MAJOR ARTICLE

# The Optimal Anatomic Sites for Sampling Heterosexual Men for Human Papillomavirus (HPV) Detection: The HPV Detection in Men Study

**Anna R. Giuliano,[1] Carrie M. Nielson,[2] Roberto Flores,[1] Eileen F. Dunne,[3] Martha Abrahamsen,[1] Mary R. Papenfuss,[1] Lauri E. Markowitz,[3] Danelle Smith,[1] and Robin B. Harris[2]**

[1]H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida; [2]Arizona Cancer Center and Mel and Enid Zuckerman College of Public Health, Tucson; [3]Division of Sexually Transmitted Disease Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

**(See the article by Nielson et al., on pages 1137–45.)**

***Background.*** Human papillomavirus (HPV) infection in men contributes to infection and cervical disease in women as well as to disease in men. This study aimed to determine the optimal anatomic site(s) for HPV detection in heterosexual men.

***Methods.*** A cross-sectional study of HPV infection was conducted in 463 men from 2003 to 2006. Urethral, glans penis/coronal sulcus, penile shaft/prepuce, scrotal, perianal, anal canal, semen, and urine samples were obtained. Samples were analyzed for sample adequacy and HPV DNA by polymerase chain reaction and genotyping. To determine the optimal sites for estimating HPV prevalence, site-specific prevalences were calculated and compared with the overall prevalence. Sites and combinations of sites were excluded until a recalculated prevalence was reduced by <5% from the overall prevalence.

***Results.*** The overall prevalence of HPV was 65.4%. HPV detection was highest at the penile shaft (49.9% for the full cohort and 47.9% for the subcohort of men with complete sampling), followed by the glans penis/coronal sulcus (35.8% and 32.8%) and scrotum (34.2% and 32.8%). Detection was lowest in urethra (10.1% and 10.2%) and semen (5.3% and 4.8%) samples. Exclusion of urethra, semen, and either perianal, scrotal, or anal samples resulted in a <5% reduction in prevalence.

***Conclusions.*** At a minimum, the penile shaft and the glans penis/coronal sulcus should be sampled in heterosexual men. A scrotal, perianal, or anal sample should also be included for optimal HPV detection.

Human papillomavirus (HPV) infection is the necessary etiologic agent for cervical carcinogenesis, with HPV infection in men significantly contributing to infection and subsequent cervical disease in women as well as to disease in men [1–4]. Case-control studies of women with cervical cancer and their husbands have demonstrated that men's sexual behavior affects women's risk of cervical neoplasia, even when controlling for female sexual activity. In areas with a high incidence of cervical cancer, men's sexual behavior is a risk factor for cervical neoplasia [5].

Available information regarding penile HPV infection is primarily derived from 3 sources: (1) studies of husbands of women with cervical cancer [6–9]; (2) cross-sectional studies of select populations, such as patients with sexually transmitted diseases (STDs) and military recruits [10, 11]; and (3) small prospective studies [12, 13] (see [14] for a recent review of the literature).

The reported prevalence of HPV infection in men varies widely. Early studies used acetowhitening of the

Received 14 March 2007; accepted 3 May 2007; electronically published 17 September 2007.

Reprints or correspondence: Dr. Anna Giuliano, Risk Assessment, Detection, and Intervention Program, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Dr., MRC-CANCONT, Tampa, FL 33612 (Anna.Giuliano@moffitt.org).

**The Journal of Infectious Diseases 2007;196:1146–52**

0022-1899/2007/19608-0006$15.00
DOI: 10.1086/521629

Potential conflicts of interest: A.R.G. is on the speakers' bureau of Merck and has served as a consultant for the male human papillomavirus vaccine program at Merck. All other authors report no potential conflicts.

Presented in part: 22nd International Papillomavirus Conference, Vancouver, 30 April–6 May 2005 (abstract P-323).

Financial support: Centers for Disease Control and Prevention, through the Association of American Medical Colleges (AAMC) (cooperative agreement [grant U36/CCU319276; AAMC identification number MM-0579-03/03]); National Cancer Institute (R25 CA078447 to C.M.N.).

Publication and report contents are solely the responsibility of the authors and do not necessarily represent the official views of the Association of American Medical Colleges or the Centers for Disease Control and Prevention.

penis as a diagnostic marker for HPV infection and examined men for epithelial lesions [15]. Although HPV is significantly associated with acetowhite penile lesions [16], many other genital conditions are also associated with these lesions [17, 18], resulting in poor specificity for HPV detection. To accurately assess HPV infection in men, molecular techniques must be used. Recent studies using polymerase chain reaction (PCR), a method sensitive enough to detect 10–100 copies of viral DNA, have found HPV detection in men to be as high as in their female counterparts [19].

