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Low False-Negative Rate of PCR Analysis for Detecting Human Papillomavirus-Related Cervical Lesions

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Although PCR analysis is a sensitive test for detection of human papillomavirus (HPV) in the cervix, the proportion of cases of cervical dysplasia missed, or the false-negative rate, has been unknown. We determined the accuracy of PCR analysis for HPV DNA as a predictor of HPV-related cervical lesions in a cross-sectional study of sexually active women, aged 18 to 50 years, from the University of Michigan Family Medicine HPV study. Of 133 eligible participants, 41 underwent colposcopy because of a positive result for HPV of the cervix by the PCR method and 92 underwent screening colposcopy with biopsy prior to knowing the HPV PCR results. Twenty-four of those screened were subsequently found to also be HPV DNA positive. In those found to be HPV positive, histological studies revealed the presence of condyloma or cervical intraepithelial neoplasia in 16 women (24.6%) and changes suggestive of condyloma in 5 (7.6%). No HPV-negative woman had an abnormal biopsy or cytology report ($P = 0.000001$). The false-negative rate (1 − sensitivity) for HPV PCR analysis for detection of the presence of a cervical HPV-related lesion was 0% (95% confidence interval, 0 to 0.047), and the specificity was 60.7%. In summary, PCR analysis for HPV DNA had a very low false-negative rate for predicting HPV-related lesions of the cervix in a community-based population. This supports the validity of using the absence of HPV at the cervix, as determined by PCR testing, as an inclusion criterion for patients in control groups in studies dealing with low-grade cervical lesions.

Human papillomavirus (HPV) infection of the cervix is common in sexually active women, even in those traditionally thought to be at low risk for having a sexually transmitted infection (2, 32, 45). Certain HPV types are etiologically associated with cervical cancer and with its precursor, squamous intraepithelial lesions (4, 36). HPV infections are typically asymptomatic (1a, 32), and clinical examination and Papanicolaou smears are insensitive screening tests for detecting the virus and HPV-related cervical lesions (6, 19, 23, 31, 32, 38).

Highly sensitive methods for detecting HPV DNA have been recently developed, the most sensitive of which is the PCR method. Using PCR analysis, as little as one molecule of HPV DNA in 10^5 cells can be detected in vivo (43). This added sensitivity has clinical relevance, because women with a positive PCR test for HPV DNA have an increased prevalence of pathological changes on colposcopically directed biopsy specimens, despite having normal cervical cytology (19, 45).

Despite the high prevalence of HPV infection in women with known precancerous cervical lesions detected because of a clinical abnormality (an abnormal Papanicolaou smear or a cervical lesion), data on whether PCR analysis misses women with HPV-related lesions that are found only by colposcopic evaluation and biopsy are lacking. This unknown false-negative rate has limited our ability to use the absence of HPV, as determined by the PCR method, as an indication that no subclinical lesions are present. To determine the accuracy of this test (sensitivity, specificity, negative predictive value, and positive predictive value) for the diagnosis of disease, studies of groups of women with negative as well as positive PCR tests, including colposcopically directed biopsies of women regardless of HPV status, who have no other known indication for such an evaluation must be conducted. The results would provide data on (i) how often patients with cytologically unrecognized HPV-related cervical changes are missed by PCR analysis (false-negative rate), (ii) the proportion of women with a negative PCR test for HPV DNA who have no HPV-related cervical lesions on colposcopically directed biopsy specimens (negative predictive value), (iii) the proportion of women with positive PCR tests that are predictive of abnormal histology (predictive value of a positive test), and (iv) the proportion of women with negative histology who also have a negative test for HPV DNA (specificity). Only with this information can we evaluate the clinical value of PCR analysis for HPV DNA, as well as its potential role and appropriate use in research, in screening, and in follow-up for precancerous or cancerous lesions of the cervix.

