# Collection Devices for Human Papillomavirus

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**BACKGROUND.** Human papillomavirus (HPV) testing has relied to date on samples collected by experienced health professionals. Self-administered testing devices could allow HPV testing to occur in large-scale epidemiologic studies of primary care screening populations. The purpose of this study is to determine whether a self-collection device for cervicovaginal HPV infection could be developed.

**METHODS.** A prospective randomized trial of a consecutive sampling of 93 women, 18 years or older, receiving routine cervical cancer screening and colposcopy in the urban gynecologic clinics in Philadelphia, Pennsylvania, were randomized into 2 arms. Women in arm 1 used a self-administered tampon before the physician-directed swabs of the cervix; in arm 2, women underwent the physician-directed swab testing before using the self-administered tampon. The concordance of HPV DNA positivity between sampling methods detected by a Hybrid Capture HPV tube test for both low- and high-risk types of HPV was the main outcome measure.

**RESULTS.** The concordance rate (ie, women whose cultures were classified as negative on both tests or positive on both tests) for arms 1 and 2 were similar: 78.3% and 80.9%, respectively.

**CONCLUSIONS.** The tampon was equivalent to the physician-directed swab in HPV detection and suggests its feasibility in long-term primary care studies of screening populations.

**KEY WORDS.** Vaginal smears; detection methods; human papillomavirus; hybrid capture [non-MESH]. *(J Fam Pract 1999; 48:531-535)*

Human papillomavirus (HPV) is recognized as an important cause of cervical cancer.[1,2] Research continues in the areas of the natural history of HPV infection, the effectiveness of treatment, the development of vaccines against HPV, and laboratory techniques to improve detection of HPV DNA for clinical use. The research methods to date relied on the testing of cervicovaginal samples collected in clinic settings by experienced health professionals. The development of a valid and reliable self-administered method for cervicovaginal cell sampling would broaden researchers' abilities to conduct large-scale, home-based epidemiologic studies of HPV and HPV-associated diseases in large primary care screening populations.

Little work has been conducted to validate self-administered cervicovaginal cell collection methods.[3-7] The purpose of our study was to determine whether it is possible to use a self-collection device to detect cervicovaginal HPV infection.

*Submitted, revised, April 30, 1999.*
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## METHODS

### Patient Population

Participating in the study were women attending gynecologic clinics affiliated with the Graduate Hospital in Philadelphia, Pennsylvania, for routine care or colposcopy. Eligible women were 18 years of age or older, not pregnant, and had neither a prior hysterectomy nor a history of treatment for cervical disease in the previous year. Twelve women had been referred for follow-up after an abnormal Papanicolaou's (Pap) test in the previous 6 months. The remaining 85 women participated as part of a routine examination.

### Study Design

We conducted a prospective randomized trial of 97 women after obtaining permission from the Institutional Review Board. Each woman was asked to participate before her examination. If she consented, the woman was randomized into 1 of 2 study arms to determine if any trends in the order of sampling were evident. In arm 1 women were instructed to collect a self-administered cervicovaginal sample using a tampon of regular size, after thoroughly washing their hands. They were instructed to insert the tampon using the directions in the box provided by the manufacturer. The women were then instructed to remove the tampon within a few minutes, place it in a 50-mL conical tube, recap, and close the tube tightly. The women assigned to arm 1 then underwent the physician sampling for

HPV. The physician used 2 Dacron swabs: one directed at the endocervix and the other at the ectocervix. Each swab was placed in specimen transport medium (STM) in a separate standard Hybrid Capture collection tube. In arm 2 the physician-directed swab collection was followed by the self-directed tampon collection.

Tampon results for 4 women were not processed because of mislabeling of the samples, giving us a total of 93 samples available for analysis (46 in arm 1 and 47 in arm 2).