Most studies of HPV infection in men have sampled penile skin—specifically, the coronal sulcus and glans penis—and the urethra [6–9, 11–13, 20–23]. Many studies combined specimens from the skin and urethra to report HPV DNA prevalence. In 2 studies, urine was collected [21, 24]; however, neither reported HPV DNA results. There is some consensus that sampling the coronal sulcus and glans penis is necessary for assessing HPV status in men because of direct contact with the cervix [14]. There is less consensus regarding urethral sampling, particularly among asymptomatic men. In addition to potentially not yielding informative data, urethral sampling may decrease study participation, particularly in prospective studies that require repetitive sampling of anatomic sites. Several studies have evaluated the scrotum and semen for the presence of HPV DNA [25–38]; however, the majority did not also evaluate the presence of HPV at other anatomic sites [25, 27, 29–31, 34, 36]. As a result, it is unclear whether sampling the scrotum and semen contribute meaningful information to the estimation of HPV prevalence. Twelve studies have attempted to evaluate HPV sampling at multiple anatomic sites [12, 21, 26, 28, 33, 37, 39–44].

The purpose of the present study was to determine the optimal anatomic site(s) for the detection of HPV infection among heterosexual men by assessing HPV DNA in urethral, glans penis/coronal sulcus, penile shaft/prepuce, scrotal, perianal, anal canal, semen, and urine samples.

## METHODS

***Study design.*** A cross-sectional study was conducted in Tucson, Arizona, from 2003 to 2006 and in Tampa, Florida, in 2005. A total of 463 men provided samples for HPV testing, 259 of whom provided samples for each anatomic site and specimen of interest. Of these, 186 men had samples that were deemed to be adequate for all 7 sites. Men were eligible for the study if they (1) were between 18 and 40 years old, (2) had had sexual intercourse with a woman within the past year, (3) acknowledged no previous diagnosis of genital warts or penile or anal cancer, (4) had no current penile discharge or pain during urination, and (5) had no current diagnosis of an STD. Primary methods of recruitment were through advertisements in city and university newspapers, flyers in public places, and in-person recruitment at the local air force base and the county health department STD clinic.

All participants gave written informed consent, and all procedures were approved by the University of Arizona Human Subjects Protection Program, the Centers for Disease Control and Prevention Institutional Review Board, the US Department of Defense, and the University of South Florida Institutional Review Board.

***Clinical sampling.*** Men were asked to (1) not have sex 24 h before the clinical visit, so as to avoid detection of HPV from partners, and (2) not wash the genitals the morning of the visit. The study clinician used a calcium alginate or Dacron urethral swab to sample the urethral epithelium. The swab was inserted ~2 cm into the urethra and rotated 360 degrees while removing it. The clinician sampled other anogenital sites by rubbing separate saline-wetted Dacron swabs to sample the entire surface of the (1) glans penis/coronal sulcus, (2) penile shaft (including the prepuce, if present), (3) scrotum, and (4) perianal area. Another saline-wetted Dacron swab was inserted into the anal canal up to the anal verge. Each of these 6 swabs was placed into a separate collection tube filled with either 250 $\mu$L (urethral swabs) or 350 $\mu$L (all other swabs) of specimen transport medium (STM; Digene Corporation). Men were instructed to collect a semen sample by masturbation 12–36 h before the clinical sampling visit, to not touch the inside of the cup, and to refrigerate the sample until the visit. Collection tubes were labeled with the participant's study identification number, date of collection, and sample type and were stored at −20°C. Men were asked to provide a first-catch urine sample of ~30 mL in a sterile urine cup. Urine cups were shaken to redistribute sediment, split into two 15-mL conical centrifuge tubes for processing, and refrigerated at 4°C before storage at −80°C until HPV analyses. Urine samples from 226 men indicated that only 51.3% were $\beta$-globin positive, and only 1 sample was HPV positive. Given the poor level of sample adequacy and HPV detection, the analyses of urine samples were stopped.

Samples were collected by 1 of 5 study clinicians: 74% of men were sampled by the primary clinician in Tucson, 21% were sampled by the primary clinician in Tampa, and 5% were sampled by 3 additional clinicians in Tucson.

