3038 (96%) were positive for β-globin. The mean number of self-collected vaginal samples per subject was 21.7 (range, 9.00–36.00 samples). The mean percentage of β-globin positive samples per subject was 97.5% (range, 76.9–100%). Seventy one percent of β-globin positive samples (2150/3038 samples) were positive for at least one HPV type. A mean of 1.19 HR-HPV types (SD, 1.19; range, 0–8 types) and 0.73 LR-HPV types per sample (SD, 0.98, range, 0–5 types) were detected.

Incidence and Prevalence of Type-Specific HPV Infections

One hundred and forty of 146 subjects (95.9%) had samples positive for at least one HR-HPV type at any point in the study; and 133/146 (91.1%) had samples positive for at least one LR-HPV type. The mean number of HPV types detected per subject was 1.92 (SD, 1.95; range, 0–12.0 types). The most frequently detected HR-HPV type was HPV 16 (70/146 subjects, 47.9%) followed by HR-HPV types 59 (67/146, 45.9%) and 66 (65/146, 44.5%). The most frequently detected LR-HPV type was HPV 84 (63/146 subjects, 43.2%) followed by HPV 62 (60/146, 41.1%) and HPV 6 (59/146, 40.4%).

A high percentage of subjects had HR and LR-HPV types detected at enrollment. Table 2 shows the prevalence at enrollment, the final visit, and the cumulative prevalence during the entire study (“all” time points). Detection of both HR and LR-HPV types increased from enrollment to the final visit (Table 2). Thirty-two of 146 subjects (21.9%) had at least one of the HPV types included in the quadrivalent vaccine (i.e. HPV types 6, 11, 16, and 18) detected at enrollment. At enrollment, the prevalence of HPV types 6, 11, 16, and 18 were 5.5%, 2.1%, 9.6%, and 7.5%, respectively. At the end of the study, HPV types 6, 11, 16, and

18 were detected in 9.6%, 2.1%, 15.8%, and 3.4%, respectively. Cumulative prevalence of HPV types 6, 11, 16, and 18 were 40.4%, 6.2%, 48%, and 24%, respectively.

Duration of Type-Specific HPV Detection

The median duration of the period of detection was calculated for each HPV type. The median duration of detection for all HPV types during the study was 423 days (95% CI 393, 435). The median duration of detection of all LR-HPV types was 414 days (95% CI 338, 435 and428 days (95% CI 407, 466) for all HR-HPV types. A survival analysis was performed to determine the significance of the observed difference in duration of detection between LR and HR-HPV types. Time to non-detection for LR-HPV types was significantly shorter than that for HR-HPV types (HR 0.85, 95% CI 0.73, 0.98). The prevalence and duration of all HPV types detected in the study are shown in Table 3.

Antibody Detection

Total IgG LIA Results—For 95 of 146 subjects, paired serology samples of sufficient volume were available from enrollment and a second time near the study conclusion. The median time between collection of the two sera was 5.19 years (range 0.23–8.20). Antibodies against the L1 major capsid protein were measured, and the median antibody titers of nine HPV types at enrollment and the second collection were determined as described above (Table 4). Specifically, for the types represented in the quadrivalent vaccine, the seropositivity at enrollment for HPV types 6, 11, 16, and 18 were 23.2%, 4.2%, 38.9%, and 30.5%, respectively. At the second collection, the seropositivity for HPV types 6, 11, 16, and 18 were 50.5%, 17.9%, 60%, and 42.1%, respectively.

Median titers increased during the study for all nine HPV types except in the cases of HPV types 33, 52, and 58. The rate of seroconversion for each HPV type was calculated among all subjects included in the serology analysis regardless of their HPV DNA type results. The rates of seroconversion are listed for each of the nine types assayed by the Total IgG in Table 4.

cLIA Results—The same serologic samples from the 95 subjects described above were tested for the presence of neutralizing antibodies against HPV types 6, 11, 16, and 18 in the cLIA. The assay cutoff values, median titer at enrollment and at the second collection, and rate of seropositivity at enrollment and at the second collection are presented in Table 4. The seroprevalence at enrollment was 24.2%, 2.1%, 6.3%, and 6.3% for HPV types 6, 11, 16, and 18, respectively. The seroprevalence at the second collection was 48.4%, 12.6%, 35.8%, and 20.0% for HPV types 6, 11, 16, and 18, respectively.