### Sample Preparation

*Tampon.* Processing was performed in a laminar flow hood. Samples were removed from the freezer where they were stored and allowed to thaw in the hood. We added 35 mL of 1X PBS buffer to each 50-mL conical tube. Tubes were shaken for 30 minutes at room temperature at 1100 rpm. Using a pipette, liquid and cells were transferred into another 50-mL conical tube by alternatively squeezing and pipetting the liquid. As precaution against contamination, one sample was handled at a time and gloves were changed between samples. The sample was then centrifuged for 10 minutes at room temperature at 2000 rpm. We discarded the supernatant and added 1 mL of PBS buffer to the pellet. The pellet was vortexed and the cell suspension transferred to a 1.8-mL hybridization tube. The hybridization tube was centrifuged for 10 minutes at 1200 rpm in a microcentrifuge at room temperature. The supernatant was discarded and 1 mL 1X STM plus 1 drop of buffered protease solution was added to each tube and mixed well. Samples were then incubated at 37°C for 30 to 60 minutes, vortexed, and stored at -70°C until ready for testing.

*Dacron swab.* We used the standard Hybrid Capture tube test processing method with a laminar flow hood. Samples from the ectocervical and endocervical samples were combined during preparation.

### HPV Analysis and Statistical Methods

The Hybrid Capture tube test as described by Cope et al[8] was used to detect HPV for both the tampon and the swab. Briefly, initial testing involved 2 separate tests, one for the low-risk HPV types (6, 11, 42, 43, and 44) and one for the high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58). Positive test results for high-risk HPV types were then tested for the following 3 groups: HPV 16 alone; HPV 18, 45, or 56; and HPV 31, 33, 35, 39, 51, 52, and 58. Then the positive results within each group were typed for individual HPV types.

The percent concordance of the 2 collection methods was calculated by summing the number of negative and positive results for HPV DNA on both tests and dividing by the total number of women. The concordance or agreement in test results was assessed with the $\kappa$ and McNemar tests. We assumed that neither method could be considered a gold standard, so the relative sensitivity of each method was calculated by dividing the number of positive test results with each method by the total number of positive results on either test.

## RESULTS

The women ranged in age from 18 to 70 years (mean = 25.6, standard deviation = 6.5). The prevalence of HPV DNA by either collection method was 34.6% in the group of women seen as part of a routine examination, compared with 58.3% for the women with a history of a recent abnormal Pap test result. The overall prevalence of HPV DNA detected by the tampon and swabs was not significantly different (29% and 25.8%, respectively).

The overall concordance rate was similar for each arm of the study: arm 1 = 78.3%; arm 2 = 80.9% ($P = 0.8$). Using a positive test result as a gold standard with either method, the overall relative sensitivity was 77.1% for the tampon and 68.6% for the swab. The relative sensitivity of the tampon was 75.0% and 78.9% for arms 1 and 2, respectively ($P = 1.0$). The relative sensitivity of the swab was 62.5% and 73.7% for arms 1 and 2, respectively ($P = 0.7$).

The distribution of test results for each collection method is shown in Table 1. The overall concordance rate for the 2 collection methods was 79.6%; that is, 58 of 93 women were classified as having a negative result on both tests and 16 of 93 women were classified as having a positive result on both tests. Nineteen women were positive for HPV DNA on one test and not the other. Evaluating the agreement between results of HPV DNA detection by the 2 collection methods yielded a $\kappa$ of 0.489 (fair agreement). The McNemar test, which relies only on discordant cells, suggests that there was not a systematic difference in concordance; ie, over- or under-identification of HPV DNA with one particular method ($\chi^2 = 0.21$, $P > 0.1$).

HPV subtypes were determined for 15 of the 22 specimens that were positive on either collection method in arm 1; the remaining 7 positive specimens had unidentified HPV DNA. In arm 2, HPV subtypes were determined for 20 of the 29 specimens which were positive on either collection method; the remaining 9 specimens had unidentified HPV DNA.

