Original article

Diagnosis of human papillomavirus infection by dry vaginal swabs in military women

Keerti V Shah, Richard W Daniel, Marie K Tennant, Nina Shah, Kelly T McKee Jr, Charlotte A Gaydos, Joel C Gaydos, Anne Rompalo

Objective: Human papillomavirus (HPV) assays are likely to be used with increasing frequency in clinical management of women with abnormal Papanicolaou smears and in cervical cancer screening. Our objective was to simplify the method of collection of female genital tract specimens. The utility of vaginal dry swabs for HPV diagnosis was evaluated.
Methods: Specimens for cytology and for HPV identification were collected by a clinician from 189 female soldiers attending a military clinic. Three methods of specimen collection for HPV identification were compared: a vaginal dry swab (v-DRY), and vaginal and cervical swabs placed into specimen transport medium (v-STM and c-STM). Swabs were shipped to a STD laboratory for processing. Specific HPV types were identified by a consensus primer based PCR based method. Results from 165 women were evaluable.
Results: HPV prevalence by the three methods was similar and ranged from 44.8% to 50.9%. 53 (32.1%) women were HPV positive and 60 (36.4%) women were HPV negative by all three collection methods. With respect to the risk categories of specific HPV types, there was greater agreement between the results from the two vaginal (v-DRY and v-STM) samples (kappa values of 0.69–0.81) than between the cervical (c-STM) and either of the vaginal samples (kappa values of 0.37–0.55). The HPV yield from c-STM was somewhat greater than that from the vaginal specimens but the correlation between cytological abnormalities and HPV was high for all three methods.
Conclusion: A dry vaginal swab may be an acceptable method of specimen collection for HPV diagnosis.
(*Sex Transm Inf* 2001;77:260–264)

Keywords: human papillomaviruses; diagnostic assays; dry swabs

Johns Hopkins University School of Public Health and Hygiene, Baltimore, MD, USA
K V Shah
R W Daniel
N Shah

Womack Army Medical Center, Fort Bragg, NC, USA
M K Tennant
K T McKee Jr

Johns Hopkins University School of Medicine, Baltimore, MD, USA
C A Gaydos
A Rompalo

US Department of Defense Global Emerging Infections Surveillance and Response System, Silver Spring, MD, USA
J C Gaydos

Correspondence to:
Joel C Gaydos, US Department of Defense, Global Emerging Infections Surveillance and Response System, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910–7500, USA
Joel.Gaydos@na.amedd.army.mil

Accepted for publication
3 April 2001

Introduction

Infection with genital tract human papillomaviruses (HPVs) is now widely accepted as the necessary event in the pathogenesis of invasive squamous cell carcinoma of the cervix and of the precursor lesions that precede invasive disease.[1 2] This observation has led to an examination of the utility of HPV assays in primary cervical cancer screening[3] and in the clinical management of women with abnormal Papanicolaou smears.[4] In primary cervical cancer screening, HPV assays have greater sensitivity but lesser specificity than Papanicolaou smears for detection of cervical neoplasia.[3] It is clear that women who have atypical squamous cells of uncertain significance (ASCUS) on their Papanicolaou smears and are HPV positive are more likely to have cervical neoplasia than women who have ASCUS but are HPV negative.[5]

One of the obstacles to universal and regular cervical cancer screening is the requirement for specimen collection by a trained clinician and the need for a pelvic examination. In the conventional collection method for an HPV assay, a clinician, during a pelvic examination, obtains cells from the cervical transformation zone with a swab or spatula and places them in specimen transport medium. After processing, the specimen is tested for the presence of HPV DNA by hybridisation assays.[6 7]

Recently, vaginal swabs, self collected by women and placed in transport medium, have been found to be suitable for HPV diagnosis.[8–10] A dry vaginal swab, which could be mailed to the laboratory, offers potential advantages in terms of collection, shipment, and cost over a vaginal swab placed in transport medium at the collection point. We report here the results of a comparison of three methods of specimen collection by a clinician for HPV diagnoses in female US army soldiers.