The median titer increased for HPV types 6 and 18, but decreased for types 11 and 16. The rate of seroconversion was calculated for each of these four HPV types. The rate of seroconversion was 28.4%, 9.5%,28.4%, and 14.7%for types 6, 11, 16, and 18, respectively.

Discussion

This longitudinal study was performed to gain new insights into the natural history of genital tract HPV infections in a group of adolescent women at high risk for STIs, but unvaccinated

against HPV. This unique cohort, while modest in size, was very closely followed for about six years on average; some for up to nine years. Multiple, sequential samples from each subject were analyzed for HPV, and serologic samples from approximately two-thirds of the subjects were also analyzed for antibodies against HPV, including vaccine types and non-vaccine types. A high percentage of subjects had HR and LR-HPV types detected at enrollment. Additionally, 21.9% had at least one vaccine type detected at enrollment. There was a wide range of detection of specific HPV types during the entire study period. Nearly all subjects had evidence of infections with both HR and LR-HPV types at some point during the study. HPV 16, the causative agent of nearly half of all cervical cancers, was detected at some point in nearly 48% of these young women. Detection of both HR and LR-HPV types increased from enrollment to the final visit, and a high cumulative prevalence of any of the four vaccine types among the study subjects was found.

Given that this cohort of adolescent women represents one with close, long-term follow-up, and was unvaccinated against HPV, it was informative to examine the presence of antibodies during natural infection. In contrast to HPV DNA detection, which has been shown to wax and wane over time, the antibody response to infection remains relatively constant over time, and may provide a better overall gauge of exposure in cohorts that would be at risk for repeated HPV exposure[28, 29]. As in prior studies, failure to seroconvert during the study period could have been due to the presence of very transient infections[12]. However, cases in which a specific HPV type was detected on only one occasion were removed from our analysis in order to provide a better estimation of the true prevalence of HPV infections.

At the beginning of the study, seropositivity as measured in the nine-type Total IgG LIA was highest for HPV types 6 and 16. Seropositivity against most HPV types increased during the study period similar to the results noted above in both the Total IgG LIA and cLIA. Results of the two serologic assays were comparable (for types represented in both assays), which was best illustrated in the case of HPV 16 with seroconversion rates of 29.5% and 28.4% for the Total IgG and cLIA assays, respectively. The increases in the seroprevalence for all of the HPV types tested (except in the case of HPV 45) indicated a high degree of past exposure to oncogenic HPV types during the study period. Even though the focus of this analysis is descriptive, the serological evidence suggests that antibody responses may rise and fall (to the point of non-detection) with time in natural infection as demonstrated by the fall in titers for certain types at the second collection point.

It is difficult to compare rates of seropositivity found in this cohort to those described in other studies due to variations in study populations and assays used. For example, Safaeian*et al*., measured seropositivity against HPV 16 and 18 in a population of women 18 to 25 years of age, using an ELISA, and found rates of 24.8% for both HPV types at enrollment[30]. In another study, a lower rate of seropositivity was found in a population-based study examining seroprevalence against the four vaccine types using the cLIA[31]. These authors found seropositivity rates of 5.3%, 1.9%, 4.0%, and 0.9% for HPV types 6, 11, 16, and 18, respectively within the 14 to 19 year old age group[31]. However, a similar population based study conducted in the Netherlands noted a higher seroprevalence for seven HPV types (HPV 16, 18, 31, 33, 45, 52, and 58) of 25.2% in young women[32]. Specifically for HPV 16

and 18, seroprevalence was 13%. The higher percentage of seropositive women in our study may be due to the high number of lifetime partners.

Serologic testing for HPV may underestimate exposure to HPV as many women do not develop an antibody response. In our cohort for example, serology appeared to reflect overall exposure given that 60% of the subjects were seropositive for HPV 16 based on the Total IgG assay at the second collection. This correlates to the percentage of subjects (48%) who were found to have HPV 16 infections based on DNA testing. When compared to testing by cLIA, the number of subjects positive for HPV 16 was below that of the Total IgG result and HPV DNA testing (35.8% vs. 60%). This would be expected given that not all women would be expected to develop the neutralizing antibodies measured by this assay.