Among the 16 women in both arms who tested positive by both sampling methods, HPV type-specific agreement was examined. There were 5 women who had unidentified HPV types on both collection devices. There was 1 woman who had HPV type 16 present on the swabs but unidentified types present on her tampon sample. Among the 10 women with typeable HPV DNA present on both collection devices, 2 (20%) had exact type agreement, 6 (60%) had at least one HPV type in common between specimens, and 2 (20%) had complete disagreement on viral types between the 2 collection devices.

Further analyses were performed after defining those

**TABLE 1**

**Detection of HPV DNA by Collection Method and Arm of Study (N = 93)**

| | | Swab Result | | | | |
|---|---|---|---|---|---|---|
| | **Arm 1*** | **Negative** | **Low Risk** | **Oncogenic** | **Unidentified** | **Total** |
| **Tampon Result** | Negative | 30 | 1 | 2 | 1 | 34 |
| | Low Risk | 2 | 0 | 0 | 0 | 2 |
| | Oncogenic | 3 | 0 | 3 | 0 | 6 |
| | Unidentified | 1 | 0 | 1 | 2 | 4 |
| | Total | 36 | 1 | 6 | 3 | 46 |

| | | Swab Result | | | | |
|---|---|---|---|---|---|---|
| | **Arm 2†** | **Negative** | **Low Risk** | **Oncogenic** | **Unidentified** | **Total** |
| **Tampon Result** | Negative | 28 | 1 | 1 | 2 | 32 |
| | Low Risk | 0 | 1 | 0 | 0 | 1 |
| | Oncogenic | 4 | 0 | 6 | 0 | 10 |
| | Unidentified | 1 | 0 | 0 | 3 | 4 |
| | Total | 33 | 2 | 7 | 5 | 47 |

HPV denotes human papillomavirus.
*Women in arm 1 used a self-administered tampon before the physician-directed swab of the cervix.
†Women in arm 2 underwent the physician-directed swab before using the self-administered tampon.
Note: Low-risk types included HPV 6, 11, 42, 43, 44 only. Oncogenic included intermediate- and high-risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 types, even if a low-risk type was present.

women with positive test results for an intermediate- or high-risk type of HPV. These oncogenic HPV DNA types were combined because they are likely to be clinically significant for predicting high-grade cervical intraepithelial disease and squamous cervical cancers. When oncogenic HPV DNA types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) were used to define a positive result for HPV, those infected with low-risk HPV types only (6, 11, 40, 42, 43, 44) were classified as negative, and when women with unidentifiable HPV types were omitted, the overall concordance rate of oncogenic HPV positivity between collection devices was 87.8%. The relative sensitivity for detecting oncogenic types of HPV DNA by the tampon for arms 1 and 2 were 75% and 90.9%, respectively. The relative sensitivity for detecting oncogenic types of HPV DNA by the swab for arms 1 and 2 were 62.5% and 63.6%, respectively. Overall, the relative sensitivity of the tampon and swab was 84.2% and 63.2%, respectively. The agreement of results between the 2 collection methods yielded a $\kappa$ of less than 0.5. The McNemar test did not detect an over- or under-identification of oncogenic HPV DNA with one particular collection method ($\chi^2 = 0.9$, $P = .34$).

Test results and HPV subtypes are shown in Table 2 for the 12 women referred after a recent abnormal Pap test result. The overall concordance rate in this high-risk group was 75%. Among these women the relative sensitivity was 100% for the tampon self-collected method and 57% for the swab method. In evaluating the pattern of discordance, the McNemar test ($\chi^2 = 1.33$, $P > .1$) again suggests that neither collection method is superior or inferior to the other in ability to detect HPV DNA, although the number of women in this subgroup is small.