The specific aim of our study was to determine prospectively the diagnostic performance characteristics of PCR analysis for HPV DNA as a predictor of HPV-related cervical lesions in sexually active women seen in community primary-care practices.

MATERIALS AND METHODS

Study population. All patients who enrolled between 1 March 1990 and 30 June 1993 in a prospective case control study on vaginitis that was being conducted in two community-based offices in southeastern Michigan were also offered the opportunity to participate in the University of Michigan Family Medicine HPV study. One office was in a college town of 100,000 people; the other was in a rural town of approximately 4,000 persons. Participating women were aged 18 to 50, were sexually active within the past 2 months, and presented either because of vaginal symptoms (itching, burning, or odor) or for a routine pelvic examination for some other reason (such as for cytology smear testing or contraception).

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Clinical evaluation. After written informed consent was obtained, patients completed a self-administered questionnaire evaluating numerous known or potential risk factors for HPV infection or HPV-related cervical disease and for vaginal infection (32). A standardized pelvic examination was performed by the clinician, and specimens for a cervical cytology smear and PCR testing for HPV DNA, and microbiological cultures for other vaginal and cervical pathogens were obtained.

Laboratory tests. All laboratory tests were performed at regional reference laboratories by standard techniques as previously described (45). A specimen for PCR analysis for HPV DNA was collected from the external cervical os of each patient by the use of a single Dacron swab and placed in Virapap transport medium. The specimens for HPV PCR analysis were frozen at −70°C and were batched and transferred from the clinics to the laboratories involved. Clinical specimens for use in testing for other vaginal and cervical pathogens were sent directly to the laboratories performing the tests. All laboratory personnel were blinded to clinical information about the patients.

PCR was performed as described previously (45). Briefly, cellular DNA was precipitated from the Virapap transport medium with isopropanol and resuspended in water. DNA quality was evaluated by testing an aliquot by PCR with primers specific for anonymous unique human genomic sequences. Samples yielding the expected 350-bp fragment on ethidium bromide-stained gels were analyzed for the presence of HPV sequences. The amplification of HPV DNA sequences was performed with the following generic primers, located in the E1 open reading frame: IU (5′-TIIRIRIIYTAAAACGAAAGT-3′) and IWDO (5′-RTCRWAIGCCCAYTGIACCAT-3′) (where I = inosine and other letters are the IUPAC single-letter codes) (17). Amplified reaction products were electrophoresed in agarose gels, transferred to nylon membranes (Hybond; Amersham), and hybridized to three separate probe mixes containing either HPV types 6 and 11 or types 16, 18, 31, 33, 35, and 52 under nonstringent conditions (T_m − 40°C). Samples giving a positive signal were rehybridized under stringent conditions (T_m − 10°C) against single probes (HPV type 6, 11, 16, 18, 31, 33, 35, or 52). Samples positive for hybridization at T_m − 40°C but negative under stringent conditions (T_m − 10°C) were considered to contain an unknown HPV type. Positive and negative controls were included with all PCR series and were analyzed in parallel with the clinical specimens.

Papanicolaou smear. A cellular sample was obtained from each participant by scraping the squamocolumnar junction of the cervix with a wooden Ayres spatula; this was followed by scraping of the endocervix with a CytoSoft cytology brush. Both specimens were submitted on one slide. Within 10 s of sample application, the slides were fixed with cytologic fixative, transported within 8 h to the University of Michigan Hospital cytopathology laboratories, processed, and examined by a qualified cytotechnologist and a cytopathologist by a standardized protocol that included a standard narrative method of interpretation. (The Bethesda System was not being used at the University of Michigan Hospitals during the time period of this study.) A cytology smear was considered positive if abnormal cells consistent with HPV infection, condyloma, dysplasia, carcinoma in situ, or invasive cervical cancer were reported.