Methods

STUDY SUBJECTS

The study was conducted between March and September 1997. Consecutive active duty military women, 18–59 years of age (median age 24 years) attending the Epidemiology and Disease Control (EDC) Clinic at Womack Army Medical Center (WAMC), Fort Bragg, NC, USA, were invited to participate in the study. Any woman who presented to the EDC Clinic for evaluation of genitourinary symptoms, for therapy as a known contact of an individual with a diagnosed STD, or for routine STD screening because of a self perceived risk, was approached in a confidential manner for study enrolment. Eighty seven per cent of the women who were approached volunteered to join the study. The study protocol was approved by the institutional review boards for research on human subjects at the Johns Hopkins Medical Institutions and WAMC, and the human subjects research review board of the US army surgeon general.

Key messages

- The frequencies and types of human papillomavirus (HPV) detected in vaginal dry swabs were similar to those found in vaginal swabs and cervical swabs placed in transport media.
- The HPV prevalence was high (44.8%–50.9%) in young military women seeking health care by all three specimen collection methods.
- There was good correlation between abnormal cervical cytology (Papanicolaou smears) and HPV detection by each of the three collection techniques.

CLINICAL PROCEDURES

Three different methods for genital specimen collection were used by clinicians in this study: a dry vaginal swab (v-DRY), a vaginal swab in specimen transport medium (v-STM), and a cervical swab in transport medium (c-STM). The clinician inserted the v-DRY swab into the vaginal canal when the woman was in the lithotomy position. The swab was then removed and placed in a sterile tube. The v-STM swab was then collected as described above but placed immediately in 1 ml of Roche (Roche Molecular Systems, Branchburg, NJ, USA) specimen transport medium. The c-STM swab from the cervical transformation zone was collected during speculum examination and placed in 1 ml of the Roche transport medium. At the completion of the clinical examination all swabs were refrigerated at 4°C, then shipped on wet ice so as to reach the Johns Hopkins STD Research Laboratory in Baltimore, MD, within 4 days of collection. In the STD laboratory, 1 ml of Roche specimen transport medium was added to the tube containing the v-DRY swab. The swab was swirled in the medium and then removed. Papanicolaou smears were collected using a cytobrush (Hardwood Products Company, Guilford, ME, USA) and submitted to the WAMC department of pathology for interpretation according to the Bethesda system.[11]

HPV DNA IDENTIFICATION BY POLYMERASE CHAIN REACTION (PCR)

In the STD laboratory all specimens were processed according to the manufacturer's instructions for the Amplicor Chlamydia kit (Roche) by adding 1 ml of specimen diluent buffer. Processed specimens were stored at −80°C until tested. DNA from 100 µl of the sample was precipitated by adding 2.5 µl glycogen (20 mg/ml), 33 µl of 10M sodium acetate, and 339 µl of ethanol. After centrifugation at room temperature for 30 minutes, the supernatant was decanted and discarded and the pellet washed once with 70% ethanol (500 µl). The pellet was dried and rehydrated in 100 µl of TRIS EDTA. Ten µl of the rehydrated specimen was employed for HPV diagnoses by PCR. The procedures for HPV amplification and detection have been described elsewhere.[6 12] Briefly, HPV primers MY09/11/HMBO1 were used in conjunction with the β globin primers PC04/GH20 to amplify, respectively, a 450 bp segment of the HPV L1 region and a 268 bp segment of the cellular β globin gene. Globin amplification served as a control for the suitability of the cellular DNA for PCR. Forty cycles of amplification (95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds) were followed by a 5 minute extension at 72°C. The PCR products were then transferred to nylon filters in a 96 well format where they were hybridised with biotinylated probes. The probes included a generic HPV probe capable of detecting most HPV types, type specific probes for 32 HPVs, and a β globin probe. Hybridisation was detected using Amersham's Enhanced Chemiluminescence (ECL) system (Amersham Pharmacia Biotech, Incorporated, Piscataway, NJ, USA).