## DISCUSSION

To study and assess the burden of HPV disease in our communities, a simple, accurate, and inexpensive method must be developed and tested for reliability and validity. Currently, most studies of HPV are clinic based:

**TABLE 2**

**Test Results for Women with Recent History of Abnormal Papanicolaou's Test Cytology (N = 12)**

| Patient | Tampon HPV Result | Swab HPV Result | Tampon HPV Subtypes | Swab HPV Subtypes |
|---|---|---|---|---|
| 1 | Negative | Negative | None | None |
| 2 | Negative | Negative | None | None |
| 3 | Negative | Negative | None | None |
| 4 | Negative | Negative | None | None |
| 5 | Negative | Negative | None | None |
| 6 | Positive | Negative | 43, 44, 56 | None |
| 7 | Positive | Negative | 16, 42, 43, 52 | None |
| 8 | Positive | Negative | 44 | None |
| 9 | Positive | Positive | 16, 31, 51 | 16, 39 |
| 10 | Positive | Positive | 39 | 16, 39 |
| 11 | Positive | Positive | 16 | 16 |
| 12 | Positive | Positive | 16, 31, 33, 52, 56 | 6, 11, 31, 56 |

a woman must go to a health care facility for a pelvic examination each time she is followed up for HPV disease. This makes true community-based HPV prevalence difficult to ascertain and study.

Our results show that neither the tampon nor the swab is significantly superior in HPV DNA detection, but there may be a trend toward increased relative sensitivity with the tampon. In the first analysis, where all positive test results of HPV DNA were grouped together, there was a trend toward increased relative sensitivity of the swab when the swab samples were collected first (arm 2). This may suggest a possible order effect. This possible order effect is not seen in the second analysis when only oncogenic HPV types are used to define positive HPV test results, omitting the unidentified types and placing the low-risk types in the negative group. In this analysis, the relative sensitivity of the swab remains constant regardless of the order in which the samples were collected.

Our study results are in agreement with Fairley and colleagues,[3] who have used the tampon for HPV detection in aboriginal Australian populations. Both studies indicate that the tampon is a sensitive instrument for cervicovaginal HPV DNA detection. Our concordance rate of 80% to 90% for HPV DNA detection by the tampon and physician-directed swab is comparable with rates others have reported for the tampon, cervicovaginal lavage, or self-directed swab. The concordance of exact HPV types is also similar.[3-7] The finding that the $\kappa$, which controls for chance agreement, was .49 when the 2 collection methods were compared is of some concern, however, and suggests that further improvements are needed before self-sampling for HPV testing is implemented in a research or public health setting.

Differences in HPV detection between the tampon and swab collection methods have been ascribed to differential cellular viral shedding, localized HPV infections, or an incomplete sampling of the infected tissue. The laboratory method used for HPV detection is also an important determinant of a positive HPV DNA test result. In our study, we used the Hybrid Capture HPV testing method, the only method currently approved by the FDA for clinical use. Future studies will need to address the issue of agreement between clinician and self-administered collection methods using the more sensitive polymerase chain reaction–based HPV assays and the next generation Hybrid Capture Microtiter testing method.

There are at least 4 implications for the clinical use of a self-administered HPV test: (1) to triage a woman with HPV testing who already has an ASCUS cytology prior to colposcopy without the additional cost of another physician visit; (2) to evaluate the self-administered HPV test as a primary screening for cervical cancer, especially in those populations wherein access to health care is difficult; (3) for self-monitoring of sexually transmitted diseases that would require further diagnostic work-up or treatment; and (4) to monitor the HPV infection rates of women in HPV vaccine trials.

## CONCLUSIONS

The multifocal nature of anogenital papillomavirus infection supports the use of the more broadly targeted self-collection devices as an indicator of HPV infection. Our results show that neither the tampon nor the swab is superior to the other in HPV DNA detection. Our data suggest that the development of a reliable cervicovaginal cell self-

collection device that would broaden our ability to screen underserved populations could reach those who would not make an office visit for physician-directed screening.

**ACKNOWLEDGMENTS**

The Robert Wood Johnson Foundation supported Dr Harper's work on this project. We gratefully acknowledge the following contributors to the project: Jackie King for aid with shipment, storage, and sample preparation; Alice Corrigan for help in HPV testing; and Robin Seitzman and Bobbi Robbins for assistance in collecting data and samples at the clinics.

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