Colposcopy. Colposcopies were performed on patients in two groups. The first group consisted of all patients enrolled in the original vaginitis-HPV study from 1 March 1990 to 15 June 1992 who either tested positive for HPV DNA by PCR analysis or had a positive Papanicolaou smear and who agreed to colposcopy; these patients were charged for the colposcopy procedure. The second group consisted of women enrolling in the vaginitis-HPV study from 16 June 1992 to 31 July 1993 whose HPV statuses were not known at the time of enrollment and who agreed to have free-of-charge colposcopy with biopsy in order to assess the specificity and negative predictive value of the PCR analysis.

Prior to all colposcopies, written informed consent was obtained. Colposcopic examinations of the perianal, vulvar, and vaginal areas were performed at a magnification of between ×4 and ×25. The uterine cervix was similarly evaluated, but that evaluation also included a green filter review and a review after application of a 4% acetic acid solution and Lugol's solution. A cervical biopsy was performed on each woman; any areas suspicious for HPV-associated disease were included, or it was done at random at the squamocolumnar margin if no suspicious areas were present. Women with known HPV infections, and with an area suggestive of HPV-related changes, were subjected to endocervical curettage.

Histology. Cervical biopsy and endocervical curettage specimens obtained from patients with HPV infection detected prior to colposcopy were read at the University of Michigan pathology laboratory by certified pathologists. Specimens obtained from patients who were enrolled in the colposcopy screening portion of the study, whose HPV statuses were not known at the time of colposcopy, were read by one of the authors (A.F.). In addition, a subset of the specimens previously read at the University of Michigan pathology laboratory was sent to the second pathologist (A.F.) for blinded review to assess the interlaboratory diagnostic agreement rate.

Statistical methods. All data were encoded and entered on an IBM-compatible computer equipped with Statistical Package for the Social Sciences data entry software. Frequencies of outcome and risk variables were determined. Chi-square analysis was used to assess univariate associations between HPV DNA positivity, as detected by PCR analysis, and the presence of abnormal histologic findings on colposcopic biopsy specimens, as well as the associations of both PCR analysis and histology with other potential risk or confounding factors.

TABLE 1. HPV types identified

HPV type	No. (%) identified by:		Overall % identified
	HPV screening	Colposcopic screening	
None	223 (76)	98 (65)	72.3
6 or 11	6 (2)	1 (1)	1.6
16	17 (6)	4 (3)	4.7
18	5 (2)	5 (3)	2.2
31	8 (3)	5 (3)	2.9
33	2 (1)	0 (0)	0.5
35	7 (2)	8 (5)	3.4
52	1 (0)	0 (0)	0.2
Unknown type	12 (4)	15 (10)	6.1
Inhibition of PCR	11 (4)	5 (3)	3.6
Insufficient quantity of DNA or PCR not done	2 (1)	9 (6)	2.5
Total	294 (101)[a]	150 (104)[a]	100.0

[a] Percentages do not sum to 100% because of rounding.

Agreement between pathological diagnoses of a subset of biopsies at the two pathology laboratories was determined by using the kappa statistic.

RESULTS

All 456 patients in the concurrent vaginitis study enrolled in the HPV study between 23 January 1990 and 31 July 1993. Participation in the vaginitis study among those offered enrollment was greater than 90% for symptomatic women and approximately 35% for asymptomatic women, with the need to return for four follow-up visits in the concurrent vaginitis study cited as the reason for declining in >80% of the refusals. No demographic information is available on the women declining participation.

HPV-positive and HPV-negative groups. Of the 306 women enrolled prior to the availability of screening colposcopy, 294 had HPV DNA testing performed. Of these 294 women, 13 were excluded because of either inhibition of the PCR test by an unknown substance(s) (n = 11) or a lack of DNA in the sample specimen (n = 2). Of the remaining 281 women tested, 58 (20.6%) had a positive test for HPV DNA (Table 1). Of these, 41 women (70.7%) returned for colposcopic examination.