A preliminary analysis revealed that the urethral and semen samples did not significantly contribute to the overall HPV prevalence and were eliminated in the third year of the study. The anal canal sample was added after the study began; therefore, this sample was not provided by the first 58 men. A total of 552 men enrolled in the study, and 499 (90.4%) completed the sample collection visit. Samples from 36 men were not included in the present analysis because of consumption of sample by laboratory optimization assays or determination of study ineligibility, resulting in a sample size of 463 men, 186

**Table 1. Sociodemographic characteristics of the men in the Human Papillomavirus Detection in Men Study.**

| Characteristic | Full cohort ($n$ = 463) | Subcohort with complete sampling[a] ($n$ = 186) |
|---|---|---|
| Residence[b] | | |
| Tucson, Arizona | 359 (77.5) | 122 (65.6) |
| Tampa, Florida | 104 (22.5) | 64 (34.4) |
| Age, mean ± SD, years | 27.2 ± 6.5 | 26.7 ± 6.3 |
| Race | | |
| White | 324 (70.0) | 122 (65.6) |
| Black | 33 (7.1) | 10 (5.4) |
| Asian/Pacific Islander | 19 (4.1) | 8 (4.3) |
| American Indian/Alaska Native | 9 (1.9) | 6 (3.2) |
| Other/unknown | 78 (16.9) | 40 (21.5) |
| Ethnicity | | |
| Hispanic | 79 (17.1) | 40 (21.5) |
| Non-Hispanic | 376 (81.2) | 145 (78.0) |
| Marital status | | |
| Single, never married | 327 (70.6) | 124 (66.7) |
| Married | 59 (12.7) | 29 (15.6) |
| Cohabiting | 29 (6.3) | 14 (7.5) |
| Divorced/separated | 35 (7.5) | 12 (6.5) |
| Education | | |
| <12 years | 23 (5.0) | 7 (3.8) |
| 12 years | 94 (20.3) | 40 (21.9) |
| 13–16 years | 240 (51.8) | 102 (55.7) |
| ⩾17 years | 101 (21.8) | 34 (18.6) |

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. All categories do not total 463 or 186 because of refused or missing data.

[a] Men with adequate samples at all 7 sites.

[b] Significant differences by clinic site ($P < .05$).

of whom provided adequate samples for each anatomic site and specimen of interest.

***HPV DNA detection and genotyping.*** HPV testing of swabbed cellular material and semen was conducted using PCR for amplification of a fragment of the L1 gene [45]. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen), in accordance with the instructions of the manufacturer. Samples were tested for the presence of HPV by amplifying 5 $\mu$L of the DNA extracts with the PGMY09/11 L1 consensus primer system [45] and AmpliTaq Gold polymerase (Perkin-Elmer). The GH20/PC04 human $\beta$-globin target was coamplified using the B_PCO4 and B_GH20 primers, along with HPV consensus primer amplification. To control for possible contamination and accuracy, a negative control ($H_2O$) and a positive control (CaSki Cells DNA) were run for every PCR plate. Samples were amplified using the Perkin-Elmer GeneAmp PCR System 9700.

HPV genotyping was conducted using the reverse line blot method [46] on all samples, regardless of HPV PCR result. This method uses HPV L1 consensus PCR products labeled with biotin to detect 37 HPV types. The HPV genotype strip contains 39 probe lines, detecting 37 individual HPV genotypes and 2 concentrations of the $\beta$-globin control probe (Roche Molecular Diagnostics). Strips were interpreted with a labeled overlay, with lines indicating the position of each probe relative to the reference mark.

***Statistical analysis.*** The presence of adequate DNA to allow for HPV testing was assessed by the detection of $\beta$-globin or HPV in the sample. The prevalence of HPV DNA at each anatomical site was calculated as the proportion of samples from the anatomical site that had HPV DNA present by PCR or genotyping. The overall prevalence of HPV was calculated as the proportion of men positive at any site for both the full cohort ($n$ = 463) and the cohort of men with complete sampling ($n$ = 186). Along with the proportions, 95% confidence intervals were calculated. To determine the optimal site(s) for detection, the overall prevalence for each cohort was recalculated after excluding specific sites and comparing the recalculated prevalence to the original overall prevalence estimate. We evaluated the effect of omitting each site individually and then combination of sites. The anatomic sites associated with a small change in prevalence were omitted until the overall prevalence was reduced by a maximum of 5%, with the remaining sites deemed to be optimal. A particular sampling site was considered to be suboptimal either because its rate of positivity for HPV DNA was low or because of joint positivity with multiple sampling sites. All analyses were conducted using Intercooled Stata (version 9.1; StataCorp).

## RESULTS

Men 18–40 years old were enrolled in the study; the mean ± SD age was 27.2 ± 6.5 years (table 1). The majority of study participants were white (70.0%), 70.6% reported having never been married, and 73.6% had more than a high school education. Sociodemographic characteristics for the cohort of men with complete sampling (hereafter, "subcohort") are also reported in table 1. There were no significant differences between the full cohort and the subcohort with respect to race, ethnicity, marital status, and level of education.