This study has some limitations. While the overall number of subjects was small, these young women were followed closely for many years. Second, there was little racial diversity among the subjects, which does not reflect the diversity in the population of the United States. It does however accurately reflect the demographics of the areas where the primary care clinics are located. Third, the young women in this study had a high number of sexual partners per subject; thus, the findings presented here cannot be directly extrapolated to all other ages or populations. Lastly, the evaluation of the antibody response to natural HPV infection was limited by the sample size, as only two-thirds of subjects had serum available for analysis.

In conclusion, we found that nearly all subjects in a longitudinally followed cohort of adolescent women were infected with multiple HPV types, including oncogenic types and types represented in the HPV vaccines. Serologic analysis confirmed the results of the HPV DNA, thus contributing to the evolving comprehension of HPV epidemiology. Correlation of various characteristics of HPV DNA detection (duration and viral load) to the development of antibody is planned for future analyses. Our findings support the need to vaccinate of young women against HPV before they become sexually active, rather than waiting until an age at which they may become exposed to multiple HPV types, including HPV 16, the type responsible for half of all cervical cancer.

Acknowledgments

Thank you to Pharmaceutical Product Development, Inc. for providing the serology testing for this study.

Financial Support. This work was supported by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health [Grant Number NIH R01 AI072020-01A2 to D. Brown]; Merck Investigator Initiated Studies Program (D. Brown). Serology testing was performed by Pharmaceutical Product Development, Incorporated.

References

1. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. Journal of Pathology. 1999; 189:12–19. [PubMed: 10451482]
2. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England journal of medicine. 2003; 348:518–527. [PubMed: 12571259]
3. zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology. 2009; 384:260–265. [PubMed: 19135222]

4. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest. 2011; 121:4593–4599. [PubMed: 22133884]
5. Smith JS, Melendy A, Rana RK, et al. Age-specific prevalence of infection with human papillomavirus in females: a global review. J Adolesc Health. 2008; 43:S5–S25. S e1–S e41. [PubMed: 18809145]
6. Moscicki AB, Schiffman M, Burchell A, et al. Updating the natural history of human papillomavirus and anogenital cancers. Vaccine. 2012; 30(Suppl 5):F24–F33. [PubMed: 23199964]
7. Winer RL, Hughes JP, Feng Q, et al. Early natural history of incident, type-specific human papillomavirus infections in newly sexually active young women. Cancer Epidemiol Biomarkers Prev. 2011; 20:699–707. [PubMed: 21173170]
8. Woodman CB, Collins S, Winter H, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet. 2001; 357:1831–1836. [PubMed: 11410191]
9. Bratti MC, Rodriguez AC, Schiffman M, et al. Description of a seven-year prospective study of human papillomavirus infection and cervical neoplasia among 10000 women in Guanacaste, Costa Rica. Revista panamericana de salud publica = Pan American journal of public health. 2004; 15:75–89. [PubMed: 15030652]
10. Stanley M. Immune responses to human papillomavirus. Vaccine. 2006; 24(Suppl 1):S16–S22. [PubMed: 16219398]
11. Brown DR, Garland SM, Ferris DG, et al. The humoral response to Gardasil over four years as defined by total IgG and competitive Luminex immunoassay. Hum Vaccin. 2011; 7:230–238. [PubMed: 21307649]
12. Carter JJ, Koutsky LA, Wipf GC, et al. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J Infect Dis. 1996; 174:927–936. [PubMed: 8896492]
13. Tu W, Batteiger BE, Wiehe S, et al. Time from first intercourse to first sexually transmitted infection diagnosis among adolescent women. Archives of pediatrics & adolescent medicine. 2009; 163:1106–1111. [PubMed: 19996047]
14. Gravitt PE, Lacey JV Jr, Brinton LA, et al. Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol Biomarkers Prev. 2001; 10:95–100. [PubMed: 11219778]
15. Lorenzato FR, Singer A, Ho L, et al. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. American journal of obstetrics and gynecology. 2002; 186:962–968. [PubMed: 12015522]
16. Van Der Pol B, Quinn TC, Gaydos CA, et al. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of Chlamydia trachomatis. J Clin Microbiol. 2000; 38:1105–1112. [PubMed: 10699004]
17. Van Der Pol B, Martin DH, Schachter J, et al. Enhancing the specificity of the COBAS AMPLICOR CT/NG test for Neisseria gonorrhoeae by retesting specimens with equivocal results. J Clin Microbiol. 2001; 39:3092–3098. [PubMed: 11526134]
18. Van Der Pol B, Kraft CS, Williams JA. Use of an adaptation of a commercially available PCR assay aimed at diagnosis of chlamydia and gonorrhea to detect Trichomonas vaginalis in urogenital specimens. J Clin Microbiol. 2006; 44:366–373. [PubMed: 16455885]
19. Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. The Journal of Infectious Diseases. 2005; 191:182–192. [PubMed: 15609227]
20. Castle PE, Carolina Porras, Quint Wim G, et al. Comparison of two PCR-based human papillomavirus genotyping methods. Journal of Clinical Microbiology. 2008; 46:3437–3445. [PubMed: 18716224]
21. Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis. 2005; 191:182–192. [PubMed: 15609227]