Of the 150 women enrolled after 15 June 1992 who were offered screening colposcopy, 93 (62.0%) had screening colposcopies and random biopsies within 3 months of enrollment. Of the remaining 57, 54 declined to have a biopsy, 1 was excluded due to pregnancy, 1 was excluded due to having had HPV diagnosed in the previous year, and 1 planned to undergo colposcopy elsewhere. One woman had an inadequate biopsy sample and was excluded from the analysis. Valid HPV DNA results were obtained for 136 of the 150 women (with 14 patients being excluded due to either inhibition of the PCR test [n = 5] or an inadvertent failure to do PCR testing [n = 9]; of those tested, 38 (27.9%) were positive for HPV DNA (Table 1). Of the 92 women who also had the screening colposcopy and an adequate biopsy specimen, 24 (26.1%) were subsequently found to be HPV positive. These 24 women plus the 41 women previously identified (before 15 June 1992) made up the HPV-positive group. The HPV-negative group was comprised of the 68 women screened by colposcopy who were negative for HPV by the PCR test.

There was no significant difference in HPV types of HPV-positive patients identified during the screening phase and

TABLE 2. Characteristics of women enrolled in this study

Characteristic	HPV-positive participants (n = 65)	HPV-negative participants (n = 68)
Age (yr ± SD)	29.4 ± 7.0	31.9 ± 7.1
Mean level of education (yr ± SD)	14.5 ± 2.4	15.3 ± 2.4[a]
Age at first intercourse (yr ± SD)	17.5 ± 3.7	17.8 ± 2.7
No. of sexual partners ever ± SD	9.2 ± 10.8	7.6 ± 9.1
No. of months participant has known current sexual partner ± SD	71.7 ± 75.0	102.9 ± 109.2[a]
Use oral contraceptives	34.4%	38.5%
Ever smoked	39.1%	39.3%
Single	51.6%	37.4%[b]
Caucasian	84.4%	85.7%
Household income (per yr)		
≤$14,000	15.9%	11.2%
>$49,000	30.1%	38.9%
History of abnormal cervical cytology	34.4%	27.0%
History of genital warts	17.5%	16.9%

[a] $P < 0.05$.
[b] $P = 0.08$.

those identified prior to the implementation of the screening protocol (Table 1).

Demographics. Characteristics of the 133 patients with complete HPV and colposcopic data are described in Table 2. Women with HPV infection were found to have had less formal education ($P = 0.04$), to have known their current partner a shorter length of time ($P = 0.05$), and to be more likely to be single ($P = 0.08$) than HPV-negative women. When stratified analysis was performed, the associations between marital status and HPV infection as well as educational level and HPV infection were present only for those who had known their partner for less than 24 months. No one had clinically apparent genital warts or other abnormal genital lesions.

Cytology. Of all participating women, only five exhibited abnormal cervical cytology (one atypia, one mild dysplasia, and three condyloma). All five of these participants were HPV DNA positive by PCR testing ($P = 0.006$).

Microbiological test results. Microbiological cultures revealed few sexually transmitted diseases among the participants. There were no patients with active herpes simplex virus infection, 4 patients with *Chlamydia trachomatis* infection of the cervix, 2 patients with *Neisseria gonorrhoeae*, 62 patients with *Ureaplasma urealyticum*, 16 with *Mycoplasma hominis*, 57 patients with symptomatic *Candida* vulvovaginitis (culture positive), 7 with bacterial vaginosis (as determined by using the Amsel criteria [1]), and 1 patient with *Trichomonas vaginalis*. The HPV-positive and HPV-negative groups differed significantly only in the prevalence of *M. hominis* (20.0 and 6.6% for the HPV-infected and the uninfected groups, respectively; $P = 0.03$); there was borderline significance for the prevalence of *U. urealyticum* (59.0 and 42.6%, respectively; $P = 0.07$).