STATISTICAL ANALYSIS

Differences in proportions of women positive between collection methods, to include proportions positive for any HPV or specific HPV types, were assessed using the χ^2 test. Agreement between collection methods and HPV risk categories was measured using the kappa statistic (measure of agreement is 0 when the amount of agreement is what would be expected by chance and 1 when there is perfect agreement).[13] Analyses were done using standard methods in Statistical Analysis System (SAS Institute, Cary, NC, USA).

Results

All three types of specimens were collected and tested from each of 189 women. β Globin was not amplified from 10 (5.3%) of v-DRY, 12 (6.4%) of v-STM, and from seven (3.7%) of c-STM specimens. These specimens were called unsatisfactory. Twenty four women who contributed one or more of these unsatisfactory specimens were excluded from analyses. Therefore, the analysis was restricted to the specimens (n=495) from 165 women for whom all three specimens tested satisfactorily.

HPV prevalence (any HPV) was similar by the three methods: 47.3% for v-DRY, 44.8% for v-STM, and 50.9% for c-STM specimens (p=0.54). The distribution of the specimen results of all three assays for any HPV (positive by generic probe) is shown in table 1. Identical results were obtained by all three methods for 113 (68.5%) of the 165 women; 53 (32.1%)

Table 1 Correlation of results (any HPV) for the three types of collection methods

Collection method			
v-DRY	*v-STM*	*c-STM*	*Any HPV, n (%)*
+	+	+	53 (32.1)
−	+	+	6 (3.6)
+	+	−	9 (5.5)
−	+	−	6 (3.6)
+	−	+	10 (6.1)
−	−	+	15 (9.1)
+	−	−	6 (3.6)
−	−	−	60 (36.4)
			165 (100)

HPV = human papillomavirus; v-DRY = dry vaginal swab; c-STM = cervical swab in specimen transport medium; v-STM = vaginal swab in specimen transport medium.

Table 2 Generic and type specific HPV prevalence for three collection methods

	Specimens, n (%)		
HPV types	*v-DRY*	*v-STM*	*c-STM*
Any HPV	78 (47.3)	74 (44.8)	84 (50.9)
High cancer risk			
16	9	8	11
18	4	3	10
31	7	8	8
45	4	3	3
Any high risk	21 (12.7)	20 (12.1)	23 (13.9)
Intermediate cancer risk			
33	3	2	3
35	2	1	2
39	1	4	1
51	8	8	4
52	8	10	5
56	3	2	3
58	4	4	8
59	1	0	4
68	3	2	5
Any intermediate risk	28 (17.0)	26 (15.8)	29 (17.6)
Low cancer risk			
6	8	8	9
11	2	2	6
26	1	0	5
32	0	0	1
40	0	0	0
42	0	0	0
53	4	5	5
54	5	1	2
55	0	1	1
57	0	0	0
61	3	2	0
66	4	2	3
69	0	0	0
70	1	2	5
72	1	1	0
73	2	1	0
MM4	0	4	0
MM7	6	5	7
MM8	5	5	3
CP8304	3	2	2
IS039	0	0	0
Untyped	12	12	19
Any low risk	48 (29.1)	34 (20.6)	54 (32.7)

HPV = human papillomavirus; v-DRY = dry vaginal swab; c-STM = cervical swab in specimen transport medium; v-STM = vaginal swab in specimen transport medium.

Table 3 Distribution of specimens by the number of HPV types detected by each collection method (n=495 specimens)

	Specimens		
Number of types	*v-DRY n (%)*	*v-STM n (%)*	*c-STM n (%)*
0	87 (52.7)	91 (55.2)	81 (49.1)
1	55 (33.3)	55 (33.3)	60 (36.4)
2	15 (9.1)	12 (7.3)	11 (6.7)
3	5 (3.0)	2 (1.2)	7 (4.2)
4	1 (0.6)	3 (1.8)	2 (1.2)
5	2 (1.2)	1 (0.6)	2 (1.2)
6	0	1 (0.6)	1 (0.6)
7	0	0	0
8	0	0	1 (0.6)
⩾Two types	23 (13.9)	19 (11.5)	24 (14.5)

HPV = human papillomavirus; v-DRY = dry vaginal swab; c-STM = cervical swab in specimen transport medium; v-STM = vaginal swab in specimen transport medium.