The number of men contributing samples per anatomic site differed because of variable willingness to provide a sample and study decisions to terminate the collection of semen and urethra samples (table 2). There were 463 samples available for HPV detection at the penile shaft, glans penis/coronal sulcus, scrotum, and perianal area. Four hundred five men provided anal canal samples, 331 provided urethral samples, and 344 provided a semen sample. A total of 186 men provided adequate samples for each sample type. The majority of external anatomic sites tested (e.g., penile shaft, glans penis, scrotum, and perianal area) were deemed to be adequate, with a range of $\beta$-globin positivity of 94.2%–97.0%. More than ninety-five percent of anal canal

**Table 2. Prevalence of β-globin and human papillomavirus (HPV), by anatomic sampling site.**

| Category | Subjects | Site | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Penile shaft | Glans penis/coronal sulcus | Scrotum | Perianal area | Anal canal | Urethra | Semen |
| Full cohort | | | | | | | | |
| Sampled | 463 | 463 | 463 | 463 | 463 | 405 | 331 | 344 |
| Adequate[a] | 463 | 449 (97.0) | 444 (95.9) | 441 (95.2) | 436 (94.2) | 386 (95.3) | 278 (84.0) | 337 (98.0) |
| Any HPV | 303 (65.4) | 224 (49.9) | 159 (35.8) | 151 (34.2) | 87 (20.0) | 68 (17.6) | 28 (10.1) | 18 (5.3) |
| Subcohort with complete sampling[b] | | | | | | | | |
| Adequate[a] | 186 | 186 | 186 | 186 | 186 | 186 | 186 | 186 |
| Any HPV | 126 (67.7) | 89 (47.9) | 61 (32.8) | 61 (32.8) | 42 (22.6) | 34 (18.3) | 19 (10.2) | 9 (4.8) |

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated.

[a] β-globin or HPV positive by polymerase chain reaction, genotyping, or both.

[b] Men with adequate samples from all 7 sites.

(95.3%) and 98% of semen samples were β-globin positive. The adequacy of the urethral samples was lowest, at 84% β-globin positivity. Overall, 65.4% of men in the full cohort and 67.7% of men in the subcohort were HPV positive at 1 or more anatomic sites. Regardless of cohort, HPV detection was highest at the penile shaft (49.9% for the full cohort and 47.9% for the subcohort), followed by the glans penis/coronal sulcus (35.8% and 32.8%) and the scrotum (34.2% and 32.8%). Detection of HPV was lower in the perianal area (20.0% and 22.6%) and in the anal canal (17.6% and 18.3%). HPV detection was lowest in urethral (10.1% and 10.2%) and semen (5.3% and 4.8%) samples.

To determine the optimal combination of anatomic sites for the assessment of HPV prevalence in men, we excluded specific anatomic samples and recalculated the overall prevalence, starting with sites that had the lowest site-specific HPV prevalence for both cohorts (table 3). In the first step, we recalculated the overall prevalence after the elimination of samples from one specific site at a time. In the full cohort, the decrease in HPV prevalence ranged from 0% after eliminating urethral samples to 8.4% after eliminating penile shaft samples (6.4% in the subcohort) (table 3). Because HPV detection at one sampling site was not independent from detection at another, we then evaluated the effect of excluding multiple sites on the overall HPV prevalence. When semen and urethral samples were excluded, HPV prevalence decreased by 0.6% in the full cohort and by 0% in the subcohort. As additional individual sample types were excluded, HPV prevalence decreased. Several different combinations of samples provided similar overall HPV prevalence estimates. If semen and samples of the urethra and glans penis/coronal sulcus were eliminated, overall HPV prevalence was reduced by 2.5% and 1.6% in the full cohort and subcohort, respectively. A decrease of ~3.0% in HPV prevalence was observed in both cohorts by 2 different scenarios: (1) if semen and samples of the urethra and perianal region were excluded and (2) if the combination of semen and samples of the urethra and scrotum were excluded. A total decrease of 3.2% and 3.7% in HPV prevalence was observed if semen and samples of the urethra and anal canal were excluded in the full cohort and subcohort, respectively. All other combinations resulted in a 5% decrease or greater in prevalence. At a minimum, the penile shaft and the glans penis/coronal sulcus should be included in the estimation of HPV prevalence in heterosexual men. Our data suggest that, for optimal detection, a scrotal, perianal, or anal sample should also be included in the sampling protocol.

## DISCUSSION

This is the first study to systematically evaluate the combination of anatomic sampling sites needed for assessing HPV DNA prevalence in men. This topic is of great importance, given that increasing attention is being placed on understanding HPV infection in men to improve strategies for prevention of infection and disease in women as well as their male counterparts. Because of variability in anatomic sites sampled, in devices used to sample, and in laboratory methods for analyses of samples, there is tremendous variability in HPV prevalence estimates in male reported in the literature [14]. Some of this variability is due to differences in populations, but much of the variability is likely due to incomplete anogenital sampling of men.