Histology. Upon histological examination, 21 of the 133 women (15.8%) were found to have HPV-related lesions; 19 of these participants had had colposcopies after obtaining positive HPV test results, and 2 were screened-group members who were eventually found to be HPV positive. Examination of the biopsy specimens of the five women who had abnormal cytological smears revealed mild cervical intraepithelial dysplasia (CIN1) in two, CIN2 in one, and no evidence of HPV-related lesions in the other two women. The 18 remaining abnormal biopsy results (85.7%) occurred in women with normal cytology reports.

TABLE 4. HPV types identified on cervical biopsy of women with HPV-related lesions

HPV type[a]	No. with biopsy result of:			
	Changes suggestive of condyloma	Condyloma	CIN1	CIN2
16	2	3	1	
31			1	1
35	2	1		
52				1
Unknown type	1	6	1	1
Total	5	10	3	3

[a] None of the patients with abnormal colposcopically directed biopsy results was found to have HPV type 6 or 11, 18, 33, or 45.

The cervical biopsy results are shown in Table 3. In the combined HPV-positive group, 16 women (24.6%) had biopsy-confirmed evidence of HPV-related lesions (condyloma or CIN) and 5 (7.9%) others had changes suggestive of condyloma. None of the HPV-negative women had histology results suggestive of an HPV-related lesion ($P = 0.00001$). The HPV types identified in the women with HPV-related lesions on cervical biopsy are shown in Table 4.

An index of concordance (kappa statistic) of 0.52 (moderate concordance) (16) was found when 27 biopsy specimens of women who had had colposcopies during the first part of the study (i.e., only after testing HPV positive) were also read by the pathologist (A.F.) who read the biopsy specimens of those who were screened by colposcopy prior to knowing their HPV results.

Test characteristics of HPV DNA testing. Data from the women screened by colposcopically directed biopsy indicate that the false-negative rate of PCR analysis for HPV DNA for predicting HPV-related lesions of the cervix was 0% (95% confidence interval [CI], 0 to 0.047); conversely, all women with HPV-related lesions in this low-risk population had positive PCR test results (sensitivity, 100%). However, of those without lesions, 42 (38.2%) were positive for HPV DNA by the PCR method. The predictive value of a positive HPV test was 33.3%, and the predictive value of a negative test was 100% (i.e., all women with negative HPV test results lacked cervical lesions).

DISCUSSION

Despite the implementation of protocols for cervical cytological screening for precursors of invasive cervical cancer and

TABLE 3. Cervical biopsy results for patients with and without HPV infection as detected by PCR ($P = 0.00001$)

Patient HPV status	No. (%) with biopsy result of:							Total no.
	Normal	Cervicitis	Changes suggestive of condyloma	Condyloma	CIN1	CIN2	None[a]	
Positive	20 (30.8)	22 (33.8)	5 (7.7)	10 (15.4)	3 (4.6)	3 (4.6)	2 (3.1)	65
Negative	41 (60.3)	27 (39.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	68

a dramatic decrease in the number of deaths from cervical cancer, this malignancy remains a significant cause of death worldwide (8). A much larger number of women have precancerous cervical lesions. It remains unclear who is at increased risk for progression to cancer and how high- and low-risk groups should be evaluated, followed, or treated. Accurate information about the presence or absence of HPV-related lesions is critical for future studies.

Cytological smears have a substantial false-negative rate for predicting squamous intraepithelial lesions (13, 19, 40, 41). We previously reported that among low-risk women, 75% of those whose cervical biopsy specimens showed HPV-related lesions had negative cytological smears (45). Similarly, cervical cancer is not uniformly identified by cervical cytological examination. Cox et al. found that repeat cytological smears had sensitivities of 60 and 73% for low- and high-grade lesions, respectively (11). Moreover, from one-third to two-thirds of women with invasive cervical cancer had normal cytological smears within 3 to 5 years before the malignancy was found (9, 29). This poor predictive value of a negative cytological result suggests the need for better methods of detecting the presence of HPV-related lesions of the cervix.