22. Shew ML, Fortenberry JD, Tu W, et al. Association of condom use, sexual behaviors, and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women. Arch Pediatr Adolesc Med. 2006; 160:151–156. [PubMed: 16461870]

23. Opalka D, Matys K, Bojczuk P, et al. Multiplexed serologic assay for nine anogenital human papillomavirus types. Clin Vaccine Immunol. 17:818–827. [PubMed: 20237197]

24. Opalka D, Lachman CE, MacMullen SA, et al. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. Clinical & Diagnostic Laboratory Immunology. 2003; 10:108–115. [PubMed: 12522048]

25. Dias D, Van Doren J, Schlottmann S, et al. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. Clin Diagn Lab Immunol. 2005; 12:959–969. [PubMed: 16085914]

26. Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol. 2005; 6:271–278. [PubMed: 15863374]

27. Villa LL, Costa RL, Petta CA, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. Br J Cancer. 2006; 95:1459–1466. [PubMed: 17117182]

28. af Geijersstam V, Kibur M, Wang Z, et al. Stability over time of serum antibody levels to human papillomavirus type 16. J Infect Dis. 1998; 177:1710–1714. [PubMed: 9607854]

29. Silins I, Kallings I, Dillner J. Correlates of the spread of human papillomavirus infection. Cancer Epidemiol Biomarkers Prev. 2000; 9:953–959. [PubMed: 11008914]

30. Safaeian M, Porras C, Schiffman M, et al. Epidemiological study of anti-HPV16/18 seropositivity and subsequent risk of HPV16 and -18 infections. J Natl Cancer Inst. 2010; 102:1653–1662. [PubMed: 20944077]

31. Markowitz LE, Sternberg M, Dunne EF, et al. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003–2004. J Infect Dis. 2009; 200:1059–1067. [PubMed: 19719390]

32. Scherpenisse M, Mollers M, Schepp RM, et al. Seroprevalence of seven high-risk HPV types in The Netherlands. Vaccine. 2012; 30:6686–6693. [PubMed: 22959981]

Table 1

Study subject characteristics. Time point of acquisition of data is dependent upon the characteristic mentioned. N=146

Characteristic	Number (%) or mean (SD)
Age, at enrollment	15.40 (1.03)
Race	
African American	138 (94.5)
White	8 (5.5)
Sexual Experience	
Enrollment visit	124 (84.9)
Final Visit	145 (99.3)
Age at first sex	14.4 (1.7)
Number of partners*	
Enrollment visit	2.88 (3.7)
Last Visit	10.6 (6.8)
History of STI**	
Enrollment visit	22 (15.1)
Last Visit	124 (84.9)
Duration of follow-up	5.81 (1.5) yrs.

*Mean number of unique cumulative lifetime partners within 2 months of enrollment and at the end of the study.

**STI= cumulative number of sexually transmitted infections other than HPV at enrollment and at the last visit.

These include Chlamydia trachomatis (CT), Neisseria gonorrhea (GC), and Trichomonas vaginalis (TV).

Table 2

Point and cumulative prevalence of HPV DNA detected. N= 146.

