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

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Abstract

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

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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 acetowhiting of the 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 μ L (urethral swabs) or 350 μ 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 β -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 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 μ L of the DNA extracts with the PGMY09/11 L1 consensus primer system [45] and AmpliTaq Gold polymerase (Perkin-Elmer). The GH20/PC04 human β -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 β -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 β -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 \pm 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 β -globin positivity of 94.2%–97.0%. More than ninety-five percent of anal canal (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 men 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 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 β -globin detection rates and HPV estimates varied in these studies, with ranges of 12.9%–100% for β -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 β -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.

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Table 1

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

Characteristic	Full cohort (<i>n</i> = 463)	Subcohort with complete sampling ^a (<i>n</i> = 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.

^aMen with adequate samples at all 7 sites.

^bSignificant differences by clinic site ($P < .05$).

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.

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%.