In the present study, samples of the penile shaft/prepuce, glans penis/coronal sulcus, scrotum, perianal, anal canal, urethra, and semen were obtained and analyzed. To our knowledge, the present study is the only one to include this wide a range of anatomic sites, and, in combination with the application of sensitive laboratory methods, this contributed to the higher overall HPV prevalence detected, compared with that in previously published studies. This is also the only study we are aware of that applied statistical methods for determining the

**Table 3. Effect on overall human papillomavirus (HPV) prevalence of exclusion of specific sampling sites or combinations of sites.**

| Category | Subcohort with complete sampling[a] ($n$ = 186) | | Full cohort ($n$ = 463) | |
|---|---|---|---|---|
| | HPV prevalence, no. (%) | Decrease in prevalence, % | HPV prevalence, no. (%) | Decrease in prevalence, % |
| Overall prevalence[b] | 126 (67.7) | … | 303 (65.4) | … |
| Individual exclusion of | | | | |
| Urethra | 126 (67.7) | 0.0 | 303 (65.4) | 0.0 |
| Semen | 126 (67.7) | 0.0 | 301 (65.0) | 0.4 |
| Anal canal | 120 (64.5) | 3.2 | 294 (63.5) | 1.9 |
| Glans penis/coronal sulcus | 123 (66.1) | 1.6 | 294 (63.5) | 1.9 |
| Perianal area | 121 (65.1) | 2.6 | 293 (63.3) | 2.1 |
| Scrotum | 122 (65.6) | 2.1 | 293 (63.3) | 2.1 |
| Penile shaft | 114 (61.3) | 6.4 | 264 (57.0) | 8.4 |
| Site combination[c] | | | | |
| Penile shaft, glans penis/coronal sulcus, scrotum, perianal area, anal canal | 126 (67.7) | 0.0 | 300 (64.8) | 0.6 |
| Penile shaft, scrotum, perianal area, anal canal | 123 (66.1) | 1.6 | 291 (62.9) | 2.5 |
| Penile shaft, glans penis/coronal sulcus, scrotum, anal canal | 120 (64.5) | 3.2 | 289 (62.4) | 3.0 |
| Penile shaft, glans penis/coronal sulcus, perianal area, anal canal | 121 (65.1) | 2.6 | 289 (62.4) | 3.0 |
| Penile shaft, glans penis/coronal sulcus, scrotum, perianal area | 119 (64.0) | 3.7 | 288 (62.2) | 3.2 |
| Penile shaft, scrotum, perianal area | 117 (62.9) | 4.8 | 278 (60.0) | 5.4 |

[a] Men with adequate samples from all 7 sites.
[b] Positive for HPV at any site.
[c] All other possible combinations of sites resulted in a decrease in prevalence of >5%.

minimal number of anatomical sites to sample for estimating HPV prevalence in men. The penile shaft, prepuce, glans penis/coronal sulcus, and scrotum are generally the anatomic sites with the highest published prevalences of HPV [14]. The relative importance of each of these sites varies from study to study. The highest site-specific HPV prevalence in the present study was observed for the penile shaft (49.9% and 47.9% for the full cohort and the subcohort, respectively). Similar to our results, a study by Hernandez et al. [28] observed the highest HPV prevalence in penile shaft samples regardless of whether a physician collected the sample or the participant collected the sample (51.2% and 51.5%, respectively). Although overall HPV prevalence was lower in a study by Weaver et al. [37], the shaft sample provided the second highest HPV prevalence (24.8%) after the prepuce sample (28.1%). Only one other study examined samples from the penile shaft [26]; however, only 20 samples from this anatomic site were evaluated, and only 1 sample was HPV positive.

HPV prevalence at the glans penis/coronal sulcus in the present study was 35.8% for the full cohort and 32.8% for the subcohort. Although many published studies have sampled solely from the glans penis/coronal sulcus, only 8 published studies compared HPV prevalence at this site with that at other anatomic sites. In studies by Fife et al. [26] and Lazcano-Ponce et al. [21], the glans penis/coronal sulcus was the site with the highest detectable HPV prevalence. However, Lazcano-Ponce et al. [21] combined the glans penis/coronal sulcus with a deep urethral sample; therefore, it is not possible to attribute all of the detectable HPV solely to this site. Although the scrotum and the prepuce yielded higher HPV prevalence estimates than those in the study by Hernandez et al. [28], their reported estimate of ~32% prevalence at the glans penis/coronal sulcus was similar to estimates obtained in the present study.

Five published studies have reported HPV prevalence estimates for scrotal samples, with estimates ranging from 7.1% [26] to 46.2% [28] by use of self-collected samples. Combined samples of the penis and scrotum yielded an HPV prevalence estimate of 46.4% in a study by Aguilar et al. [39]. In the present study, we detected HPV DNA in 32.8% (subcohort) and 34.2% (full cohort) of scrotal samples, and this anatomic site had the third highest HPV prevalence estimate. Recent reports of HPV detection in scrotal samples are notable, because they indicate that transmission to women could occur despite consistent use of barrier contraceptive methods, such as condoms.