Time Point(s)	Number of subjects positive, number (%)			
	HPV any type	HR-HPV	LR-HPV	Any Vaccine Type*
Enrollment visit	68 (46.6)	55 (37.7)	45 (30.8)	32 (21.9)
Final visit	108 (74)	92 (63)	76 (52)	40 (27.4)
All visits (cumulative prevalence)	140 (95.9)	140 (95.9)	133 (91.1)	106 (72.6)

*Any Vaccine Type includes HPV types 6, 11, 16, and 18.

Table 3

Number of subjects, median durations and confidence intervals of DNA detection of each HPV type tested.

HPV Type	Subjects Positive for each Type N (%)	Median Duration (Days)	95% CI (Days)
16	70 (47.9)	841	508–1109
18	35 (24)	423	305–1575
26	6 (4.1)	392.5	85–431
31	30 (20.5)	428	173–757
33	7 (4.8)	575	85–1163
35	38 (26)	431	253–735
39	46 (31.5)	337	206–431
45	34 (23.3)	420	337–781
51	60 (41.1)	498	253–897
52	57 (39)	596	288–946
53	50 (34.2)	512	323–705
56	35 (24)	253	169–421
58	33 (22.6)	930	421–1677
59	67 (45.9)	372	286–644
66	65 (44.5)	533	337–617
67	28 (19.2)	169	155–262
68	33 (22.6)	337	197–512
69*	2 (1.4)	113	N/A
70	15 (10.3)	1072	274–1989
73	33 (22.6)	260	169–424
82	21 (14.4)	536	92–786
82(IS39)	4 (2.7)	165.5	85–267
6	59 (40.4)	420	173–498
11	9 (6.2)	267	18–533
40	24 (16.4)	346	169–533
42	38 (26)	183	155–260
54	42 (28.8)	435	337–939
55	29 (19.9)	331	252–519
61	38 (26)	589	367–1275
62	60 (41.1)	477	336–590
64*	1 (0.7)	83	N/A
72	8 (5.5)	256.5	85–512
81	20 (13.7)	456	394–1168
83	42 (28.8)	731	505–883
84	63 (43.2)	277	169–428
89	49 (33.6)	260	169–435

* Confidence interval could not be calculated as there was only one case.

Table 4

Number of subjects with positive serology to the nine types assayed by the Total IgG and cLIA assays. N=95

Assay Type	HPV Type	Assay Cutoff**	Number Positive at enrollment, N (%)	Number Positive at second collection N (%)	Median titer at enrollment*	Median titer at second collection*	Rate of sero-conversion*** among all subjects N (%)
IgG	6	>15	22 (23.2)	48 (50.5)	31.0	52.5	29 (30.5)
IgG	11	>15	4 (4.2)	17 (17.9)	32.6	48.2	15 (15.8)
IgG	16	>7	37 (38.9)	57 (60)	10.6	49.0	28 (29.5)
IgG	18	>10	29 (30.5)	40 (42.1)	15.5	22.7	24 (25.3)
IgG	31	>6	9 (9.5)	26 (27.4)	10.3	16.5	20 (21.5)
IgG	33	>6	7 (7.4)	14 (14.7)	17.8	12.9	8 (8.4)
IgG	45	>17	5 (5.3)	3 (3.2)	18.5	45.7	3 (3.2)
IgG	52	>8	7 (7.4)	23 (24.2)	19.1	18.5	19 (20.0)
IgG	58	>6	9 (9.5)	21 (22.1)	18.7	18.5	16 (16.8)
cLIA	6	>20	23 (24.2)	46 (48.4)	44	123.5	27 (28.4)
cLIA	11	>16	2 (2.1)	12 (12.6)	81.5	37.0	9 (9.5)
cLIA	16	>20	6 (6.3)	34 (35.8)	315.0	184.5	27 (28.4)
cLIA	18	>24	6 (6.3)	19 (20.0)	52.0	77.0	14 (14.7)

* Median titer was calculated after excluding all negative serologies.

** Any value greater than the number listed for that type-specific assay was considered positive.

*** Seroconversion was defined as any value below the cut-off for that type specific assay at enrollment and any value greater than the assay cut-off at the second collection.

IgG= Total IgG for each HPV type and cLIA= competitive luminex immunoassay for each HPV type listed.