*Table 5 Correlation between Papanicolaou smear results and HPV prevalence by the three collection methods**

		HPV+ specimens, n (%)		
Pap result	*Total*	*v-DRY*	*v-STM*	*c-STM*
Normal	137	56 (40.9)	52 (38.0)	65 (47.4)
ASCUS	17	12 (70.6)	12 (70.6)	11 (64.7)
LSIL	7	7 (100)	7 (100)	6 (85.7)

*Results from four unsatisfactory Papanicolaou smears are excluded.
HPV = human papillomavirus; HPV+ = positive for HPV; v-DRY = dry vaginal swab; c-STM = cervical swab in specimen transport medium; v-STM = vaginal swab in specimen transport medium; ASCUS = atypical squamous cells of uncertain significance; LSIL = low grade squamous intraepithelial lesions.

women were positive and 60 (36.4%) women were negative for any HPV by all three collection methods. The numbers of specimens positive exclusively by one assay were six (3.6%) for v-DRY, six (3.6%) for v-STM, and 15 (9.1%) for c-STM.

The type specific diagnoses from each of the three kinds of specimens are shown in table 2. For most HPV types, c-STM specimens yielded equal or greater numbers of positive specimens, compared with v-DRY and v-STM specimens. The proportion of specimens that had multiple types was similar, ranging from 11.5% to 14.5% for the three collection methods (p=0.69) (table 3). Eight (1.6%) of the 495 specimens contained five or more HPV types (table 3).

Agreement between specimens collected by different methods for different HPV cancer risk categories was generally good (table 4). Agreements between the two vaginal specimens (v-DRY and v-STM) were in the range of 89.7–95.8% with kappa values of 0.69–0.81. However, there was less agreement between vaginal (v-DRY and v-STM) and cervical (c-STM) specimens (range 81.8–87.3%) with kappa values of 0.37 to 0.55.

Almost 80% of cervical cancers are attributed to the four high risk HPV types, 16, 18, 31, and 45.[2] A total of 35 women (21.2 %) were positive for one or more of these types by at least one of the collection methods. Among these 35 women, the cervical specimens (c-STM) were positive more often (65.7%) than vaginal specimens (60.0% for v-DRY and 57.1% for v-STM). These differences were not statistically significant (p=0.76).

The correlation between Papanicolaou smear results and HPV diagnoses from the three types of specimens is shown in table 5. The three collection methods gave comparable results. Overall, the HPV prevalence was higher in women with abnormal cytology. When the results of the three specimen types were combined, HPV assays were positive for 20 of

Table 4 Comparison of collection methods for diagnoses by HPV cancer risk categories

	v-DRY v v-STM		*v-DRY v c-STM*		*v-STM v c-STM*	
HPV cancer risk category	*% agreement*	*kappa*	*% agreement*	*kappa*	*% agreement*	*kappa*
Low	89.7	0.69	82.4	0.48	81.8	0.46
Intermediate	91.5	0.69	87.3	0.55	82.4	0.37
High	95.8	0.81	86.7	0.42	87.3	0.44

HPV = human papillomavirus; v-DRY = dry vaginal swab; c-STM = cervical swab in specimen transport medium; v-STM = vaginal swab in specimen transport medium.