Few published studies have obtained samples from the anal canals [12, 33] of heterosexual men. In studies by Nicolau et al. [33] and Van Doornum et al. [12], the anal canal yielded the lowest HPV DNA prevalence estimates, compared with

other anatomic sites sampled. In contrast, a study conducted among men who have sex with men (MSM) [43] reported that 32.8% of anal canal samples had detectable HPV DNA, a proportion 2 times higher than that reported for the glans penis/coronal sulcus of participants. In the present study of heterosexual men, 17.6% (full cohort) and 18.3% (subcohort) of anal canal samples and 20.0% (full cohort) and 22.6% (subcohort) of perianal samples were HPV DNA positive.

Urethral and semen samples yielded the lowest HPV DNA prevalence estimates in the present study, 10.1% and 10.2% for urethral samples and 5.3% and 4.8% for semen samples for the full cohort and the subcohort, respectively. These are also the samples that are more difficult to obtain in research studies. Deep urethral sample collection is painful and semen samples require additional time or, as in the present study, an additional study visit. Nine published studies have reported HPV DNA estimates for semen, with detection rates ranging from 2.2% to 82.9% [25, 27, 29–31, 34, 36, 40, 42]. A larger number of studies have compared HPV detection in urethral samples with other anogenital samples [14]. Both $\beta$-globin detection rates and HPV estimates varied in these studies, with ranges of 12.9%–100% for $\beta$-globin and 8.7%–44.4% for HPV DNA. In the present study, 84.0% of urethral samples were deemed to be adequate on the basis of $\beta$-globin positivity. Both urethra and semen samples had the poorest sampling accessibility and acceptability and low HPV DNA prevalence. Elimination of these 2 samples resulted in a negligible reduction in prevalence (0.6% for the full cohort and 0% for the subcohort). Therefore, these 2 sample types were deemed to be suboptimal and were removed from the sampling scheme.

In the analyses presented here, several different combinations of samples were likely to yield similar HPV prevalence estimates, because HPV infection is often observed at multiple anatomic sites within the same individual. The challenge is to select the smallest combination of anatomic sites for sampling that will yield reliable HPV prevalence estimates and be accessible and acceptable to men. A priori, we established a maximal tolerated loss of HPV prevalence of 5%. Using this threshold, we systematically evaluated the effect of excluding individual anatomic sites and combinations of sites on the overall HPV prevalence. All acceptable scenarios included the elimination of semen and urethral sampling. In addition to eliminating these 2 sites, acceptable prevalences were observed if either the glans penis/coronal sulcus, perianal, scrotal, or anal canal samples were eliminated. In light of previously published findings on the importance of these sites for HPV transmission to women and the acceptability of sampling, we recommend sampling from the penile shaft, glans penis/coronal sulcus, and scrotum in heterosexual men. It is important to note that these are also the sites with the highest prevalences of multiple HPV infections [47]. However, equivalent HPV prevalence estimates will probably be obtained if the scrotal sample is replaced by either a perianal or an anal canal sample. Among MSM, anal canal and perianal sampling is essential, because the prevalence of HPV infection and disease is high at these anatomic sites.

The present study has several limitations. First, the prepuce was sampled as part of the penile shaft, not in a separate collection. Therefore, it is impossible to assess the individual contribution of the prepuce to the overall HPV prevalence. Second, this study included a convenience sample of men from the broader communities of Tucson and Tampa. Enrollment was based on interest in the study and willingness to comply with study procedures; therefore, the HPV prevalence estimates noted in this study may not represent the true underlying population prevalence of these communities. The selection of interested men for this study, however, should not have biased the relative importance of the different anatomic sites to the evaluation of HPV prevalence. Third, men who acknowledged a history of genital warts or current symptoms or diagnosis of an STD were excluded from this study. It is possible that MSM, men with genital warts, and men with other symptomatic sexually transmitted infections would have a different anatomic distribution of HPV infection. Fourth, the clinical significance of HPV detected in this study is unknown. The strengths of this study were the relatively large sample size, the thorough sampling, the sampling of the majority of participants by 2 providers, the use of consistent methods, and the centralized laboratory analysis of samples.

In summary, the present study detected HPV DNA infection in a high proportion of men 18–40 years old, with prevalence being highest at the penile shaft. On the basis of data obtained in this study, we conclude that optimal sampling for estimating HPV prevalence in heterosexual men includes the collection of penile shaft, glans penis/coronal sulcus, scrotal, anal, or perianal samples.