Previous measures performed as adjuncts to cytology, including visualization techniques such as acetic acid staining, chemiluminescence (28), and cervicography (18, 33), have increased the sensitivity of screening for HPV-related lesions. More recently, DNA detection methods have resulted in a dramatic increase in the sensitivity for detecting the presence of HPV in cervical cells. Early studies of women with documented cervical lesions, using the Southern blot method, indicated a sensitivity of approximately 69 to 79% (19, 27, 35). The Virapap DNA test increased the sensitivity of cytological screening; 31.6% of women with a negative cytological smear but a positive Virapap test had low- or high-grade lesions on biopsy, whereas only 5.4% of persons for whom both tests were negative had these lesions (10).

PCR is even more sensitive for detecting HPV in cervical tissue, due to the amplification procedure that greatly increases the amount of DNA present (17, 43). Using this method, HPV has been detected in over 90% of condylomas, dysplasias, and cervical cancers (4, 42). We previously reported that although abnormal cytological smears occurred in only 3% of our community-based population, 20.3% percent of the women were HPV positive by PCR analysis, and of these, 52.9% had HPV-related lesions which were detected (condyloma to CIN2) on colposcopically directed biopsy (45). Others found similar results (13). However, in no study have HPV-negative women been systematically assessed colposcopically and biopsied to verify the lack of precancerous lesions of the cervix. This screening is necessary to determine whether a substantial false-negative rate (or insufficient sensitivity) is inherent in the PCR method. If present, this bias would increase the classification error in studies using the test and would minimize any differences in outcomes seen between the groups compared. Thus, knowledge of the false-negative rate for HPV testing by PCR analysis is necessary in order to interpret and design studies for the prevention, monitoring, management, and treatment of cervical lesions.

In this study, we assessed the false-negative rate for HPV testing (by the PCR method) for precancerous lesions of the cervix, using cervical biopsy as the "gold standard." Our population exhibited a low rate of abnormal cytological smears and a substantial rate of HPV infection as detected by PCR (20.3%), and undetected HPV-related lesions were evident in approximately 50% of infected individuals (45). We found the false-negative rate to be quite low (0%; 95% CI, 0 to 0.047), indicating that very few women with HPV-related cervical lesions in our low-risk population were missed when screened by PCR testing.

The specificity of the HPV DNA test for identifying HPV-related lesions was 61.8% in this study; i.e., 61.8% of the women whose biopsy specimens did not show evidence of HPV-related lesions were HPV DNA negative. The remaining 38.2% of the women might be classified as false positives; however, these women may comprise a group at higher risk for the development and progression of those lesions. Others have demonstrated that the presence of HPV predicts the development of lesions (25, 26) and that the presence of HPV type 16 or of multiple HPV types is a risk factor for progression of HPV-related lesions (22). As with all screening tests, only a proportion of those with positive tests will actually have the disease, but a higher-risk group for whom further testing might be indicated is identified.

We did find that a substantial proportion of the HPV cases detected (26.2%) were not one of the specific types identified, i.e., type 6 (or 11), 16, 18, 31, 33, 35, or 52. This proportion of unknown HPV types is not unusual. Kotloff et al. found that 22.0% of the HPV isolates in their study of college women were nontypeable with their 25 type-specific probes (24). Further work by one of us (L.G.), involving the cloning of the E1-amplified products from four of the unknown-HPV-type cases followed by sequence analysis, indicated that three of the four were of known but unmeasured types (types 51, 58, and 30) (16a).