Table 6 HPV types recovered from LSIL patients by the three methods

LSIL patient	Age	HPV type(s) detected	Collection method		
			v-DRY	v-STM	c-STM
1	19	33	33	33	33
2	23	11, 66	11, 66	11, 66	11, 66
3	20	56	56	56	—
4	19	39, 45, 54, 61, cp8304	39, 45, 54, 61, cp8304	39	39
5	20	Pap291	Pap291	Pap291	Pap291
6	24	51	51	51	51
7	20	11, 16, 18, 66, 68	16, 68	16	11, 16, 18, 66, 68

HPV = human papillomavirus; LSIL = low grade squamous intraepithelial lesions; v-DRY = dry vaginal swab; c-STM = cervical swab in specimen transport medium; v-STM = vaginal swab in specimen transport medium.

the 21 specimens (95.2%) derived from the women who had low grade squamous intraepithelial lesions (LSIL), from 35 of the 51 specimens (68.6%) from women with ASCUS, and from 173 of the 411 specimens (42.1%) from women with normal cytology (LSIL *v* normal, p=0.0001; ASCUS *v* normal, p=0.0003).

The type specific HPV diagnoses by each of the three collection methods for the seven cases of LSIL are shown in table 6. Multiple infections were common and were detected in specimens collected by all three methods. High risk HPVs were found in specimens of two, intermediate risk types in two and low risk types in three of these seven women.

Discussion

The HPV prevalence was high (44.8%–50.9%) in this group of young military women by all three specimen collection methods. This was not surprising because the women were considered to be at a high risk of sexually transmitted infections, by virtue of their attendance at the WAMC EDC Clinic. Infection with multiple HPV types was common in our study, a finding previously described by others.[14]

Our data show that the frequencies and types of HPV detected in vaginal dry swabs (v-DRY) were similar to those found in vaginal swabs placed in transport media (v-STM) and those from cervical swabs, which were also placed in transport media (c-STM). Overall HPV prevalence was not significantly different among the three specimen collection methods. There was no difference in the proportion of specimens yielding multiple HPV types, and the numbers of high risk HPV types detected by each method were statistically similar. Moreover, there was good correlation between abnormal cervical cytology (Papanicolaou smears) and HPV detection by each of the collection techniques. In a study of intralaboratory reproducibility of HPV PCR assays, we found that when the same specimen was tested twice, the agreement between the results of the two tests for the presence of HPV was 95% with a kappa value of 0.89.[15] The agreement between v-DRY (47.3% positive) and v-STM (44.8% positive) was somewhat lower in this study, with kappa values ranging from 0.69 to 0.81 for the three risk categories of HPV. However, this degree of difference may not be meaningful.

The HPV yield from both types of vaginal specimens was lower than that from cervical swabs placed immediately into transport medium. This may be because the distribution of HPVs varies at different sites in the genital tract. However, HPV diagnosis is clinically useful because of its relation to Papanicolaou smear abnormalities. In this respect, the vaginal specimens as well the cervical specimens had a nearly 100% sensitivity for detection of SIL. Similarly, the three collection methods identified the virus in 64.7–70.6% of women with ASCUS. We had no women with high grade SIL in our study population.

A major advantage of a vaginal specimen over a cervical specimen is that it can be self collected. Previous studies have shown that self collection is an acceptable alternative to clinician collection for HPV diagnoses.[16 17] Self collection could improve access to health care, reduce healthcare costs, and save time for patients and providers. In the present study, the vaginal as well as the cervical specimens were collected by the clinician. However, we have shown that with respect to the identification of chlamydial and gonococcal infections, self collected vaginal dry swabs are equivalent to clinician collected vaginal specimens.[18] Therefore, we expect that the HPV results from self collected and clinician collected dry swabs will be comparable, but this needs to be verified. Dry swabs may not require refrigeration but this needs to be evaluated as well.

Self collected dry vaginal swabs for HPV diagnosis may be useful in several circumstances. Military women who are on active duty in places away from medical clinics could use this method when HPV diagnosis is required or desired. The same swab can be utilised for the PCR detection of several different sexually transmitted organism.[18] The method may also be useful in clinical management—for example, in monitoring HPV shedding in women who have been treated for clinical lesions. Clearing of the HPV infection would be supporting evidence of successful treatment.[19] The method would also be useful in screening surveys of genital tract infections where pelvic examinations are impractical. More importantly, availability of methods like this will give women the opportunity to take a greater role in meeting their own health needs.