## Acknowledgments

We thank the staff members of the University of Arizona Health Research Clinic (Tucson, Arizona); the Men's Research Clinic at the Lifetime Cancer Screening and Prevention Center, H. Lee Moffitt Cancer Center (Tampa, Florida); the staff members of the Molecular Epidemiology Laboratory at the H. Lee Moffitt Cancer Center; Digene Corporation (Gaithersburg, Maryland); and Melody Schiaffino for editorial assistance.

## References

1. Agarwal SS, Sehgal A, Sardana S, Kumar A, Luthra UK. Role of male behavior in cervical carcinogenesis among women with one lifetime sexual partner. Cancer **1993**; 72:1666–9.
2. Buckley JD, Harris RW, Doll R, Vessey MP, Williams PT. Case-control study of the husbands of women with dysplasia or carcinoma of the cervix uteri. Lancet **1981**; 2:1010–5.
3. Thomas DB, Ray RM, Pardthaisong T, et al. Prostitution, condom use, and invasive squamous cell cervical cancer in Thailand. Am J Epidemiol **1996**; 143:779–86.

4. Zunzunegui MV, King MC, Coria CF, Charlet J. Male influences on cervical cancer risk. Am J Epidemiol **1986**; 123:302–7.
5. Shah KV. Human papillomaviruses and anogenital cancers. N Engl J Med **1997**; 337:1386–8.
6. Bosch FX, Castellsague X, Munoz N, et al. Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J Natl Cancer Inst **1996**; 88:1060–7.
7. Castellsague X, Ghaffari A, Daniel RW, Bosch FX, Munoz N, Shah KV. Prevalence of penile human papillomavirus DNA in husbands of women with and without cervical neoplasia: a study in Spain and Colombia. J Infect Dis **1997**; 176:353–61.
8. Munoz N, Castellsague X, Bosch FX, et al. Difficulty in elucidating the male role in cervical cancer in Colombia, a high-risk area for the disease. J Natl Cancer Inst **1996**; 88:1068–75.
9. Thomas DB, Ray RM, Kuypers J, et al. Human papillomaviruses and cervical cancer in Bangkok. III. The role of husbands and commercial sex workers. Am J Epidemiol **2001**; 153:740–8.
10. Hippelainen M, Syrjanen S, Hippelainen M, et al. Prevalence and risk factors of genital human papillomavirus (HPV) infections in healthy males: a study on Finnish conscripts. Sex Transm Dis **1993**; 20:321–8.
11. Strand A, Rylander E, Evander M, Wadell G. Genital human papillomavirus infection among patients attending an STD clinic. Genitourin Med **1993**; 69:446–9.
12. Van Doornum GJ, Prins M, Juffermans LH, et al. Regional distribution and incidence of human papillomavirus infections among heterosexual men and women with multiple sexual partners: a prospective study. Genitourin Med **1994**; 70:240–6.
13. Wikstrom A, Popescu C, Forslund O. Asymptomatic penile HPV infection: a prospective study. Int J STD AIDS **2000**; 11:80–4.
14. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: a systematic review of the literature. J Infect Dis **2006**; 194:1044–57.
15. Krebs HB, Schneider V. Human papillomavirus-associated lesions of the penis: colposcopy, cytology, and histology. Obstet Gynecol **1987**; 70:299–304.
16. Voog E, Ricksten A, Olofsson S, et al. Demonstration of Epstein-Barr virus DNA and human papillomavirus DNA in acetowhite lesions of the penile skin and the oral mucosa. Int J STD AIDS **1997**; 8:772–5.
17. Pinto PA, Mellinger BC. HPV in the male patient. Urol Clin North Am **1999**; 26:797–807, ix.
18. Richart RM, Barrasso R, Ferenczy A. Examining male partners of women who have abnormal smears. Contemp Ob Gyn **1988**; 31: 157–72.
19. Baken LA, Koutsky LA, Kuypers J, et al. Genital human papillomavirus infection among male and female sex partners: prevalence and type-specific concordance. J Infect Dis **1995**; 171:429–32.
20. Castellsague X, Bosch FX, Munoz N, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med **2002**; 346:1105–12.
21. Lazcano-Ponce E, Herrero R, Munoz N, et al. High prevalence of human papillomavirus infection in Mexican males: comparative study of penile-urethral swabs and urine samples. Sex Transm Dis **2001**; 28: 277–80.
22. Franceschi S, Castellsague X, Dal Maso L, et al. Prevalence and determinants of human papillomavirus genital infection in men. Br J Cancer **2002**; 86:705–11.
23. Svare EI, Kjaer SK, Nonnenmacher B, et al. Seroreactivity to human papillomavirus type 16 virus-like particles is lower in high-risk men than in high-risk women. J Infect Dis **1997**; 176:876–83.