Previously, we reported three obstacles to the use of PCR analysis for routine screening for cervical HPV infection in low-risk populations (45). The first was the lack of data regarding the false-negative rate of the test. The data presented here indicate that this rate is low. Second, PCR analysis is time intensive, requires diligent attention to cross-contamination risks, and has not previously been widely available. Since that previous report, the use of PCR testing in research and clinical settings has increased dramatically, and this technology is becoming more readily available. However, cross-contamination remains a concern, and laboratories performing this test must address this issue. Third, the natural histories of women with HPV, as determined by PCR testing, were unknown. Although studies suggest that the rate of conversion from latent infection to clinically significant disease is substantial (25, 26), factors identifying which HPV-infected women will have progression of disease to cervical cancer, even among those with higher-risk subtypes of HPV present or higher viral loads, are unknown. Most cervical lesions are low grade and either regress or persist without progression to more advanced changes (7, 34), and the majority of HPV infections are transient (15, 22, 30). However, the persistence of HPV infection as determined by PCR identifies a subpopulation at increased risk of persistence (20) or progression (34, 37) of cervical lesions. The clinical value of detecting all lesions as they develop, compared to the amount of morbidity, mortality, and costs generated by assessing the large number of transient lesions diagnosed by colposcopically directed biopsy, has not been determined. Further research is needed to determine whether there is public health value in the recognition of HPV infection at a time when the cytology is negative, to ensure cancer prevention and/or to allow recognition of infection status to prevent its spread. The data reported here on the low false-negative rate of PCR testing for HPV in women with normal cytology suggest that PCR testing is a reasonable tool for use in these studies.

There are limitations of our study that warrant highlighting. First, the women enrolled in the colposcopic screening study

had been enrolled in a case control study comparing women with vaginal symptoms (itching, discharge, or odor) to women presenting for a pelvic examination for an unrelated reason (usually for routine cytological smear or contraception). For the vaginitis study, all needed to be sexually active in the previous 2 months and to have a partner who might participate in the study; a sizable proportion (71%) had initial vaginal symptoms. Our population also consisted of women who were more likely to be married and were more educated than in most previous HPV studies in high-risk populations, reflecting a more typical community-based population (32). Our prevalence of HPV infection (20.6%), however, was similar to that reported from other community-based studies (5, 21), suggesting that our population was typical of that seen in community medical offices.

Second, although all lesions detected on biopsy were associated with a positive PCR test for HPV DNA, most lesions were low grade, as would be expected in this population. Cytology may be more sensitive with higher-grade lesions than with low-grade lesions (13). Data on quantitative HPV assessment in women with dysplasia indicate that high-grade lesions (CIN3 or CIN2) are associated with high copy numbers of HPV, suggesting that the sensitivity of HPV testing should remain high in this group (3, 12).

In addition, cervical biopsy specimens were interpreted at two different laboratories during the study. To assess the potential differences in interpretations between the two laboratories, a subset of the slides from the first laboratory were blindly reviewed by the coinvestigator who performed the histological analyses for the screening colposcopies (A.F.) to assess interobserver reliability; this was found to be moderate (kappa statistic = 0.52). Previous studies assessing the rate of interobserver variation found similar or less concordance between expert cytopathologists (14, 39, 44). The differences inherent in interpretation do suggest that the association between HPV testing and cervical biopsy findings is an estimate that is in part dependent on the variability in pathological interpretation. However, the moderate correlation between the interpretations of observers suggests that the degree of change in results would not be expected to be substantial.

The test characteristics of PCR analysis for HPV DNA as a method for identifying women with HPV-related lesions of the cervix were shown to be excellent, with a false-negative rate in this low-risk population of 0% (95% CI, 0 to 0.047). Although colposcopy with biopsy should continue to be performed in women with clinically evident abnormalities or abnormal cytological smears regardless of HPV testing, PCR testing for HPV DNA increases the accuracy of detection of HPV-related lesions and decreases the risk of false-negative categorizations that occur when using cytology and physical examination alone. Whether the improved detection available with the addition of this test is clinically necessary or cost-effective for preventing invasive cervical cancer in community-based populations, as opposed to using routine cytological smears alone, is unclear and needs further study. However, the use of this test in research protocols will improve the accuracy and reliability of the results reported.

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