Funding and disclaimer: Funding was provided by a Women's Health Defense Initiative grant from the Department of the Army (DAMD17-1-6309). The opinions presented are those of the authors and do not necessarily represent the policies or positions of the US Department of Defense, US Army or the institutions with which the authors are affiliated.

Contributors: KVS, KTM, Jr, CAG, JCG, and AR designed the study; RWD directed the laboratory work; MKT conducted the fieldwork; NS did the statistical analysis. All authors participated in the preparation of the manuscript.

1 International Agency for Research on Cancer. *IARC monograph on the evaluation of carcinogenic risks to humans. Vol 64: Human papillomaviruses*. Lyons: IARC, 1995.
2 Walboomers JMM, Jacobs MV, Manos MM, *et al.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;**189**:12–19.
3 Cuzick J, Sasieni P, Davies P, *et al.* A systematic review of the role of human papillomavirus testing within a cervical screening programme. *Health Technol Assess* 1999;**3**:1–204.
4 Schiffman MH, Bauer HM, Hoover RN, *et al.* Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1993;**85**:958–64.
5 Cox JT, Lorincz AT, Schiffman MH, *et al.* Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical

squamous cells of undetermined significance. *Am J Obstet Gynecol* 1995;**172**:946–54.
6 Manos MM, Ting Y, Wright DK, *et al.* The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 1989;7:209–14.
7 Lorincz A. Hybrid Capture™ method for detection of human papillomavirus DNA in clinical specimens. *Papillomavirus Rep* 1996;7:1–5.
8 Moscicki A-B. Comparison between methods for human papillomavirus DNA testing: a model for self-testing in young women. *J Infect Dis* 1993;**167**:723–5.
9 Morrison EAB, Goldberg GL, Hagan RJ, *et al.* Self-administered home cervicovaginal lavage: a novel tool for the clinical-epidemiologic investigations of genital human papillomavirus infections. *Am J Obstet Gynecol* 1992;**167**:104–7.
10 Wright TC, Denny L, Kuhn L, *et al.* HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. *JAMA* 2000;**283**:81–6.
11 National Cancer Institute Workshop. The Bethesda System for reporting cervical/vaginal cytologic diagnoses. *JAMA* 1989;**262**:931–4.
12 Hildesheim A, Schiffman MH, Gravitt PE, *et al.* Persistence of type-specific human papillomavirus infection among cytologically normal women in Portland, Oregon. *J Infect Dis* 1994;**169**:235–40.
13 Landis JR, Koch GG: The measurement of observer agreement for categorical data. *Biometrics* 1977;**33**:159–74.
14 Thomas KT, Hughes JP, Kuypers JM, *et al.* Concurrent and sequential acquisition of different genital human papillomavirus types. *J Infect Dis* 2000;**182**:1097–102.
15 Daniel RW, Ahdieh L, Hayden D, *et al.* Intra-laboratory reproducibility of human papillomavirus identification in cervical specimens by a polymerase chain reaction-based assay. *J Clin Virol* 2000;**19**:187–93.
16 Sellors JW, Lorincz AT, Mahony JB, *et al.* Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. *CMAJ* 2000;**163**:513–18.
17 Wright TC, Denny L, Kuhn L, *et al.* HPV testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. *JAMA* 2000;**283**:81–6.
18 Rompalo A, Gaydos C, Crotchfelt K, *et al.* Evaluation of a novel approach to the diagnosis of genital infections in female soldiers. Abstract No 416 presented at the 13th Annual Meeting of the International Society for Sexually Transmitted Research, 11–14 July 1999, Denver, Colorado, USA.
19 Kjellberg L, Wadell G, Bergman F, *et al.* The human papillomavirus genome regularly disappears after conization of cervical dysplasia by carbon dioxide laser. Abstract No 196 presented at the 18th International Papillomavirus Conference, 23–28 July 2000, Barcelona, Spain.