24. Levine RU, Crum CP, Herman E, Silvers D, Ferenczy A, Richart RM. Cervical papillomavirus infection and intraepithelial neoplasia: a study of male sexual partners. Obstet Gynecol **1984**; 64:16–20.
25. Aynaud O, Poveda JD, Huynh B, Guillemotonia A, Barrasso R. Frequency of herpes simplex virus, cytomegalovirus and human papillomavirus DNA in semen. Int J STD AIDS **2002**; 13:547–50.
26. Fife KH, Coplan PM, Jansen KU, et al. Poor sensitivity of polymerase chain reaction assays of genital skin swabs and urine to detect HPV 6 and 11 DNA in men. Sex Transm Dis **2003**; 30:246–8.
27. Green J, Monteiro E, Bolton VN, Sanders P, Gibson PE. Detection of human papillomavirus DNA by PCR in semen from patients with and without penile warts. Genitourin Med **1991**; 67:207–10.
28. Hernandez BY, McDuffie K, Goodman MT, et al. Comparison of physician- and self-collected genital specimens for detection of human papillomavirus in men. J Clin Microbiol **2006**; 44:513–7.
29. Inoue M, Nakazawa A, Fujita M, Tanizawa O. Human papillomavirus (HPV) type 16 in semen of partners of women with HPV infection. Lancet **1992**; 339:1114–5.
30. Kyo S, Inoue M, Koyama M, Fujita M, Tanizawa O, Hakura A. Detection of high-risk human papillomavirus in the cervix and semen of sex partners. J Infect Dis **1994**; 170:682–5.
31. Lai YM, Lee JF, Huang HY, Soong YK, Yang FP, Pao CC. The effect of human papillomavirus infection on sperm cell motility. Fertil Steril **1997**; 67:1152–5.
32. Lajous M, Mueller N, Cruz-Valdez A, et al. Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. Cancer Epidemiol Biomarkers Prev **2005**; 14:1710–6.
33. Nicolau SM, Camargo CG, Stavale JN, et al. Human papillomavirus DNA detection in male sexual partners of women with genital human papillomavirus infection. Urology **2005**; 65:251–5.
34. Olatunbosun O, Deneer H, Pierson R. Human papillomavirus DNA detection in sperm using polymerase chain reaction. Obstet Gynecol **2001**; 97:357–60.
35. Svare EI, Kjaer SK, Worm AM, Osterlind A, Meijer CJ, van den Brule AJ. Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic. Sex Transm Infect **2002**; 78:215–8.
36. Tanaka H, Karube A, Kodama H, Fukuda J, Tanaka T. Mass screening for human papillomavirus type 16 infection in infertile couples. J Reprod Med **2000**; 45:907–11.
37. Weaver BA, Feng Q, Holmes KK, et al. Evaluation of genital sites and sampling techniques for detection of human papillomavirus DNA in men. J Infect Dis **2004**; 189:677–85.
38. Shin HR, Franceschi S, Vaccarella S, et al. Prevalence and determinants of genital infection with papillomavirus, in female and male university students in Busan, South Korea. J Infect Dis **2004**; 190:468–76.
39. Aguilar LV, Lazcano-Ponce E, Vaccarella S, et al. Human papillomavirus in men: comparison of different genital sites. Sex Transm Infect **2006**; 82:31–3.
40. Astori G, Pipan C, Muffato G, Botta GA. Detection of HPV-DNA in semen, urine and urethral samples by dot blot and PCR. New Microbiol **1995**; 18:143–9.
41. Forslund O, Hansson BG, Rymark P, Bjerre B. Human papillomavirus DNA in urine samples compared with that in simultaneously collected urethra and cervix samples. J Clin Microbiol **1993**; 31:1975–9.
42. Rintala MA, Pollanen PP, Nikkanen VP, Grenman SE, Syrjanen SM. Human papillomavirus DNA is found in the vas deferens. J Infect Dis **2002**; 185:1664–7.
43. van der Snoek EM, Niesters HG, Mulder PG, van Doornum GJ, Osterhaus AD, van der Meijden WI. Human papillomavirus infection in men who have sex with men participating in a Dutch gay-cohort study. Sex Transm Dis **2003**; 30:639–44.
44. Wikstrom A, Lidbrink P, Johansson B, von Krogh G. Penile human papillomavirus carriage among men attending Swedish STD clinics. Int J STD AIDS **1991**; 2:105–9.
45. Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol **2000**; 38:357–61.
46. Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol **1998**; 36:3020–7.
47. Nielson CM, Flores R, Harris RB, et al. Human papillomavirus prevalence and type distribution in male anogenital sites and semen. Cancer Epidemiol Biomarkers Prev **2007**; 16:1107–14.